**N-modification of chitosan for improved membrane permeability: Synthesis, physicochemical characterization and submicron particle formulation**

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**ABSTRACT SUMMARY**

A novel N-modified chitosan was synthesized. The N-modification of chitosan was confirmed by IR & size exclusion chromatography. Modified chitosan submicron particles were prepared by ionic gelation. The sizes of the formed particles were between 200-500 nm. The formed particles were uniform in shape and well dispersed; they are likely to increase the membrane permeability of hydrophilic drugs.

**INTRODUCTION**

Chitosan is the second most abundant polysaccharide in nature; it is widely used in the formulation of fine particles and nanoparticles mainly due to its unique properties (1). Chitosan nanoparticles were reported to be non-toxic, biodegradable, biocompatible and relatively easy to formulate (2). The positive surface charge of chitosan renders it attractive for ocular drug delivery due to the possible physical interaction with the negatively charged surface of the eye. This is likely to increase the residence time of the loaded drug and reduce the precorneal drainage, therefore, enhance ocular bioavailability (2). Furthermore, it has been previously reported that alkyl groups will increase chitosan nanoparticle’s permeation by virtue of the the lipophilic nature of such a group (3).

We report on a new method to modify chitosan; where 2,3-O-isopropylidene-D-erthronolactone (OIDE) was reacted with chitosan, thus, a short alkyl glycerol group was added to the chitosan backbone at the N position. The new chitosan derivative is likely to be more lipophilic than the parent compound and as such may improve the membrane permeability of hydrophilic drugs.

**EXPERIMENTAL METHODS**

*N-modification of chitosan (synthesis and physicochemical characterisation)*

Chitosan (500 mg) was mixed with acetic acid (50 mL, 1% w/v) and stirred for two hours. A predetermined amount of OIDE was added, followed by concentrated acetic acid (1.5 mL). The reaction was quenched by NaOH (8-10 mL, 15% w/v) and a white yellowish precipitate formed. The formed precipitate was filtered, washed with acetone and dried overnight under a stream of nitrogen.

Characterization of the modified chitosan was achieved using infrared spectroscopy (IR) (Perkin Elmer Spectrum 100. FT-IR, USA) and a Viscotek size exclusion chromatography (SEC) system, (Malvern, UK)

**Formulation of N-modified chitosan particles**

Chitosan submicron particles were formulated using ionic gelation with sodium tripolyphosphate (TPP) (1). Briefly, modified chitosan was dissolved in aqueous acetic acid in a1:1 ratio. Four different concentrations of TPP (4 mL of 0.1-0.4 mg/mL) were added to 10 mL of chitosan solution. Flasks were sealed off and sonicated for 3 hours.

**N-modified chitosan particle characterization**

Particle size and zeta potential (ζ) of the prepared particles were measured using a zetasizer (Malvern Mastersizer 3000HSA, UK). Scanning Electron Microscope (SEM) (Zeiss EVO50, Germany) was used to study and characterize the prepared particles.

**RESULTS AND DISCUSSION**

Figure 1 illustrates the fingerprint peaks for unmodified chitosan and N-modified chitosan spectra. The peaks at 2921.67 cm⁻¹ and 1423.39 cm⁻¹ correspond to the CH₂ group stretching vibration. The peak at 1156.70 cm⁻¹ is assigned to the C—O—C stretching. N-modified chitosan spectra have the same main peaks of the chitosan with some new ones. The peak at 1558.85 cm⁻¹ is attributed to the N-H bending of the secondary amide of the N-modified chitosan, while the peak at 1639.49 cm⁻¹ is related to the C=O stretching of the
secondary amide that was formed as a result of the modification. Finally, there are two peaks appearing at 3177.01 cm\(^{-1}\) and 3287.93 cm\(^{-1}\) respectively, these are attributed to the N-H stretching vibration of the secondary amide.

The molecular weight of chitosan and N-modified chitosan determined using SEC were found to be 151,439 Da and 197,754 Da respectively. As expected, the molecular weight of N-modified chitosan is higher than the unmodified form because of the extra alkyl glycerol chain fragments.

Figure 2 summarises the particle size and ζ potential results of the formulated submicron particles.

The size of the prepared particles was between 200-500 nm. These particles were prepared using a TPP concentration between 0.2-0.4 mg/mL. The ζ potential values of the prepared particles were positive, this can be attributed to the presence of the unmodified amine group of the chitosan backbone; such a group would protonate into NH\(^{3+}\). The ζ potential of the modified chitosan particles was less than the unmodified ones; this can be attributed to the reduced number of amine groups on the chitosan backbone after modification. This result is an indirect confirmation of the successful chitosan modification.

The formed submicron particles are spherical in shape and have a mean diameter of 250 nm. Furthermore, the formed particles are well dispersed, with no signs of aggregation or accumulation (Figure 3).

**CONCLUSION**

A novel N-modified chitosan derivative was successfully synthesized, characterized and fabricated into submicron particles. The alkyl glycerol modification is likely to increase the overall lipohilicity, hence the permeation of loaded model hydrophilic drugs. This would render these particles promising for several applications including topical ocular drug delivery.

**REFERENCES**


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