Synthesis and Characterization of Thermosensitive Nanoparticles Conjugated with Chitosan

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Abstract

New thermosensitive nanoparticles, based on PNIPAAm–AA core and chitosan shell structure, were designed and synthesized for the controlled release of the loaded drug. PNIPAAm nanoparticle containing carboxylic group on their surface was synthesized using emulsion polymerization. The particle size of synthesized nanoparticles was varied from 380 nm to 25 nm as temperature of the dispersed medium increased. LMWSC-conjugated nanoparticles of 2wt% MBA, crosslinking monomer, leaded to a stable aqueous dispersion at concentration of 1mg/1ml otherwise showed gel collapse temperature. The effect of temperature on the dynamic behavior of the PNIPAAm chains were also studied by dynamic light scattering and UV–Vis absorption measurements.

Keywords: LMWSC, Lower critical solution temperature, Thermosensitive

1. Introduction

A new challenge is the development of the thermosensitive nanogels with biological activity, especially for drug delivery applications. Several authors have reported the thermosensitive gel systems based on PNIPAAm conjugated with biomaterials. Kono et al. synthesized liposomes coated with a copolymers of NIPAAm and N,N-didodecylacrylamide.1 The release of calcein from the copolymer-modified liposomes was very slow below LCST, whereas the release was rapid above LCST. Katayama et al reported the thermosensitive PNIPAAm nanogels conjugating with the receptor of protein kinase A.2

Recently our previous studies focused on chemical modifications, nanoparticle preparation, and drug-release behaviors of low molecular weight water-soluble chitosan (LMWSC). In the present study, we have designed novel thermosensitive nanoparticles, based on PNIPAAm core exhibiting LCST behaviors and chitosan shell having biocompatibility, to develop the controlled release of the loaded drug. This study disclosed the synthesis, characterization and thermosensitive behaviors of nanoparticles composed of either NIPAAm-co-AA or NIPAAm-co-AA conjugated with chitosan.
2. Experimental

Materials
Low molecular weight water-soluble chitosan (LMWSC) was obtained from KITTOLIFE Co. Korea. LMWSC was modified to enhance water-solubility as described previously as a water-soluble chitosan (MW: 10,000, deacetylation degree=97%).\(^3\) NIPAAm was obtained from Fisher Scientific Inc.(Fair Lawn, NJ, USA) and recrystallized from hexane. MBA and AA were purchased from Aldrich(Milwaukee, WI, USA) and used without further purification. Dialysis tubing (MWCO=12,000) was commercially obtained from Spectrum.

Preparation of NIPAAm-co-AA nanoparticles
6.80 g of NIPAAm (60mmol), 0.5 g of SDS (1.7mmol), and 0.14 g of MBA (0.91mmol) were dissolved in 470 g of double distilled water, degassed with argon. The synthesis was carried out under argon atmosphere to exclude oxygen. After heating the solution to 75 °C, 0.5 g of KPS (1.8mmol) and 0.3 g of AA (4.2mmol) in 30 g of water was added under rigorous stirring. The reaction became turbid and the reaction proceeded for 4 h at constant temperature. The obtained NIPAAm-co-AA nanoparticles in aqueous solution were dialyzed with distilled water by using a dialysis membrane (celluSep\(^\circ\)P, molecular weight cut-off= 5,000) for 24 h in order to purify the product.

Synthesis of thermosensitive nanoparticles based on PNIPAAm core and chitosan shell structure
2 ml of NIPAAm-AA solution (1.2 wt%) was uniformly dispersed in 20ml of de-mineralized water by ultrasonic for ten minutes. The prepared NIPAAm-AA solution was slowly added into the stirred LMWSC aqueous solution (10 mg in 10 ml de-mineralized water) and preadsorbed for 2 hours at room temperature. After the preadsorb procedure, 0.5 mg of 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDAC, Aldrich), the catalyst of carboxyl-amine conjugation reaction, was added to the solution described above. The nanoparticle- LMWSC conjugation reaction was carried out for 4 hours with stirring at 25 °C. The resulting LMWSC-conjugated nanoparticle aqueous solution was dialyzed with distilled water by using a dialysis membrane(celluSep\(^\circ\)P, MWCO=12,000) for 24 h in order to purify the product.

Characterization of the thermosensitive nanoparticles
The temperature dependent particle size of NIPAAm-AA and LMWSC conjugate nanoparticles were measured by the dynamic lights cattering (DLS, Malvern, Zetasizer, 3000HS). The particle size was measured in 0.1 wt % of nanoparticles dispersed in doubled distilled water. At each temperature concerned, the solution was stand for 5 minutes to equilibrium. The gel-collapse temperature of nanoparticle solution, which is analogue to the LCST of the linear temperature, was determined by using a UV/visible spectrophotometer (Pharmacia Biotech, Ultrospec 1000E).

3. Result and discussion
Preparation of NIPAAm-co-acrylic acid nanoparticles

The NIPAAm-co-AA particles were synthesized by emulsion polymerization of

Table 1. Ingredients and Conversions for the synthesis of NIPAAm-AA particles

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Water (g)</th>
<th>NIPAAm (g)</th>
<th>AA (g)</th>
<th>MBA (g)</th>
<th>SDS (g)</th>
<th>KPS (g)</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4(2)</td>
<td>500</td>
<td>6.80 (95%)</td>
<td>0.3</td>
<td>0.14</td>
<td>0.5</td>
<td>0.5</td>
<td>98.1</td>
</tr>
<tr>
<td>5(5)</td>
<td>500</td>
<td>6.85 (91%)</td>
<td>0.3</td>
<td>0.41</td>
<td>0.5</td>
<td>0.5</td>
<td>97.8</td>
</tr>
<tr>
<td>8(5)</td>
<td>500</td>
<td>6.75 (87%)</td>
<td>0.3</td>
<td>0.41</td>
<td>0.5</td>
<td>0.5</td>
<td>98.6</td>
</tr>
<tr>
<td>12(5)</td>
<td>500</td>
<td>6.70 (83%)</td>
<td>0.42</td>
<td>0.41</td>
<td>0.5</td>
<td>0.5</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Figure 1. Scanning electron micrograph of PNIAA-4(5) nanoparticles

NIPAAm monomer in the presence of MBA as a cross-linker, KPS, as an initiator and SDS as a surfactant. The sample number and detail ingredient of the NIPAAm-AA nanoparticles were shown in Table 1. From Table 1, high conversion (>95%) of all nanoparticles were measured. The scanning electron micrograph of PNIAA-4 nanoparticle is shown in Figure 1. The electron micrograph shows the synthesized nanoparticles are spheres.

Particle Size and the Gel-collaps Temperature of NIPAAm-AA particles

The NIPAAm-AA nanoparticles exhibits different thermosensitive behaviors with particle concentration in water. Figure 2 summarized the results of NIPAAm-AA particles obtained from dynamic light scattering at various temperatures. Our results did not show significant difference within the range of 4%-12% acid contents. The particle size of NIPAAm-AA particle is almost composition independent.

Figure 2 also showed the significant decrease of particle size as the temperature increased from 10 °C to 50 °C. Results of transmittance measurement of NIPAAm-AA particles are shown in Figure 3.
particles at higher concentration (15 mg of particles / 1 ml of water) as a function of temperature are shown in Figure 3. In Figure 3, all of the NIPAAm-AA particles show similar trend of transmittance; that is, the transmittance of the particle with different AA content dropped abruptly around 32 °C, which is in consistence with the particle size measurement.

*Thermosensitive behaviors of nanoparticles based on PNIPAAm core and chitosan shell structure.*

As the NIPAAm-AA nanoparticles slowly added into a highly diluted LMWSC Solution, LMWSC might be adsorbed at the surface of the nanoparticles by carboxyl–amine interaction, and the conjugation reaction of NIPAAm-AA particle with LMWSC was carried out by using 3-(3-dimethylaminopropyl) carbodiimide (EDAC). Therefore, it seems that the resulting nanoparticles may have NIPAAm-AA core and crosslinked chitosan shell structures. Figure 4 shows the size data of PNIAA-LMWSC nanoparticles. The particles size was decreased at below LCST, increased at around LCST and re-decreased at the above LCST. The results of light transmission experiment of chitosan conjugated nanoparticles were shown in Figure 5. From the Figure 5, it is clearly shown that the cloud point of chitosan conjugated PNIPAAm-AA nanoparticles solution was elevated about 2–3 °C compared with pure particles.

4. Conclusions

We have reported the first example of thermosensitive nanoparticles of PNIPAAm-AA core and chitosan shell structure for the controlled release of the loaded drug. The thermosensitive characteristics of chitosan conjugated nanoparticles make it potentially useful in the design of a new type of intelligent drug capsules that is compatible with cells to release its pharmacological activity.

References