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Preparation of environmental-responsive chitosan-based nanoparticles by self-assembly method

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ABSTRACT

In this research, environmental-responsive nanoparticles of chitosan-*graft*-poly(*N*-isopropylacrylamide) copolymers (CS-*g*-PNIPAAm) were prepared by the self-assembly method. The copolymer was first synthesized through polymerization of NIPAAm monomer in the presence of CS in an aqueous solution using cerium ammonium nitrate as the initiator. Then, the CS-*g*-PNIPAAm solution was diluted by deionized water and heated to a proper temperature for CS-*g*-PNIPAAm to undergo self-assembly. Micelles of CS-*g*-PNIPAAm were formed, and glutaraldehyde was added to reinforce the micelle structure to form nanoparticles. TEM images showed that a porous or hollow structure of nanoparticles was developed. The synthesized nanoparticles carried positive charges on the surface and their mean diameter could be manipulated by changing the temperature of environment. These nanoparticles with environmentally sensitive properties are expected to be utilized in hydrophilic drug delivery system.

1. Introduction

In recent years, polymeric particles with hollow core morphology or cavity-containing structure have attracted great attention because of the broad potential applications (Gao, Lukyanov, Singhal, & Torchilin, 2002; Hu, Jiang, Ding, Chen, & Yang, 2004; Langer & Tirrell, 2004; Meier, 2000). Several different methods have been used to prepare polymeric hollow particles, such as the layer-by-layer deposition of polyelectrolyte on a template core, polymerizing monomers in lipid vesicles, self-assembly of block copolymers in an appropriate solvent and emulsion (or miniemulsion) polymerization (Berth, Voigt, Dautzenberg, Donath, & Mohwald, 2002; Caruso, Caruso, & Mohwald 1998; Caruso, Caruso, & Mohwald 1999; Donath, Sukhorukov, Caruso, Davis, & Mohwald, 1998; Hotz & Meier, 1998; Huang, Remsen, Kowalewski, & Wooley, 1999; Jang & Ha, 2002; Krafft et al., 2001; Stewart & Liu, 1999; Tiarks, Landfester, & Antonietti, 2001). For the biomedical applications, it would be better that polymeric hollow particles also have properties of biocompatibility, biodegradability and low cytotoxicity. Among numerous biocompatible and biodegradable materials, biopolymers are the suitable materials to synthesis hollow particles for biomedical applications because of its good biological properties.

Chitosan (CS) is a high-molecular-weight polysaccharide composed mainly of β-(1,4) linked 2-deoxy-2-amino-D-glucopyranose units and partially of β-(1,4) linked 2-deoxy-2-acetamido-D-glucopyranose. It is obtained by partial deacetylation of chitin, which is the most abundant natural biopolymer next to the cellulose and can be found in the skeletal materials of crustaceans and insects, and cell walls of bacteria and fungi. Differently from chitin, chitosan is soluble in acidic solution (pH < 6.4) as a result of the protonation of amino groups on the D-glucosamine residues (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Because of its advantageous properties including biodegradability, biocompatibility, anti-bacteria and non-toxicity, CS can be used in the fields of wastewater treatment, food processing, cosmetics, pharmaceuticals, biomaterials and agriculture (Chen & Chen, 1998; Muzzarelli, 1977; Stevens, Rao, & Chandrkrachang, 1996). In the drug delivery field, the vesicles based on chitosan and derivatives can be used for transdermal, nasal, ocular, oral and parenteral administration and other application (Illum, Jabbal-Gill, Hinchcliffe, Fisher, & Davis, 2001; Thanou, Verhoef, & Junginger, 2001; Thein-Han & Stevens, 2004). Many methods have been developed to prepare CS particles including emulsion, spray-drying and emulsion-droplet coalescence technique. Recently, Cheng, Xia, and Chan (2006) prepared CS-polypyrrole hollow spheres by using AgBr crystal as the core template. Using different shapes of AgBr crystal, the size and morphology of hollow spheres could be controlled. Hu et al. (2004) reported the preparation of CS-PAA hollow spheres by an emulsion

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polymerization method without using a core template material. It is therefore possible to synthesize CS-based hollow spheres for biomedical application.

In our previous research, we found that chitosan could play a role as a surfactant when CS-PNIPAAm and CS-PAA-PNIPAAm nanoparticles were synthesized by the soapless dispersion polymerization (Chuang, Don, & Chiu, 2009; Lee, Wen, & Chiu, 2003; Lee, Wen, Lin, & Chiu, 2004). The surface charge of nanoparticles is positive. Recently, pH-sensitive micelles self-assembled by changing medium pH value to change the hydrophilicity of two pH-sensitive polymers have been successfully developed. Dou, Jiang, Peng, Chen, and Hong (2003) reported that the hydroxyethylcellulose-poly(acrylic acid) (HEC-PAA) hollow spheres could be prepared by pH-dependent self-assembly method. Based on these reports, we aim to synthesize well dispersed chitosan-based nanoparticles by the self-assembly method.

In this study, cerium ammonium nitrate (CAN) was used to initiate the polymerization of NIPAAm monomers in the presence of chitosan to form a CS-garft-PNIPAAm copolymer (CS-*g*-PNIPAAm) solution. In appropriate concentration and temperature, because PNIPAAm became hydrophobic when the environment temperature was higher than LCST, CS-*g*-PNIPAAm aggregated to form CS-PNIPAAm micelles. After crosslinking the shell region of micelles and cooling down the temperature, the CS-PNIPAAm porous or hollow nanoparticles were obtained. Structure, morphology, particle size, surface charge, thermo- and pH-responsive properties were examined.

2. Materials and methods

2.1. Materials

CS (degree of deacetylation 95%, average molecular weight 200,000 g/mol) was obtained from Kio-Tek (Taipei, Taiwan). NIPAAm and acetic acid (HAc) were obtained from Acros (Geel, Belgium). Ceric ammonium nitrate (CAN) was purchased from SHOWA (Tokyo, Japan). Doxycycline hyclate was obtained from Sigma–Aldrich (St. Louis, MO). All the other chemicals were analytical grade or above and used as received without further purification.

2.2. Synthesis of CS-g-PNIPAAm copolymer solution

Designed amount of CS was first dissolved in 20 mL HAc aqueous solution containing specific amount of NIPAAm. The solution was purged with nitrogen and heated to 40 °C. CAN was dissolved in 5 mL of water and pre-heated to 40 °C, before it was poured into the solution. After 6 h of reaction, a solution containing CS-*g*-PNIPAAm copolymers and PNIPAAm homopolymers was obtained. The solution was poured into acetone to precipitate the CS-*g*-PNIPAAm and PNIPAAm homopolymer. The characteristic data of CS-*g*-PNIPAAm copolymer sample were listed in Table 1. The grafting ratio (GR) was calculated by the gravimetric analysis using the following equation:

$$\mathrm{GR} = \frac{(W_2 - W_{CS})}{(W_{CS})} \quad (1)$$

In the equation, W_{CS} is the initial weight of chitosan. Polymer mixture was extracted by methanol at 20 °C to remove the PNIPAAm homopolymers. After 48 h of extraction, the remaining CS-*g*-PNIPAAm copolymer was obtained by oven drying for 48 h at 60 °C in a circulation oven followed by another 48 h in a vacuum oven at 60 °C and weighted (W_2).

Molecular weight of PNIPAAm grafted on CS chains was characterized by a gel permeation chromatography (GPC) (Waters 2695, Waters, Milford, MA) analysis system with THF as the eluent at a flow rate of 1.0 mL/min and narrow-polydispersity polystyrene as the calibration standard. CS-*g*-PNIPAAm copolymer was hydrolyzed by 12 M $HCl_{(aq)}$ for 4 h, 40 °C to degrade the CS chain and obtain the grafted PNIPAAm. Grafted PNIPAAm was dissolved in THF to be analyzed by GPC. Average graft point on each CS chain was calculated by the following equation:

$$\text{Average graft point} = \frac{(W_2 - W_{CS})}{(M_w)} \div N_{CS} \quad (2)$$

In the equation, M_w was the average molecular weight of grafted PNIPAAm, measured by GPC. N_{CS} was the mole of CS chain.

2.3. Structure analysis of CS-g-PNIPAAm copolymers

Structure analysis was carried out with an NMR spectrometer (Avance-500, Bruker, Billerica, MA). CS-*g*-PNIPAAm copolymer was ground to fine powder and dissolved in 5% D-acetic acid/95% D_2O solution to prepare the sample solution (500 MHz for ^1H).

2.4. Preparation of CS-PNIPAAm nanoparticles

The dried CS-*g*-PNIPAAm copolymers were dissolved in design amount of HAc aqueous solution and the pH value of solution was 3.0. After that, the solution containing CS-*g*-PNIPAAm was heated to 40 °C for self-assembly. The solution became translucent, revealing the CS-PNIPAAm micelles were formed. After 24 h micellization, designed amount of glutaraldehyde (GA) as crosslinker was added to crosslink the micelles for 2 h at 40 °C (molar ratio of glucosamine unit in CS to glutaraldehyde, [CS]/[GA] = 2.0/1.0). After that, a dispersion solution containing CS-PNIPAAm nanoparticles was obtained. The reaction conditions and the sample code of CS-PNIPAAm nanoparticles were listed in Table 2.

2.5. Morphology observation

The morphologies of the CS-PNIPAAm nanoparticles were observed with TEM (JSM-1230 EX II, JEOL, Tokyo, Japan). One drop

Table 1
Characteristic data of CS-*g*-PNIPAAm copolymer.

Copolymers	GR		PNIPAAm grafts M_w (g/mol)	Average graft point[a]
	Gravimetric method	^1H NMR method		
CS-*g*-PNIPAAm	0.302	0.404	4725	17.8

[a] GR (gravimetric method) was used to calculate the average graft point.

Table 2
Sample code and characteristic data of CS-PNIPAAm nanoparticles.

Nanoparticle sample	Copolymer concentration (mg/mL)	Self-assembly temperature (°C)	Solution pH	Particle size (nm)[a]	Average zeta potential (mV)
CS-PNIPAAm	1.5	40	3.0	295 ± 65	16.1

[a] Based on intensity.