Nanoparticles from Chitosan

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Summary: Novel biodegradable nanoparticles were synthesized by chemical modification of the chitosan linear chain. A natural dicarboxylic acid (malic acid) was used as a crosslinking agent for intramolecular covalent condensation reaction to obtain hydrophilic nanoparticles based on chitosan. A variety of methods including, solubility studies, laser light scattering (DLS), transmission electron microscopy (TEM) and nuclear magnetic resonance (NMR) was used to characterize the crosslinked macromolecules.
The prepared biodegradable chitosan nanoparticles, soluble in aqueous media, might be useful for various biomedical applications, like injectable drug- or gene-delivery systems.

Keywords: biodegradable; chitosan; crosslinking; hydrophilic; nanoparticles

Introduction

After cellulose, chitin is the second most abundant natural biopolymer on earth. The principle derivative of chitin, chitosan, is obtained by N-deacetylation, resulting in a copolymer of β-[1→4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose.

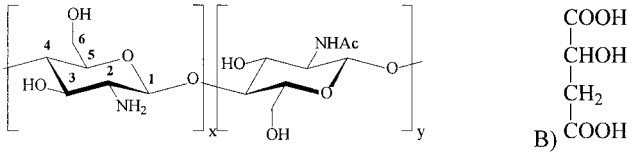

Figure 1. Chemical structure of chitosan(A) and malic acid(B).

Chitosan is a linear polysaccharide containing reactive amino groups. Because of their presence, chitosan is soluble in aqueous acidic media and forms viscous solutions [1]. A limiting factor in the modification and application of chitosan is its low solubility in aqueous media at neutral and alkali conditions and in most organic solvents. However, a variety of studies have focused on improving solubility of chitosan material by water soluble linkage [2,3].

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Chitosan, as a functional polysaccharide, offers a special set of characteristics: non-toxicity, biocompatibility, biodegradability, low immunogenicity and antibacterial properties, and has found wide application in a variety of areas, such as cosmetics, food technology [4], and pharmaceuticals [5], including tissue engineering [6] and drug- or gene-delivery systems [7,8].

Various methods have been developed for the crosslinking of chitosan, including chemical modification of linear macromolecules with difunctional molecules [9,10] or ionic crosslinking with charged ions [11] or molecules [12] to form bridges between polymeric chains. However, crosslinking of chitosan generally produces hydrogel formation. Such preparations have a number of potential biomedical applications [13,14], but intravenous injections are precluded. In the present investigation, hydrophilic nanoparticles based on chitosan were prepared by a covalently crosslinking reaction between amino groups of polysaccharide chains with a natural dicarboxylic acid at different crosslinking ratios. Chitosan nanoparticles crosslinked in this manner constitute a stable colloid system in aqueous media, and are nano-sized at neutral pH. These particles may prove to be attractive biosystems for biomedical or pharmaceutical applications as drug- or gene-delivery systems.

Experimental

Materials Chitosan (M_v = 320 kDa, degree of deacetylation = 80%) was purified by dissolving in acetic acid solution (1.0% w/w) dialyzed against deionized water and lyophilized. The molecular weight was calculated from the intrinsic viscosity using the Mark-Houwink's relation. The degree of deacetylation was determined by ^{1}H NMR spectroscopy. Malic acid was used as received without further purification. Water soluble 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide methiodide was applied as a condensation agent. All materials were purchased from the Sigma Aldrich Co.

Instrumentation. The structure of the prepared colloid system was analyzed by NMR spectroscopy using a Bruker DRX 500 MHz instrument. The particle size of dried crosslinked chitosan derivatives was characterized by a JEOL2000 FX-II transmission electron microscope (TEM) and the hydrodynamic diameter of swelled chitosan nanoparticles was gauged by using a BI-200SM Brookhaven Research Laser Light Scattering (DLS) photometer.

Synthesis of crosslinked chitosan. Malic acid was dissolved in water and then neutralized to pH 6.5 with 0.1 M sodium hydroxide. After the addition of water soluble carbodiimide,

the reaction was stirred at ambient temperature for 30 min and subsequently mixed with purified chitosan dissolved in water. The reaction mixture was stirred at room temperature for 24 h. The solution containing chitosan nanoparticles was purified by dialysis for 7 days against distilled water, and freeze dried [15]. The stoichiometric ratio of chitosan and malic acid was varied from 30 % to 240 %.

Results and Discussion

Water Solubility. The water solubility of chitosan derivatives was evaluated in an aqueous medium at pH 6.5. All samples were opaque aqueous colloid systems and stable at room temperature for several weeks. As crosslinking increased, solution opalescense increased (Figure 2.). When the stoichiometric ratio of crosslinking was 120% or higher the nanoparticles remained dispersed in water and no precipitation was observed. This is explained by protonation of residual free amino groups of the chitosan chain which were detected by NMR representing lower conversion than that of the stoichiometric ratio.

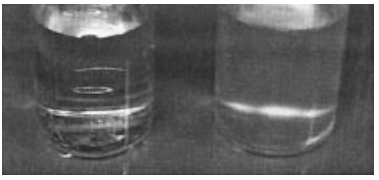

Figure 2. Image of nanoparticles crosslinked with malic acid at stoichiometric ratio of 30% and 120%.

NMR Results. The structure of the malic acid - crosslinked chitosan derivatives was characterized by NMR spectroscopy. Samples were dissolved in D_2O containing a few drops of 20% w/w DCl/D_2O.

The ^1H NMR assignments and chemical shifts (ppm) of chitosan are: δ 5.5 (H-1 of deacetylated glucose units), δ 5.0 (H-1 of acetylated glucose units) δ 3.5-4.1 (H-3, H-4, H-5, H-6), δ 3.2 (H-2), δ 2.1 ($NHCOCH_3$).

^1H NMR signals (ppm) of a modified chitosan (a 30% stoichiometric ratio) is given in Figure 3. The ^1H-NMR data of glucose units are observable in spectrum, respectively: δ 5.5 (H-1 of unmodified glucose unit), δ 4.9-5.1 (H-1 of modified glucose unit), δ 3.5-4.1 (H-3, H-4, H-5, H-6), δ 3.25 (H-2), δ 2.1 ($NHCOCH_3$). The chemical shift of crosslinking agent is given: δ 2.9 (CH_2 of malic acid), and δ 4.65 (CH of malic acid). The conversion was calculated on the basis of integral values of signals at δ 4.65 and δ 2.1 ppm.

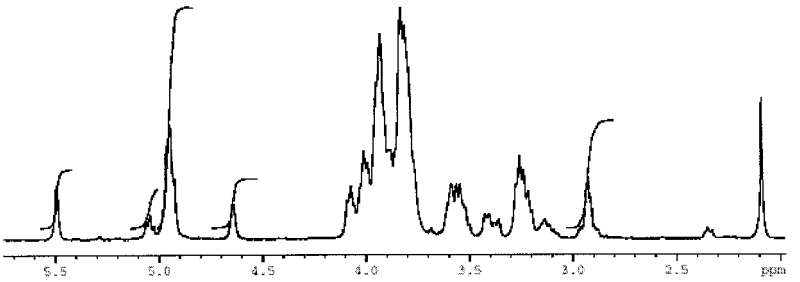

Figure 3. 500 MHz ^{1}H NMR spectrum of chitosan crosslinked with malic acid at stoichiometric ratio 30%.

Particle Size. Changing the crosslinking ratio does not affect the size of chitosan nanoparticles. However, crosslinking does influence the opacity of colloid dispersion as is shown in Figure 2.

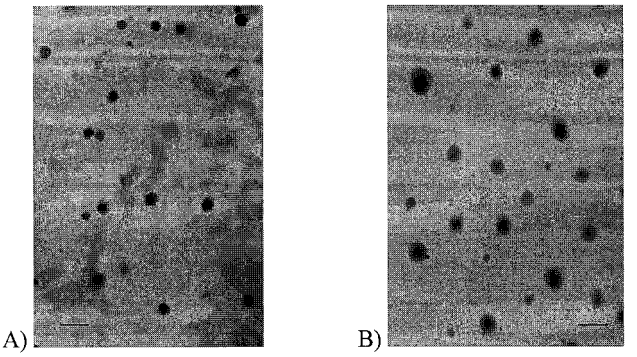

Figure 4. TEM micrographs of chitosan nanoparticles crosslinked with malic acid at stoichiometric ratio of 30% (A) and 60% (B) (bar = 500nm).

The crosslinked chitosan nanoparticles constitued separate spherical particles in aqueous environment and in dried states. TEM micrographs confirmed the nanosize of dried crosslinked chitosan particles and showed the dispersity of these derivatives.

The hydrodynamic diameter of swelled chitosan nanoparticles was measured by DLS. Samples were prepared from the reaction mixtures after dialysis; the concentration of the polysaccharide solution was 100μg/ml. The pH of the samples was adjusted with a solution of hydrochloric acid in the presence of 0.2 M sodium acetate buffer.

Hydrodymanic diameter of samples was measured at pH 7.6 and was found that no effect on size occurred with different crosslinking ratio.

At different pH, polycations were produced when chitosan was crosslinked with malic acid. The free amino groups of these macromolecules can be protonated with hydrochloric acid resulting in no increase in the hydrodynamic diameters despite the repulsive interaction of positive ions. The hydrodynamic diameter of chitosan nanoparticles crosslinked at 60% with malic acid was measured at different pH values (Table 1). It can be seen, that the hydrodynamic diameter is not affected significantly by the pH.

Table 1. Crosslinking ratios and mean hydrodynamic diameters of modified chitosan macromolecules.

Crosslinking ratio (%)		**pH**	**Mean diameter** (nm)
Stoichiom.	**Calc. by ^{1}H NMR**		**DLS**
30	23	7.6 ± 0.03	310
60	36	7.6 ± 0.03	300
60	36	6.6 ± 0.03	280
60	36	5.6 ± 0.03	310
60	36	4.6 ± 0.03	280
60	36	3.6 ± 0.03	300
60	36	2.6 ± 0.03	290
120	44	7.6 ± 0.03	300
240	57	7.6 ± 0.03	310

Mean hydrodynamic diameters of crosslinked chitosan nanoparticles are between 280nm and 310 nm. The nanoparticles appear to swell in aqueous media, independently of the pH. The framework of the polysaccharide rings likely limits the swelling. [16]

Conclusion

It has been shown that hydrophilic chitosan nanoparticles were successfully synthesized using a natural dicarboxylic acid as a crosslinking agent. The crosslinked macromolecules were found to be 280-310 nm and formed stable colloid systems in aqueous environment. These nanoparticles may prove useful as drug- or gene-delivery systems.

Acknowledgement

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