Chitosan-Nanoparticles as UV Filter and Carrier for Cosmetic Actives

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ABSTRACT

Chitosan derivative was prepared by grafting both phthaloyl groups and methoxy poly(ethylene glycol) moieties onto the polymer chain. Nanoparticles of the prepared chitosan derivative could then be obtained using solvent displacement technique. The products are stable white particles with the average size of 40-150 nm (dry particles). UV screening property, antimicrobial activity and stability of the particles were investigated. Various cosmetic actives such as ethylhexyl-*p*-methoxycinnamate, ascorbyl palmitate and astaxanthin could be successfully encapsulated into these chitosan-nanoparticles. Encapsulated cosmetic active showed significantly improved photostability comparing to the un-encapsulated compound.

Keywords: Nanoparticles / Chitosan / Encapsulation / Cosmetic actives

INTRODUCTION

During the past decades, chitosan have been developed continuously for applications in pharmaceutical, medicine and cosmetic fields (1-3). Various chitosan derivatives were prepared into micro- and nano-particles for the use as carriers in drug delivery systems (4-6). The drug incorporation could be achieved either by physical processing or by chemical conjugating methods. Recently, self assembly nanoparticles from poly(ethylene glycol)-phthaloylchitosan has been reported (7-8). In this work, nanoparticles of poly(ethylene glycol)-phthaloylchitosan was prepared. Encapsulations of some cosmetic actives including ascorbyl palmitate, ethylhexyl-*p*-methoxy-cinnamate (EHMC) and astaxanthin, into these particles were also carried out. Photostability of the encapsulated EHMC and that of the free EHMC were compared. In addition, release of the EHMC from the particle during the post application onto baby mouse skin was demonstrated.

MATERIALS AND METHODS

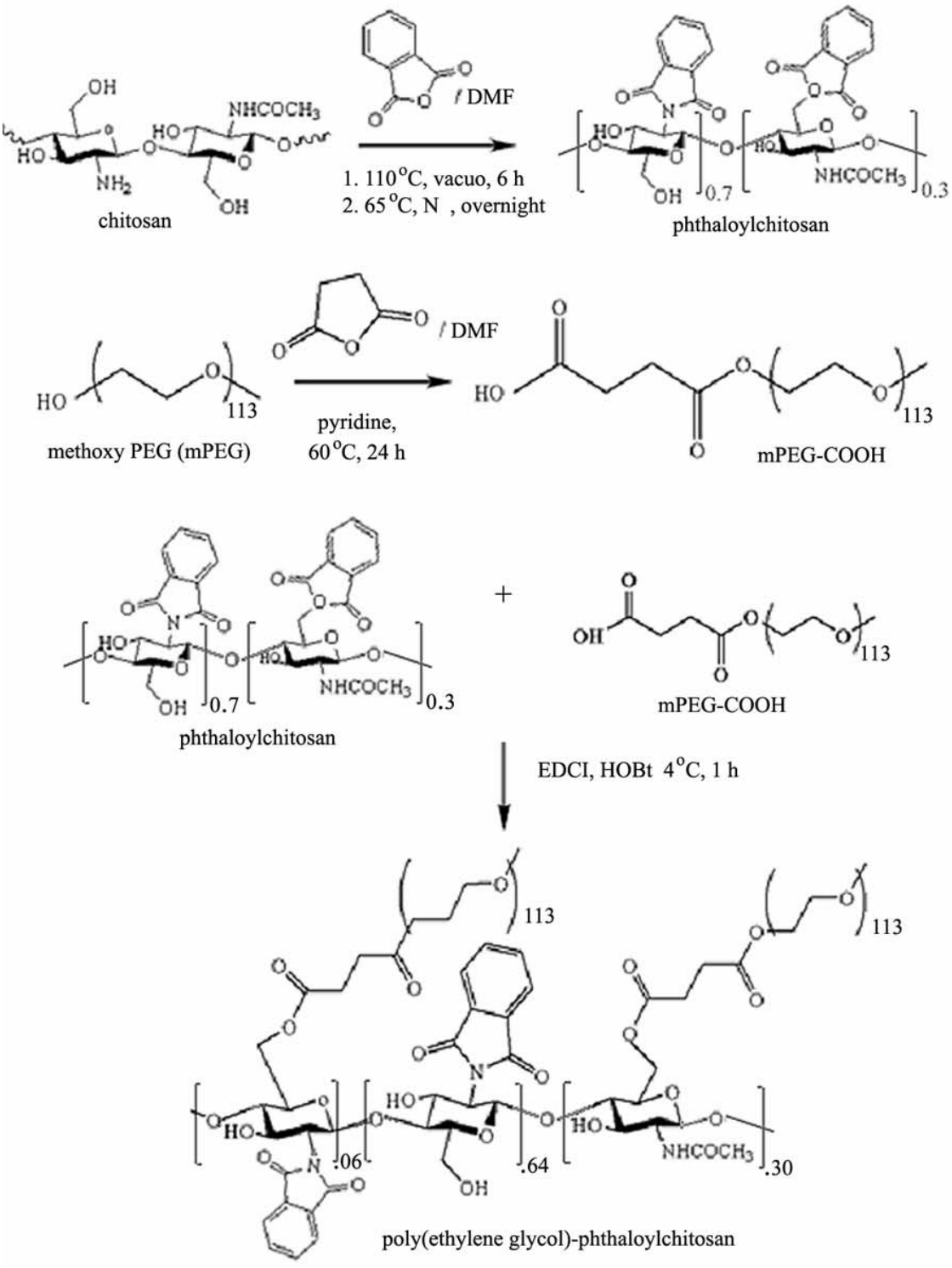
Chitosan (MW 110,000) was purchased from Seafresh Chitosan (Lab), Co., Thailand. N,N'-Dimethyl formamide and dimethyl sulfoxide were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). 1-Ethyl-3(3-dimethyl-aminopropyl)carbodiimide (EDCI), 4-methoxy-cinnamic acid and 1-hydroxy benzo-triazole (HOBt) were purchased from Acros Organics (New Jersey, USA). Methoxy poly(ethylene glycol) methyl ether (mPEG) MW of 5,000 was purchased from Fluka Chemical company (Buchs, Switzerland). Phthalic anhydride was purchased from Carlo Erba Reagent (Val de Reuil, France). Pyridine was purchased from sigma (Sigma chemical Co. Ltd, USA.). 2-Ethylhexyl-*p*-methoxycinnamate (EHMC, Eusolex 2292) was obtained from Merck Thailand Co. Ltd. (Bangkok, Thailand). Astaxanthin was obtained from Gowel Co., Ltd. (Bangkok, Thailand). Ascorbyl palmitate was obtained from Adinop Co., Ltd. (Bangkok, Thailand). Membranes used for dialysis experiments were seamless cellulose tube (MWCO 12,400, Viskase Companies, Inc., Japan). Centrifugal filter devices with MWCO 10,000 (Amicon Ultra-15) were purchased from Millipore (Ireland).

All FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared spectrophotometer: Impact 41.0 (Nicolet Instruments Technologies, Inc., WI, USA). ^{1}H-NMR spectra were obtained in DMSO-d_6 or DMF-d_7 with TMS as an internal reference using Varian Mercury spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C (Variance Company, USA). Ultraviolet absorption spectra were obtained with the aids of an HP 8453 UV/VIS spectrophotometer (Agilent Technologies, CA, USA). Broad band UVA (320-400 nm) and broad band UVB (280-320 nm) were generated by an FSX24T12/BL/HO (PUVA) and an FSX24T12/BL/HO lamps (National Biological Corporation, Twinsburg, Ohio, USA), respectively. Centrifugation was performed on Allegra 64R et Avanti 30 (Beckman Coulter, Inc., USA). TEM and SEM photographs were obtained from transmission electron microscope (JEM-2100, JEOL, Japan) and scanning electron microscope (JSM-6400, JEOL, Japan), respectively.

Phthaloylchitosan and poly(ethylene glycol)-phthaloylchitosan were prepared according to scheme 1 (7). Substitution degree of phthaloyl and PEG moieties were determined from NMR analyses. UV Absorption profile of the grafted product was acquired in DMF. Nanoparticle formation was self-induced by dialyzing the chitosan solution in DMF against water (7). Aqueous dispersibility of nanoparticle suspensions at various concentrations was monitored visually at room temperature. Encapsulation of EHMC was done by performing the nanoparticle formation of 0.6% (w/v) poly(ethylene glycol)-phthaloylchitosan solution in a presence of 0.0415 M EHMC. The obtained suspension of EHMC-encapsulated-nanoparticles in water was centrifuged and quickly washed with methanol to remove EHMC on the outer sphere. Encapsulation efficiency was determined by quantitating amounts of EHMC recovered in the dialysate and in the washing MeOH, using UV absorption spectroscopy with the aid of a calibration curve. Percentage of the encapsulated material was determined by NMR analysis using the ratio of peak area representing chitosan proton and EHMC proton. Encapsulations of ascorbyl palmitate and astaxanthin were done in the same manner.

Photostability tests of free EHMC and encapsulated EHMC were carried out by irradiating 1) the EHMC encapsulated particle suspension in aqueous 1.5% tween 20 solution and 2) the EHMC + un-encapsulated particle suspension in 1.5% tween 20 aqueous solution (free EHMC), with broad band UVB light (1.48 mW/cm^2) for 0,

15 and 30 min (corresponded to the radiant exposures of 0, 22.5 and 45.0 mJ). At such UV exposure doses, both suspensions were withdrawn, quickly dried under light proof condition, and subjected to NMR analyses.



Scheme 1 Synthesis of poly(ethylene glycol)-phthaloylchitosan

In vitro permeation studies of free EHMC and EHMC encapsulated particle suspension were conducted with vertical Franz diffusion cells using abdominal skin of 2 week old baby mice (*Mus Musculus* Linn.). The receptor medium consisted of isotonic phosphate buffered saline, pH 7.4 (PBS-buffer) with 1% w/v Tween 20 to ensure the

solubility of EHMC. The encapsulated particles which contained 34% (w/w) of EHMC, were added into the donor compartment to give a 4.4 mg/cm^2 final coverage of the sample on the skin which corresponded to 1.5 mg/cm^2 of EHMC coverage on the skin. At 0, 1, 2, 4 and 24 h, 3.4 mL of receptor fluid were withdrawn and replaced with fresh receptor medium. The concentration of EHMC in each withdrawn receptor fluid was then determined by UV/VIS spectrophotometer using calibration curve constructed with EHMC standard solution in the receptor fluid. All tests were done at least in triplicate. Since skins from different mice gave different penetration rates, penetration of each encapsulated EHMC sample was compared to the penetration rate of free EHMC (at 1.5 mg/cm^2 coverage) using skin from the same mouse.

Antibacterial activity was determined by monitoring the turbidity of the suspension containing bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) and the test sample in a triptic soy liquid broth. Test samples included the poly(ethylene glycol)-phthaloylchitosan nanoparticles, chitosan solution, clindamycin (an antibacterial standard) and water (blank). At 0, 12, 24 and 48 h of incubation, the suspension was withdrawn and subjected to optical density determination at 600 nm.

RESULTS AND DISCUSSION

Phathaloylchitosan was obtained by a classical amide/ester formation between amino/primary hydroxyl groups of chitosan and phthalic anhydride using EDCI and HOBt. The grafted polymer was characterized by ^{1}H NMR (in DMF-d7), which clearly indicated the presence of phthaloyl moieties by the appearance of signals at 7.1-7.9 ppm (4H, Ar-H of the phthaloyl groups). Substitution degree of 100% (see Figure 1) was determined from integration of these phthaloyl protons against H1-H6 protons of the glucosamine backbone (3.2-5.5 ppm, 7H, H1-H6 of glucosamine). ^{1}H-NMR integration of the N-acetyl signal (s, 2.0 ppm $COCH_3$ of the chitosan) against the H1-H6 protons of glucosamine, indicated 30% degree of N-acetylation of the material. Presence of new peaks at 1778 and 1710 cm^{-1} in the IR spectrum, referring to carbonyl anhydride, also confirmed the successful phthaloylation.

Grafting of PEG onto phthaloylchitosan could also be achieved using EDCI and HOBt. Five percent substitution degree of PEG (see Figure 1) was calculated through the increase in the integration at 3.2-5.5 ppm arised from -OCH_2CH_2O- protons of the PEG moieties (3.2-4.0 ppm), against the phthaloyl protons (7.1-7.9 ppm). Presence of new peaks at 1736 cm^{-1} for carbonyl, and 1111 cm^{-1} for ether bond (C-O-C) in the IR spectrum of poly(ethylene glycol)-phthaloylchitosan also confirmed successful grafting.

By grafting poly(ethylene glycol) onto phthaloyl-chitosan, absorption in the UVA region (320-360 nm) could be observed clearly (Fig 1). Nanoparticle formation by self assembly of the polymer using solvent displacement method (displacing DMF with water) gave spherical nanoparticles with diameter of approximately 40-150 nm when visualized by SEM (Fig 2). Stability of the particle aqueous suspension was concentration dependent. Suspensions with concentrations of less than 500 mg/L were clear and stably dispersed. However, suspensions at higher concentrations (>1000 mg/L) were opaque and precipitates of the agglomerated particles could be observed after 2-3 days.

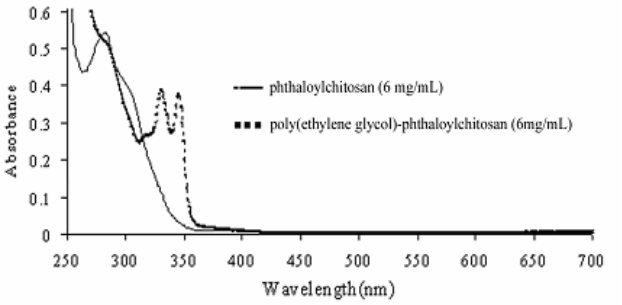


Figure 1 UV absorption spectra of phthaloylchitosan and poly(ethylene glycol)-phthaloylchitosan solutions in DMF.

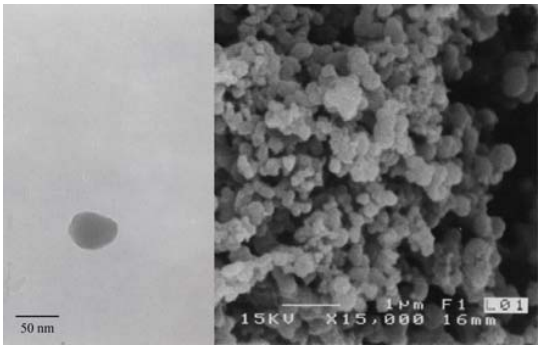
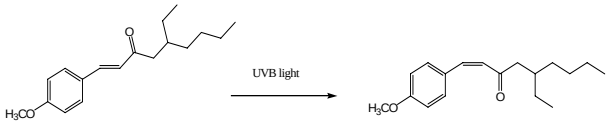


Figure 2 TEM (left) and SEM (right) images of the poly(ethylene glycol)-phthaloylchitosan particles.

The poly(ethylene glycol)-phthaloylchitosan particles showed obvious antibacterial activity against both gram positive bacteria *(Staphylococcus aureus* ATCC 25923) and gram negative bacteria *(Escherichia coli* ATCC 25922). However, at similar bacterial density, chitosan solution showed 2-3 times higher antibacterial activity than the poly(ethylene glycol)-phthaloylchitosan particle suspension.

Encapsulation of ascorbyl palmitate, ethylhexyl-*p*-methoxycinnamate (EHMC) and astaxanthin into poly(ethylene glycol)-phthaloylchitosan particles by performing the particle formation in the presence of 0.0415 M active compound using 0.6% (w/v) poly(ethyleneglycol)-phthaloylchitosan solution, resulted in 99-100 % encapsulation for all three compounds. All the actives were incorporated into the particles and no active could be detected in the dialysates. Under this condition, the resulting encapsulated particles contained 70, 38 and 37% (w/w) of the actives for ascorbyl palmitate-, EHMC- and astaxanthin-encapsulated particles, respectively. The sizes of the ascorbyl palmitate-encapsulated particles were bigger than those of the other two. This might be a result of higher percentage of incorporation.

Table 1 Percentages of *trans* and *cis* configuration in the irradiated encapsulated-EHMC and free EHMC.



trans-EHMC *cis*-EHMC

UVB exposure (mJ)	% *cis* configuration	
	EHMC-encapsulated particles	Free EHMC + unencapsulated particles
0	0	0
22.5	13	26
45.0	17	45

It has been known that EHMC can be photoisomerized from *trans* to *cis* configuration upon exposing to UVB light (9-10). To evaluate the ability of the poly(ethylene glycol)-phthaloylchitosan particles in shielding UV light from the encapsulated material, photostability of the encapsulated EHMC was compared with that of free EHMC. Both suspensions contained similar concentration of EHMC and poly(ethylene glycol)-phthaloylchitosan particle; 10 mL of the encapsulated nanoparticle suspension contained 147 mg of the EHMC-encapsulated particles which corresponded to 27 mg of EHMC and 120 mg of the poly(ethylene glycol)-phthaloylchitosan while 10 mL of the free EHMC suspension contained 27 mg EHMC and 120 mg poly(ethylene glycol)-phthaloylchitosan nanoparticles. It should be noted here that the EHMC-encapsulated particles prepared freshly for this photostability test contained less EHMC than the earlier mentioned EHMC-encapsulated particles. *Tran-cis* photoisomerization of EHMC was monitored through ^{1}H NMR using the integration of the *trans* EHMC proton (6.3 ppm, d, J = 16, CHCOO-) against that of the *cis* (5.8 ppm, d, J = 12, CHCOO-). The encapsulated EHMC was more photostable than the free EHMC (Table 1).

Further investigation on the release of the encapsulated-EHMC from the particles was carried out by comparing the skin penetration of encapsulated-EHMC to that of the free EHMC. Release of the encapsulated EHMC from the particle after the particle was applied onto the baby mouse skin could be confirmed by obvious detection of the EHMC in the receptor fluid. The release could occur without additional need of pH or temperature induction. However, at similar coverage, free EHMC could penetrate through the baby mouse skin faster than the encapsulated-EHMC (Figure 4). The encapsulated EHMC thus possessed controlled released characteristic.

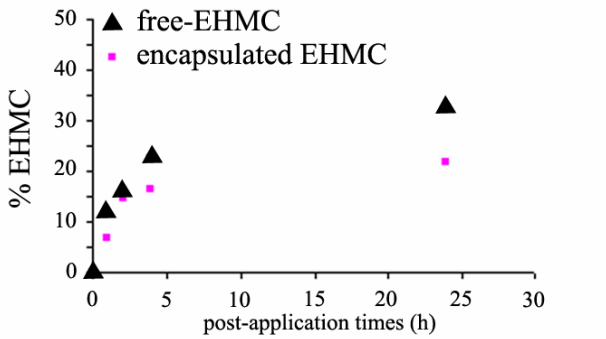


Figure 4 Amounts of EHMC detected in receptor fluid at various post-application times.

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