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# Encapsulation of ascorbyl palmitate in chitosan nanoparticles by oil-in-water emulsion and ionic gelation processes

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ABSTRACT

The encapsulation of ascorbyl palmitate (AP) in chitosan particles was carried out by droplet formation via an oil-in-water emulsion, followed by droplet solidification via ionic gelation using sodium triphosphate pentabasic (TPP) as a cross-linking agent. The success of AP encapsulation was confirmed by FT-IR, UV–vis spectrophotometry, TGA, and XRD techniques. The obtained AP-loaded chitosan particles were spherical in shape with an average diameter of 30–100 nm as observed by SEM and TEM. Loading capacity (LC) and encapsulation efficiency (EE) of AP in the nanoparticles were about 8–20% and 39–77%, respectively, when the initial AP concentration was in the range of 25–150% (w/w) of chitosan. Augmentation of the initial AP concentration led to an increase of LC and a reduction of EE. The amount of AP released from the nanoparticles in ethanol and tris buffer (pH~8.0) increased with increasing LC and decreasing TPP concentration.



## 1. Introduction

For decades, ascorbyl palmitate (AP), a fat-soluble ester form of vitamin C, has been used as a source of vitamin C and as an antioxidant for foods, pharmaceuticals, and cosmetics [1,2]. Although AP is more stable than ascorbic acid, the low chemical stability and water insolubility limit its utilizations.

Encapsulation potentially can protect active molecules from degradation by direct exposure to severe environments, e.g., light, oxygen, chemicals, etc. In other words, encapsulation can reduce the loss of activity of the active compounds. An encapsulant, or shell, frequently plays an important role as a carrier for delivery of the molecules to the target organs. In addition, the shell performs a release mechanism to control the diffusion level of active molecules under specific conditions, resulting in prolonged activities of these molecules. A few materials such as lipids [3–6], poly(D,L-lactide) [7], and poly(D,L-lactide-co-glycolide) [7] have been used as encapsulants for AP in the forms of microemulsions [3], liposomes [3,5,8], solid lipid nanoparticles [3], nanostructured lipid carriers [4,6] and nanoparticles [7]. In addition, the nano-level encapsulation would likely enhance the bioavailability of lipophilic compounds, thus increasing the degree to which those compounds become available to the target tissues.

Chitosan, a natural copolymer of N-acetyl-D-glucosamine and D-glucosamine units, is one of the polysaccharides potentially most suitable as carriers for a large number of active compounds [9–16]. Amino groups along chitosan backbones allow solubility in dilute organic acid solutions, ionic cross-linking, and chemical modification of the biopolymer to form gels, beads, films, particles, etc. In addition to its biodegradability, biocompatibility and non-toxicity, chitosan has received much attention in the development of micro- and nanoencapsulation systems [9–16].

The formation of chitosan particles by ionic gelation or polyionic coacervation has been reported for the delivery systems of various active molecules, e.g., proteins [10,15], hydrophilic and hydrophobic drugs [9,11,13,16], and vitamins [12,14,17]. Although vitamin C has been incorporated into chitosan–tripolyphosphate particles by spray-drying [12,14,17], the encapsulation of its fat-soluble derivative, AP, by chitosan has not been reported. Spray-drying is a convenient process to produce particles encapsulating active compounds; however, particles obtained are of micro-level size. In addition, the use of high temperature (e.g., 170 °C) during the microparticle preparation might induce the degradation of the active compounds.

The present work thus focuses on the fabrication of AP-loaded chitosan nanoparticles by a two-step process: emulsion formation and ionic gelation. This process is unique because it not only provides very tiny particles or nanoparticles, but also avoids the use of

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high temperature. We also clarified the successful encapsulation by Fourier transform infrared (FT-IR) and ultraviolet–visible (UV–vis) spectrophotometry, thermal gravimetry analysis (TGA), and X-ray diffraction (XRD) techniques, and determined the shape and size of the particles by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In addition, the release of AP from chitosan nanoparticles was investigated.

## 2. Materials and methods

### 2.1. Materials

Chitosan (deacetylation degree of 0.95 and molecular weight of ~700,000 Da) was purchased from the Seafresh Chitosan (Lab) Co., Ltd. (Bangkok, Thailand). Ascorbyl palmitate (AP), sodium triphosphate pentabasic (TPP), Tween 60 and Span 60 were supplied by Fluka Chemika (Buchs, Switzerland). Acetic acid was obtained from Merck (Darmstadt, Germany). Soybean oil (Jade Brand) was purchased from Lam Soon Public Co., Ltd. (Bangkok, Thailand). All chemicals were used as received without further purification.

### 2.2. Preparation of AP-loaded chitosan nanoparticles

AP-loaded chitosan nanoparticles were prepared according to a method modified from the ones described by Ko et al. [9] and Ajun et al. [16]. However, soybean oil was used instead of $CH_2Cl_2$ for preparation of the oil phase in order to avoid toxicity of the chemical residue. Briefly, aqueous and oil phase solutions were produced. Chitosan solution (1.5% (w/v)) was prepared by agitating chitosan in an aqueous acetic acid solution (1% (v/v)) at ambient temperature (ca. 25–28 °C) overnight. Tween 60 (0.45 g) was subsequently added to the solution (40 mL) and stirred at ambient temperature until the mixture was homogeneous. Soybean oil (10 mL) and Span 60 (0.05 g) were mixed at 50 °C for 2 h and then cooled to ambient temperature. AP was added to the oil mixture and agitated to achieve a homogeneous oil phase solution.

The oil–AP fraction (10 mL) was gradually dropped into the aqueous chitosan solution (40 mL) during homogenization at a speed of 16,000 rpm for 2 min to obtain an oil-in-water emulsion. TPP solution (0.5% (w/v), 40 mL) was then slowly dropped into the agitated emulsion. Agitation was continuously performed for 30 min. The formed particles were collected by centrifugation at 10,000 × *g* for 15 min at 20 °C, and subsequently washed several times with Tween 60 solution (0.1% (v/v)) and water. The particles were dried at ambient temperature under reduced pressure and stored in dry condition at 25 °C. Weight ratios of chitosan to AP (CTS:AP) of 1:0, 1:0.25, 1:0.50, 1:1.00 and 1:1.50 were used for the present study.

### 2.3. Characterization of nanoparticles

FT-IR spectra were obtained by using a Thermo Nicolet Nexus 670 spectrometer (Thermo Electron Corp., Madison WI, USA) with 32 scans at a resolution of 4 $cm^{-1}$ over a wavenumber range of 4000–400 $cm^{-1}$. XRD patterns were recorded over a $2\theta$ range of 5–50° by a JEOL JDX-3530 (JEOL Ltd., Tokyo, Japan) with a step angle of 0.04 °C/min. A Mettler-Toledo TGA/SDTA 851$^e$ thermogravimetric analyzer (Columbus OH, USA) was used, with a $N_2$ flow rate of 60 mL/min and a heating rate of 10 °C/min from 30 to 600 °C. The *Z*-average diameter of samples was determined at 20 °C by a Malvern Zetasizer (model 3600, Malvern Instruments Ltd., Worcestershire, UK) equipped with a He–Ne laser operating at 4.0 mW and 633 nm with a fixed scattering angle of 90°. SEM analysis of the products was carried out using a JEOL JSM LV-5600 at an operating voltage of 15 kV. Transmission electron micrographs were observed by a JEOL JEM-1220 at an accelerate voltage of 80 kV. UV–vis absorption spectra were recorded on a Thermo Spectronic Helios Gamma spectrophotometer (Thermo Scientific, Waltham MA, USA) with a scan speed of 60 nm/min over a wavelength range of 200–400 nm ($\lambda_{max}$ = 247 nm).

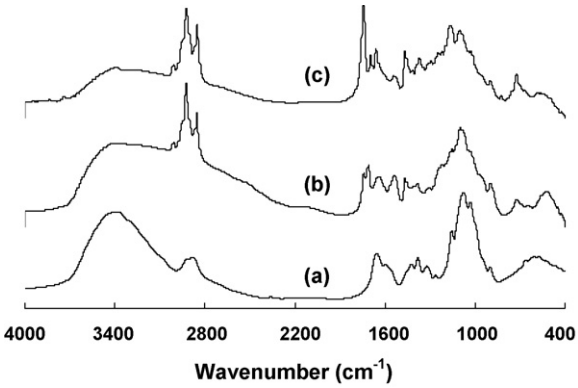


**Fig. 1.** FT-IR spectra of (a) chitosan flakes, (b) chitosan particles and (c) AP-loaded chitosan particles with CTS to AP weight ratio of 1:1.50.

### 2.4. Determination of loading capacity and encapsulation efficiency of AP

The content of AP loaded in chitosan nanoparticles was determined by TGA/DTG (derivative thermal gravimetric) technique. The amount of loaded AP per 100 g of sample—loading capacity (LC) and the amount of loaded AP per 100 g of initial AP (in feed)—encapsulation efficiency (EE) were thus calculated from Eqs. (1) and (2), respectively:

$$\%LC = \left(\frac{\text{weight of loaded AP}}{\text{weight of sample}}\right) \times 100 \quad (1)$$

$$\%EE = \left(\frac{\text{weight of loaded AP}}{\text{weight of initial AP}}\right) \times 100 \quad (2)$$

### 2.5. Study on in vitro release of AP from chitosan nanoparticles

Ethanol and tris buffer (pH ~8.4) were used as model media for an *in vitro* AP release study. Wet samples (10 mg) and media (1.2 mL) were placed in a microtube and incubated at ambient temperature. At sampling time, the incubated mixture was centrifuged and 100 μL of supernatant was collected. Evaluation of the amount of AP released was determined using a spectrophotometer at a wavelength of 247 nm. An equal volume of fresh media was then replaced in the mixture, and the same procedure was repeated for the subsequent sampling. These *in vitro* release studies were performed in triplicate for each of the samples.

## 3. Results and discussion

### 3.1. Characteristics of AP-loaded chitosan nanoparticles

AP-loaded chitosan particles were prepared by a two-step process. The first step involved the formation of oil droplets (including AP) by an oil-in-water emulsion. The second step was the solidification of the formed droplets by an ionic gelation of chitosan, enveloping the oil droplets, with TPP.

FT-IR spectra of the obtained particles are presented in Fig. 1. In general, chitosan flakes show characteristic peaks at 3382 (–OH and –$NH_2$ stretching), 2886–2854 (–CH stretching), 1634 (amide I), 1565 (amide II), 1062 (C–O–C) and 887 (pyranose ring) (Fig. 1a). For chitosan particles, the peak of amide II (–$NH_2$ bending)