Effective encapsulation of ascorbic acid bioactive in chitosan nanoparticles

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Introduction

Ascorbic acid or vitamin C is a water-soluble and strong antioxidant presented in many vegetables and fruits. Ascorbic acid is essential for collagenesis in living organism, which protect tissue and cells from oxidation reactions by free radicals and other reactive oxygen-derived species. In cosmeceutical field, ascorbic acid has been scientifically proven that it can promote the synthesis of collagen and visibly reduce the effects of skin wrinkles (Zhang 1999). Besides that it can enhance the efficacy of other fat soluble antioxidants such as α-tocopherol (vitamin E) and β-carotene (Basu 1999, Gallarate1999). Ascorbic acid is notoriously unstable when exposed to air, humidity, light, heat, oxygen and base. In addition, with its water-soluble property, the permeability of the unmodified vitamin C (an active form) through skin is obstructed by a major barrier, stratum corneum (Zhang 1999). Therefore, a carefully-designed topical delivery system for ascorbic acid is of importance for effective delivery of this compound. From the benefits of nanotechnology which are of interest in medical, pharmaceutical and cosmetic fields, preparation of ascorbic acid loaded nanoparticles could enhance ascorbic acid utility by protecting the entrapped ascorbic acid from environment, control releasing rate at releasing area or absorbing area and could be designed to be a drug delivery system to the preferred target sites such as hair follicles. In this study, chitosan (CS) and sodium carboxymethylcellulose (SCMC) were employed for the nanoparticle fabrication with the addition of polyethylene glycol (PEG) in another formula at appropriate ratios. Various factors which effected on the preparation of ascorbic acid loaded nanoparticles and the physicochemical properties of the prepared CS nanoparticles were characterized.

Experimental

Materials

Standard L(+) ascorbic acid (AR grade) was the product of Fluka, Finland. Chitosan was the product of Seafresh Chitosan (Lab) Company Limited, Thailand. It had 90 % deacetylation and molecular weight of 15,000 Da. Sodiumcarboxymethylcellulose (SCMC), Ultra low viscosity, was purchased from Fluka, Finland. Polyethylene glycol 400 (PEG400) was purchase from Srichansaha-o-sod, Bangkok, Thailand. Milli Q water was used as a solvent in aqueous system. Other ingredients were of the highest grade available.

Preparation of ascorbic acid nanoparticles

Ascorbic acid nanoparticles were prepared by using ionotropic complexation technique at room temperature. Chitosan solution was prepared in 1% acetic acid and SCMC were separately dissolved in water and mixed at appropriate ratios by stirring for about 1 hour under the stream of nitrogen. For the entrapment of ascorbic acid, the 2% ascorbic acid in the EDTA solution was added before the addition of SCMC. In another formular, PEG400 was added after the addition of SCMC. The colloidal suspension was then further centrifuged at 15,000 rpm or 10,000 rpm at 4°Cfor 10 minutes and washed twice with EDTA solution. Then a 2 ml acetate buffer was added and nanoparticles were redispersed homogeneously.

Effects of various factors on chitosan nanoparticles preparation
Various factors such as chitosan concentration, weight ratio and duration of mixing were investigated in order to obtain the optimal preparation conditions. Average size, size distribution and surface charge of the prepared nanoparticles were employed for the selection of preparation protocol. Finally, the two most optimal formula, chitosan: SCMC = 1 : 1 (w/w) and chitosan: SCMC: PEG = 1 : 1 : 0.3 (w/w/w), were compared in term of their physicochemical properties.

Measurements of average diameter and zeta potential of nanoparticles
Mean droplet size, size distribution and surface charge of the prepared nanoparticles were determined by photon correlation spectroscopy (PCS) with a Malvern / Zetasizer ZS (Malvern Instruments, Worcestershire, UK).

Determination of the entrapment efficiencies
Particles loaded with ascorbic acid were centrifuged at 15000 rpm, 4 ° C for 10 minutes. Then supernatant were collected and quantified by UV-visible Spectrophotometer (Jasco / FP-777) at 265 nm. Quantities of the entrapped ascorbic acid were expressed as weight to the initial amounts before entrapment.

Release of the entrapped ascorbic acid from nanoparticles
The prepared nanoparticles were collected by centrifugation at 10000 rpm, 4 ° C for 10 minutes. The particles pellet was then redispersed and collected in the presoaked dialysis bag prior subjecting into a beaker containing acetate buffer pH 5.5. Ascorbic acid concentrations at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 minutes were analyzed by UV-visible Spectrophotometer (Jasco/ FP-777) at 265 nm.

Results and Discussion

CS-SCMC nanoparticles can be prepared from the electrostatic interaction between positively charges of CS and negatively charges of SCMC. When ascorbic acid was added into the mixture, hydrogen bonding and van der waals interaction may be responsible for the complexation led to the formation of the ascorbic acid loaded nanoparticles. Various factors; for examples, mixing ratios and duration effected on size, size distribution and surface charges of the prepared nanoparticles as shown in Fig. 1 and 2. Appropriate conditions were selected for the further study.

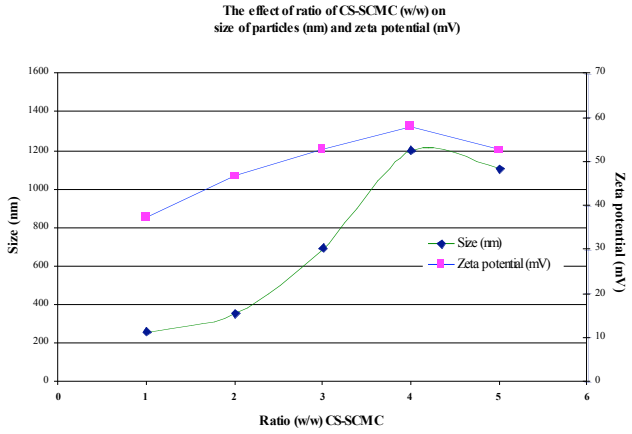

Figure 1. The effect of ratio of mixing of CS and SCMC on size, size distribution and surface-charge of the CS-SCMC nanoparticles

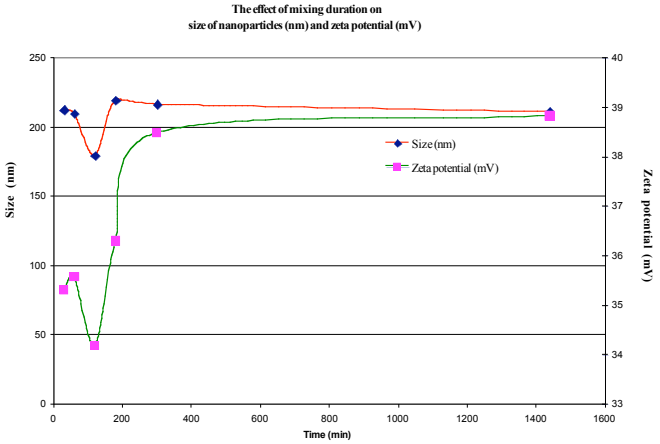

Figure 2. Effect of mixing duration of CS and SCMC on size, size distribution and surface-charge of the CS-SCMC nanoparticles

The suspension character and morphology of nanoparticles obtained from the microscope were shown in Fig. 3. The ascorbic load nanoparticles with and with out PEG were both of spherical shape. The size, size distribution and surface-charges of the particles were 135 ± 1nm (polydispersity index (PI) 0.077) and the zeta potential of 36.8 ± 3.5 mV and 152±3 nm (PI 0.118) and the zeta potential of 38.2 ± 1.0 mV for nanoparticles which prepared from chitosan/ SCMC and chitosan/ SCMC/ PEG, respectively.

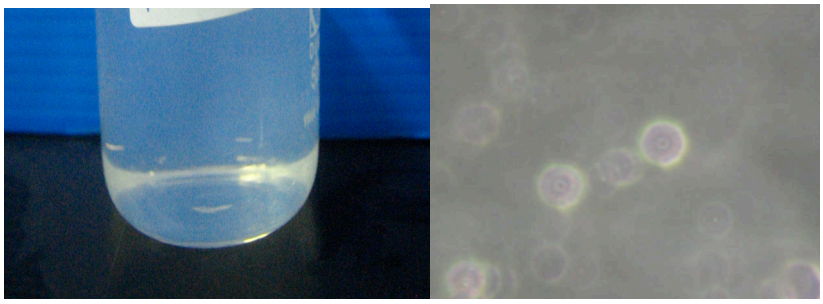

Figure 3. Physical appearance of chitosan/ SCMC nanoparticlesobserved by naked eye and under microscope

Percentages of drug entrapment (PDE) ascorbic acid in the prepared nanoparticles were determined by the indirect method using the following equation:

PDE = [Total Non-Bound Drug Loading/Total Drug Loading] x100

Efficient entrapment of ascorbic acid in the nanoparticles was achieved as shown in table 1.

Samples	Average Percentage Drug Entrapment (%)	SD	%RSD
AA/CS/SCMC	99.06	0.28	0.29
AA/CS/SCMC/PEG	97.16	0.02	0.02

Table 1. Average Percentage Drug Entrapment (PDE)

The ascorbic acid release profiles at pH 5.5 which is the pH of the human skin were demonstrated in Fig 4. The release study indicated a sustained release of ascorbic acid from the nanoparticles. In the PEG containing formulae, the release of ascorbic acid gradually increased, contrasting to the formulae without PEG which reached the plateau after 20 minutes.

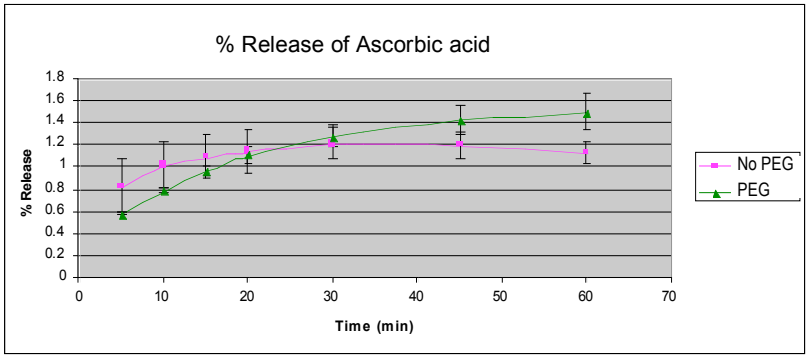

Figure 4. Percentage of released ascorbic acid from nanoparticles

Summary

Nanoparticles were prepared, based on ionotropic complexation between chitosan (CS) and sodium carboxymethylcellulose (SCMC). Optimized preparation conditions which were stirring for 1 hour in pH 5 buffer with the mixing ratios of chitosan : SCMC=1:1 and chitosan : SCMC : PEG= 1:1: 0.3, could entrap ascorbic acid effectively in the nanoparticles. This colloid performed consistent distribution also terquirization. In this condition, nanoparticles had spherical shape under microscope with polydispersity index of 0.077 and 0.118, average size of 135 ± 1nm and 152 ± 3 nm and percentage of entrapped ascorbic acid of 99.06 ± 0.28% and 97.1 6± 0.02 % for nanoparticles which prepared from chitosan/SCMC and chitosan/SCMC/PEG,respectively. They perform high value which demonstrated that preparation condition and method of nanoparticle are suitable. Moreover, from the releasing study of ascorbic acid found that the prepared nanoparticles in buffer pH 5.5 could perform prolonged releasing property which released 1.14% and 1.50% in 60 minutes for nanoparticles which prepared from Chitosan/ SCMC and Chitosan/ SCMC/ PEG, respectively. From this study, ascorbic acid could perform prolonged releasing behavior which different from burst releasing as in the study of Gupta and Ravi Kumar (2001). PEG showed the enhancement of releasing rate in this study.

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