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Retinol-encapsulated low molecular water-soluble chitosan nanoparticles

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Abstract

This aim of this study was to encapsulate retinol into chitosan nanoparticles and reconstitute it into aqueous solution. Retinol-encapsulated chitosan nanoparticles were prepared for application of cosmetic and pharmaceutical applications. Retinol-encapsulated chitosan nanoparticle has a spherical shape and its particle sizes were around 50–200 nm according to the drug contents. Particle size was increased according to the increase of drug contents. Solubility of retinol is able to increase by encapsulation into chitosan nanoparticles more than 1600-fold. It was suggested that retinol was encapsulated into chitosan nanoparticles by ion complex as a result of FT-IR spectra. Specific peak of chitosan at 1590 cm^{-1} was divided to semi-doublet due to the electrostatic interaction between amine group of chitosan and hydroxyl group of retinol. At ^1H NMR spectra, specific peaks of retinol disappeared when retinol-encapsulated chitosan nanoparticles were reconstituted into D_2O while specific peaks both of retinol and chitosan appeared at D_2O/DMSO (1/4, v/v) mixture. XRD patterns also showed that crystal peaks of retinol were disappeared by encapsulation into chitosan nanoparticles. Retinol-encapsulated nanoparticles were completely reconstituted into aqueous solution as same as original aqueous solution and zeta potential of reconstituted chitosan nanoparticles was similar to their original solution. At HPLC study, retinol was stably and efficiently encapsulated into chitosan nanoparticles.

Keywords: Water-soluble chitosan; Retinol; Polyion complex; Nanoparticles

1. Introduction

Chitosan is a natural polymer derived from chitin by deacetylation. Since chitosan is regarded as biocompatible, biodegradable, and non-toxic, it is interesting biomaterial because of its ability as a drug carrying materials and ease of modification (Hirano, 1999). Furthermore, chitosan has been reported to enhance drug delivery across the nasal or mucosal layer without damage (Fernandez-Urrusuno et al., 1999; Kotez et al., 1997). Despite of its superiority as a biomaterial, chitosan is not fully soluble in water and then soluble in acidic solution. Aqueous solubility of chitosan only in acidic solution limits its application to bioactive agents such as gene delivery carriers, peptide carriers, and drug carriers. Nah and Jang (2002) developed water-soluble chitosan (WSC) with low molecular weight and free-amine group. Water-soluble chitosan is easily soluble in neutral aqueous solution. Its advantage is ease of modification, useful as gene or peptide drug carriers, and drug carriers (Lee et al., 2001).

Retinol and its derivatives are extensively used in the pharmaceutical and cosmetic area. Especially, retinoids are recognized as being important for modern therapy of dermatological treatment of wrinkled skin (Guénin and Zatz, 1995; Varani et al., 2000). Of the retinoids, retinol and retinyl palmitate are thought to induce thickening of the epidermis and are effective for treatment of skin diseases (Tsunoda and Takabashy, 1995; Vahlquist, 1999). These functional substances, however, are known to be unstable if exposed to light or heat. A characteristic feature of retinoids is their sensitivity to ultraviolet radiation. UVB and UVA radiation reduce the Vitamin A content of the human epidermis (Andersson et al., 1999; Sorg et al., 1999). Although retinol and retinyl palmitate are less toxic than retinoic acid (which, due to its irritative properties, is acceptable only for therapeutic treatment) the ultraviolet effect on these compounds makes their use in dermatology more difficult. To increase the stability and decrease the toxicity of retinoic acid, its precursors

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retinol and retinyl esters (such as palmitate) are employed in formulations for dermatological use (Song et al., 1999).

Since retinol and its analogs have stability and solubility problem (Szuts and Harosi, 1991), various carriers or formulations are developed. Jenning et al. (2000a) used solid lipid nanoparticles (SLN) for encapsulation of retinol. They reported that retinol-incorporated solid lipid nanoparticles are incorporated in convenient topical dosage form (Jenning et al., 2000a) and high retinol concentrations were found in the upper skin layers when SLN were applied (Jenning et al., 2000b). Nanoparticles are reported to be useful formulation to solve the poor aqueous solubility of retinoids and are able to use it by intravenous injection (Lim and Kim, 2002). Thünemann reported that polyethyleneimine (PEI) complexes with retinoic acid form nanoparticles for controlled release of atRA. They showed a nanoparticulate complex between atRA and PEI has sizes ranging from 170 to 580 nm. Rodomska and Dobrucki (2000) reported that microemulsion preparation for retinol-encapsulation was stable and showed no physical changes for 6 months at 20 °C.

For this study, we prepared retinol-encapsulated chitosan nanoparticles. Preparation and their physicochemical properties of chitosan nanoparticles were investigated using various analytical equipments such as TEM, DLS, and Fourier-transform infrared (FT-IR) spectroscopy. Furthermore, in vitro solubility and stability of retinol when encapsulated into chitosan nanoparticles were performed.

2. Materials and methods

2.1. Materials

Water-soluble chitosan (M.W. = 18,000, deacetylation degree = 96%) was obtained from KITTOLIFE Co., Korea. Retinol and ethanol were purchased from Sigma Co. Ltd., USA. Dimethyl sulfoxide (DMSO)-D form and deuterium oxide (D_2O) were purchased from Sigma Co. Ltd., USA.

2.2. Preparation of retinol-encapsulated chitosan nanoparticles

A 50 mg of chitosan was dissolved in 10 ml of deionized water (0.5%, w/v) without addition of acetic acid. Retinol dissolved in 1 ml of ethanol was dropped into 10 ml of chitosan solution (0.5%, w/v) with ultrasonication (probe type sonicator, Sonic & Materials Inc., Danbury, CT, USA) at an output power of 50 W for 10 cycles of 2 s on ice. After that, ethanol was evaporated using rotary-evaporator. Resulting solution was analyzed or lyophilized for 3 days. All procedure was performed on darkened condition.

2.3. Particle size and zeta potential measurement

Particle size and zeta potential of nanoparticles were measured with a photon correlation spectroscopy (Zetasizer 3000, Malvern instruments, England) with an He–Ne laser beam at a wavelength of 633 nm at 25 °C (scattering angle of 90°). A nanoparticle solution prepared by dialysis method was used for particle size measurement (concentration: 0.1 wt.%) and measured without filtering.

2.4. Transmission electron microscope (TEM) observation

A drop of nanoparticles suspension was placed on a carbon film coated on a copper grid for TEM. Observation was done at 80 kV in a JEOL JEM-2000 FX II.

2.5. Fourier-transform-infrared spectroscopy measurement

The chemical structure and complexes formation between chitosan and atRA were analyzed by FT-IR spectroscopy (Shimadzu, FT-IR 8700, Japan). For measurement of FT-IR, polyelectrolyte complexes between chitosan and atRA were lyophilized for 3 days under dark condition.

2.6. ^1H nuclear magnetic resonance spectra study

To study retinol-encapsulation into nanoparticles, ^1H NMR spectra were measured using DMSO and D_2O. For ^1H NMR study, free retinol and chitosan were dissolved in DMSO and D_2O, respectively. Retinol-encapsulated chitosan nanoparticles were dissolved in D_2O and D_2O/DMSO (1:4, v/v) mixture.

2.7. Reconstitution test

A 10 mg of lyophilized retinol-encapsulated chitosan nanoparticles was suspended into 10 ml of deionized water and solution was vortexed for 5 s. Resulting solution was used for analysis of particle size measurement.

2.8. Solubility test

Solubility test was performed by two methods. First method is as follows: excess amount of retinol was dissolved in 1 ml of ethanol and mixed with various concentrations of 10 ml chitosan aqueous solution. Resulting solution was stirred for 1 or 3 days. After that, solution was filtered with filter paper (Whatman No. 2). Filtered solution was diluted 10–100 times and measured absorbance by UV spectrophotometer (Shimadzu UV-1601, Shimadzu Co. Ltd., Japan).

2.9. High-pressure liquid chromatography (HPLC) measurement

For analysis of retinol-encapsulated in polymeric micelle by HPLC, 5 mg of lyophilized polymeric micelle was redistributed into 0.3 ml of deionized water and then 1.2 ml of EtOH was added slowly for 10 min with sonication (bar-type sonicator, Vibracell, Sonic & Materials Inc.) at 4 °C. This solution was treated with additional sonication for 10 min (25 s pulse with 5 s intervals, 20 times) at 4 °C and magnetically stirred for 3 h. 0.1 ml of this solution was mixed with 0.9 ml of EtOH and then injected into the column.

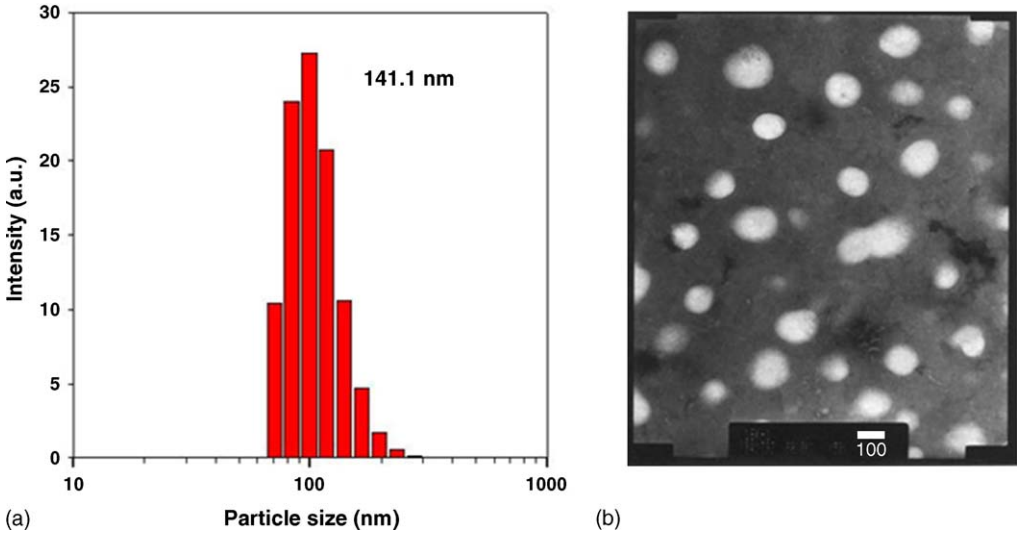

Fig. 1. Particle size distribution (a) and TEM images (b) of retinol-encapsulated chitosan nanoparticles.

Mobile phase (methanol:water = 95:5, v/v) was degassed by sonication under reduced pressure for 3 min before use. Retinol was separated by isocratic elution chromatography, at a flow rate of 1.5 ml/min and at 25 °C, using Phenomenex Sphereclone 5micro ODS(2) column (250 mm × 4.60 mm). Phenomenex sphereclone 5micro ODS(2) precolumn (30 mm × 4.60 mm) was placed between the injector and column. UV absorbance detection was at 235 nm. For standard test, retinol was dissolved in EtOH and injected 20 μl into the column. The retention time of free retinol was about 4.3 min.

3. Results and discussion

3.1. Characterization of retinol-encapsulated chitosan nanoparticles

Nanoencapsulation of retinol is thought to be an ideal approach since it is one of the highly hydrophobic and photosensitive drugs. To solve the solubility and stability problem, a lot of approaches were reported (Guénin and Zatz, 1995; Jenning et al., 2000a,b; Rodomska and Dobrucki, 2000). Among various kinds of encapsulations or formulations, polyelectrolyte complex formation should be a promised procedure to solve solubility and stability problem of retinoids analogs because of their ionic characteristics (Thünemann and Beyermann, 2000). We previously reported that water-soluble chitosan with high contents of free-amine group in the main chain (Nah and Jang, 2002) were suitable for solubilization of all-trans retinoic acid based on ion complex (Kim et al., 2005). Water-soluble chitosan is distinguished in their water-solubility, i.e. normal chitosan is needed to use an acidic aqueous solution to dissolve chitosan completely whereas WSC can dissolve in distilled water directly without use of acidic solution. These properties have great poten-

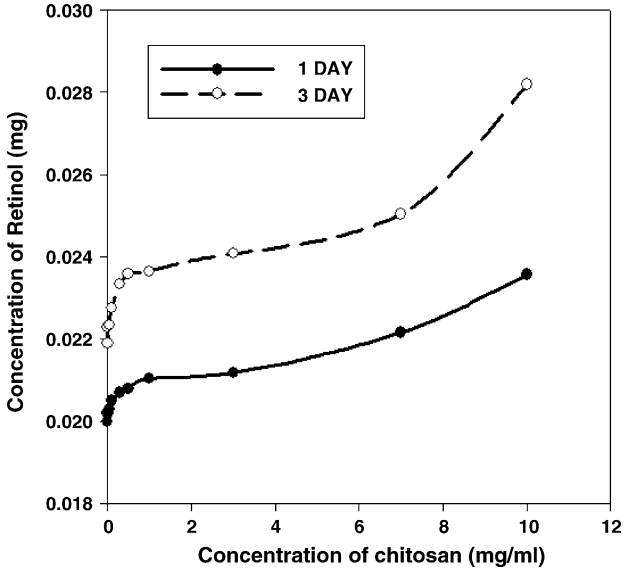

Fig. 2. Solubilization of retinol into chitosan.

Table 1
Characterization of retinol-encapsulated chitosan nanoparticles

	Chitosan/retinol weight ratio (mg/mg)	Particle size (nm)			Zeta potential (mV)
		Intensity ave.	Weight ave.	Number ave.	
18 K-2	50/2	100.6 ± 30.3	79.6 ± 21.5	67.8 ± 14.7	51.90
18 K-5	50/5	135 ± 42.4	106.2 ± 28.9	90.6 ± 19.4	55.92
18 K-10	50/10	174.2 ± 46.6	143.8 ± 35.7	124.2 ± 26.1	67.32
18 K-20	50/20	254.5 ± 67.2	208.6 ± 53.3	179.0 ± 38.1	74.86

tial of application in the drug delivery system and biomedical application since most of the drugs, proteins, peptides, and DNA drugs are sensitive to acidic solution and easy to inactivate in the high acidic environment. Actually, we showed that WSC is a good candidate for gene delivery system with high transfection efficiency (Lee et al., 2001; Nah and Jang, 2002).

Particle size distribution and TEM observation were obtained to study chitosan nanoparticle formation when retinol added into chitosan aqueous solution. As shown in Fig. 1(a), size of retinol-encapsulated chitosan nanoparticles was 141.1 nm with broad size distribution. Retinol-encapsulated chitosan nanoparticles have spherical shapes in their morphologies and showed around 100 nm in their particle size. As shown in Fig. 1(b), particle size observed in TEM photos was almost similar to the results of particle size. The reason of broad size distribution (Fig. 1(a)) was thought to be an aggregation of chitosan nanoparticles. As shown in Fig. 1(b), some nanoparticles interact with each other (arrows in Fig. 1(b)).

Retinol-encapsulated chitosan nanoparticles were prepared against retinol contents and their results were described in Table 1. As shown in Table 1, particle size and zeta potential were increased according to the contents of retinol as expected. Particle sizes of chitosan nanoparticles were increased from 70 nm up to 250 nm according to the increase of drug contents. Also,

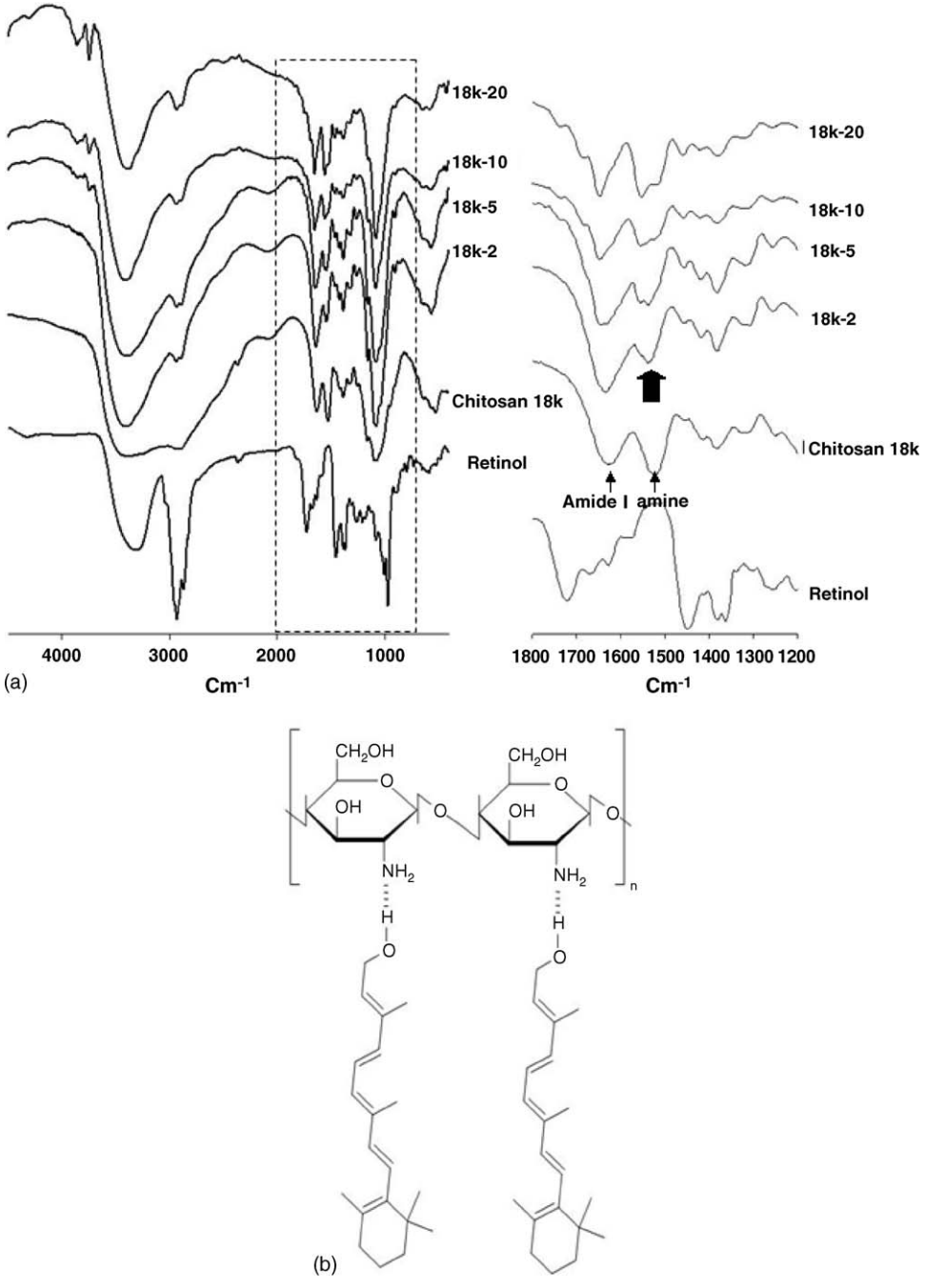

Fig. 3. (a and b) FT-IR spectra of retinol-encapsulated nanoparticles.

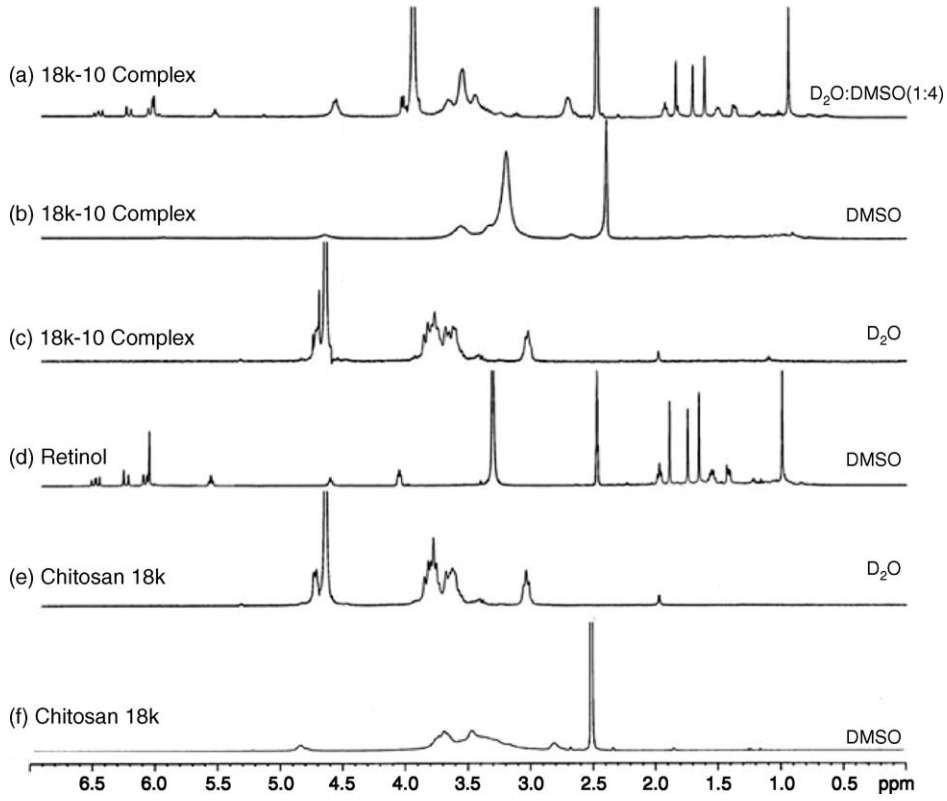

Fig. 4. ^{1}H NMR spectra of retinol-encapsulated chitosan nanoparticle in D_2O/DMSO mixtures (1:4, v/v) (a), DMSO (b), D_2O (c), free retinol in DMSO (d), chitosan in D_2O (e), and chitosan in DMSO (f).

zeta potential was increased according to the increase of drug contents.

Fig. 2 shows the potential of chitosan for retinol solubilization. As shown in Fig. 2, retinol concentration was increased over 28 μg/ml according to the increase of chitosan concentration, indicating that retinol solubility was increased at least more than about over 1600-fold since retinol aqueous solubility is known to be 0.06 μM (Szuts and Harosi, 1991), indicating that water-soluble chitosan is a superior vehicle for increasing solubility of retinol.

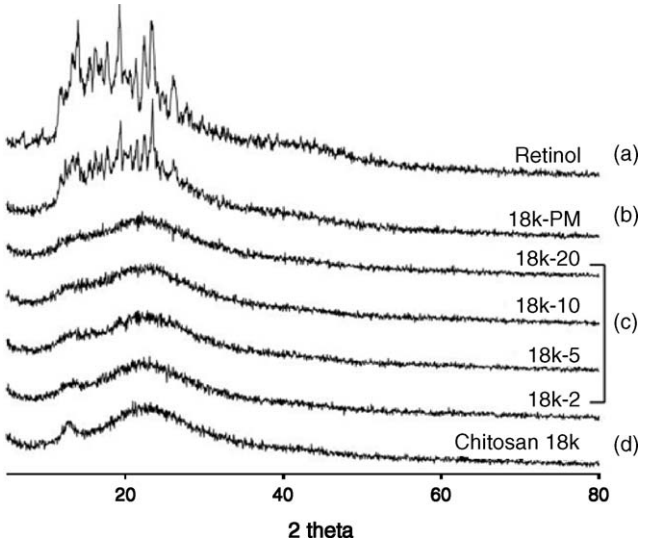

Fig. 5. X-ray powder diffraction of retinol-encapsulated chitosan nanoparticles. Free retinol (a), retinol-encapsulated chitosan nanoparticle (b), retinol/chitosan physical mixture (c), and chitosan (d).

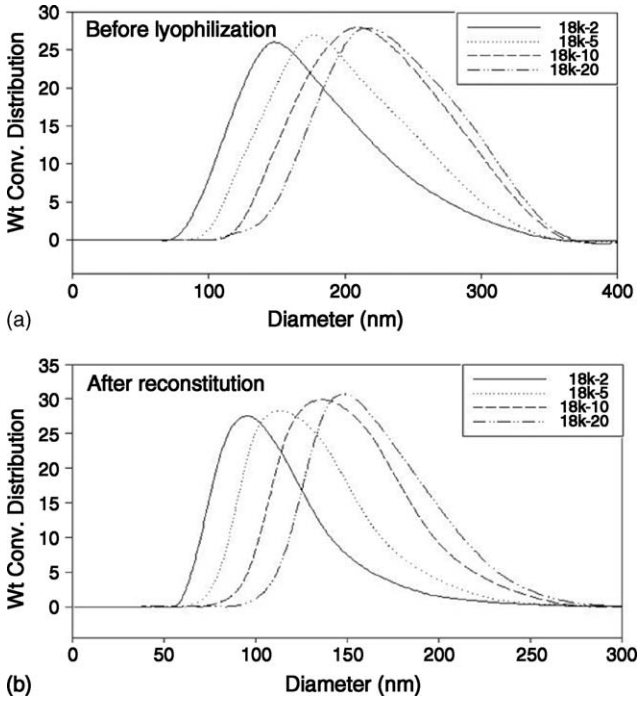

Fig. 6. Particle size distribution changes of retinol-encapsulated chiotosan nanoparticles before (a) and after (b) lyophilization.

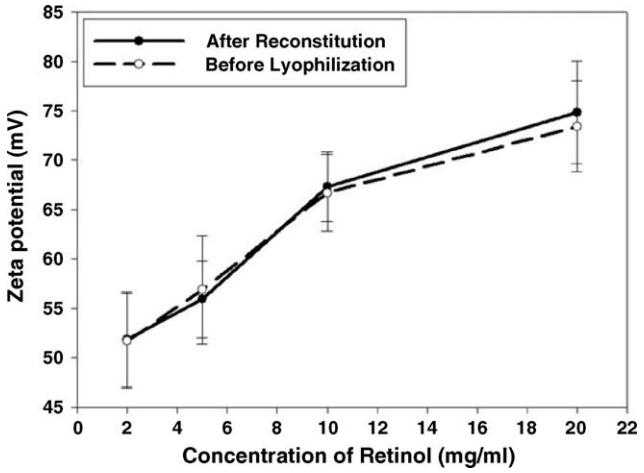

Fig. 7. Particle size and zeta potential of reconstituted retinol-encapsulated chitosan nanoparticles.

3.2. Analysis of retinol-encapsulated chitosan nanoparticles

In FT-IR analysis of chitosan, specific peaks of chitosan were observed at 1630 cm^{-1} (amide I) and 1590 cm^{-1} (amine). As shown in Fig. 3(a), specific peak of chitosan at 1590 cm^{-1} was divided to semi-doublet due to the electrostatic interaction between amine group of chitosan and hydroxyl group of retinol. Furthermore, specific peak of retinol at 1720 cm^{-1} was significantly decreased when retinol encapsulated into chitosan nanoparticles. These results indicated that retinol-encapsulation mechanism is ion complex formation between chitosan and retinol following nano-sized particle formation.

To study the properties of nanoparticle formation and retinol-encapsulation into core of chitosan nanoparticles, ^1H NMR and X-ray powder diffraction pattern were also measured. As shown in Fig. 4, retinol (Fig. 4(d)) and chitosan itself (Fig. 4(e)) showed their specific peak characteristics. When retinol-encapsulated chitosan nanoparticles were reconstituted into D_2O (Fig. 4(c)), specific peaks of retinol were disappeared at their proton spectra while specific peaks of chitosan were shown. However, specific peaks of both of retinol and chitosan were appeared when retinol-encapsulated chitosan nanoparticles dissolved into D_2O/DMSO mixtures (Fig. 4(a)). These results indicated that retinol was encapsulated in the core of the chitosan nanoparticles when nanoparticles were reconstituted in water but chitosan nanoparticles were destroyed at D_2O/DMSO (1:4, v/v) and specific peaks both of retinol and chitosan were shown at their proton spectra (Fig. 4(a)). Interestingly, specific peaks of retinol were disappeared when retinol-encapsulated chitosan nanoparticles distributed into DMSO (Fig. 4(b)). These results might be due to that chitosan itself was practically insoluble in DMSO and showed broad spectrum (Fig. 4(f)). Furthermore, it suggested that retinol in chitosan nanoparticle was not liberated from the chitosan nanoparticles at DMSO environment due to the strong electrostatic interactions between chitosan and retinol in spite of retinol itself is soluble in DMSO. These results also approved that free drug did not remain in the chitosan nanoparticle formulation. Resultantly, retinol-encapsulated chitosan nanoparticles maintained solid state in the DMSO.

Fig. 5 showed X-ray powder diffraction pattern of retinol-encapsulated chitosan nanoparticles. As shown in Fig. 5(a and d), retinol has specific sharp crystal peaks and chitosan has a specific broad peak. When retinol was encapsulated into chitosan nanoparticles, their sharp crystal peaks were disappeared whereas physical mixture of retinol and chitosan showed both of specific peaks of retinol and chitosan. Especially, specific sharp crystal peaks were not observed at highest drug contents (18 K-20) as similar as at lower drug contents, indicating that retinol was completely and successively encapsulated into core of chitosan nanoparticles.

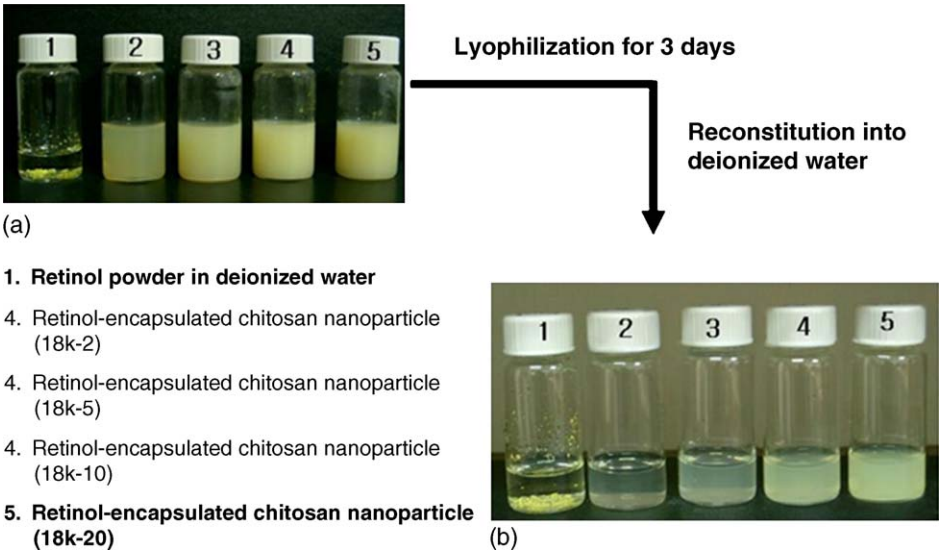

Fig. 8. Photographs of reconstituted retinol-encapsulated chitosan nanoparticles before (a) and after (b) lyophilization.

3.3. Reconstitution of retinol-encapsulated chitosan nanoparticles

In the pratical use of nanoparticles, the reconstitution of lyophilized solid nanoparticles is one of the most important factors in the whole process of nanoparticle preparation and application. For long-term storage of nanoparticles, aqueous solutions of nanoparticles are essentially required to lyophilize as solid products and lyophilized solid nanoparticles must be reconstituted into physiological solution as same as its original aqueous solution before use of it (Chacon et al., 1999; Jaeghere et al., 1999; Zimmermann et al., 2000). Konan et al. (2002) reported that sterilized sub-200 nm nanoparticles of polyester showed complete redispersion in the presence of lyoprotectants tested such as saccharides while aggregation was observed without lyoprotectant. Jaeghere et al. (1999) reported that poly(DL-lactide) (PLA)–poly(ethylene oxide) (PEO) nanoparticles were aggregated after freeze-drying and this problem could be circumvented by use of trehalose as a cryoprotectant. Generally, lyophilized nanoparticles were significantly aggregated and par-

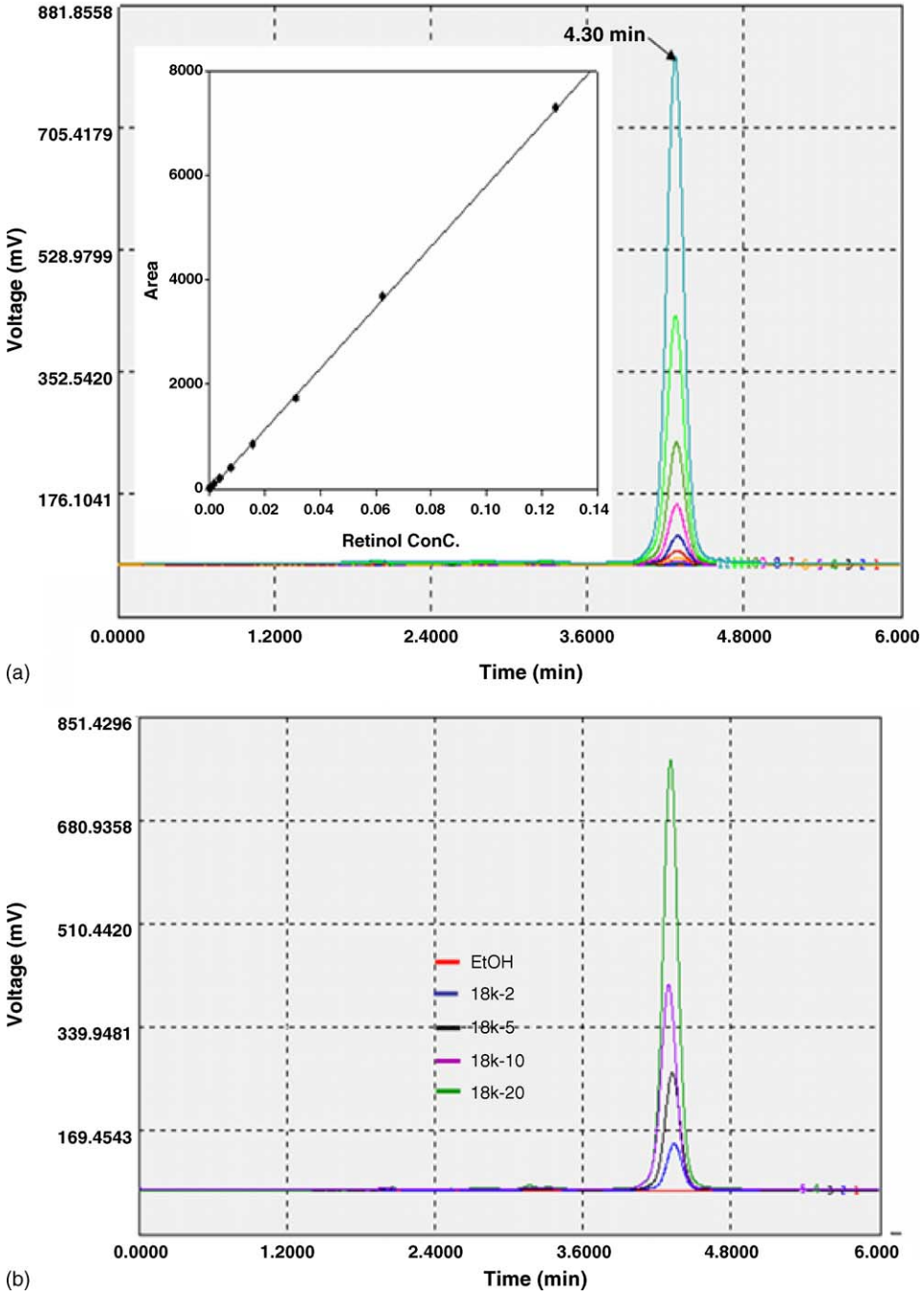

Fig. 9. HPLC chromatogram of retinol. (a) retinol—chromatogram was recorded by original retinol according to the concentration of retinol (box is a calibration curve) and (b) extracted retinol from nanoparticles—retinol was extracted by water/EtOH mixed solvent system. Precise procedure was described in Section 2.

ticle size increased when it reconstituted into aqueous solution although those nanoparticles were prepared in the presence of surfactant or emulsifier (Chacon et al., 1999; Konan et al., 2002).

In our study, chitosan is water-soluble whereas other kinds of chitosan are required acidic solution to dissolve it completely. Therefore, it suggested that lyophilized chitosan nanoparticles encapsulating retinol are able to redisperse into aqueous solution without aid of cryoprotectants. Whether or not lyophilized chitosan nanoparticles can reconstitute into aqueous solution, aqueous solution of retinol-encapsulated chitosan nanoparticles were lyophilized and reconstituted it into deionized water.

Fig. 6 showed particle size distribution before and after reconstitution of retinol-encapsulated chitosan nanoparticles. Before lyophilization, retinol-encapsulated chitosan nanoparticles showed broad size distribution at 50–400 nm at all formulation. When lyophilized nanoparticles were reconstituted into water (Fig. 6(b)), particle size distribution was practically same to original aqueous solution (Fig. 6(a)). These results indicated that retinol-encapsulated chitosan nanoparticles were completely reconstituted into aqueous solution without aid of cryoprotectants. Lyophilized retinol-encapsulated chitosan nanoparticles were successively reconstituted without any aggregation. Chitosan nanoparticles do not need cryoprotectants to lyophilize and reconstitute whereas cryoprotectants are necessary to other kind of nanoparticles. Fig. 7 showed changes of zeta potential before and after reconstitution of chitosan nanoparticles. As shown in Fig. 7, zeta potential of reconstituted chitosan nanoparticles was quite same to its original nanoparticle aqueous solution. These results indicated that surface properties of chitosan nanoparticles were not significantly changed by lyophilization and reconstitution processes. It suggested that chitosan was superior vehicle for encapsulation, storage, and reconstitution of retinol. Fig. 8 showed that photographs of retinol-encapsulated chitosan nanoparticles before (a) and after (b) lyophilization. As shown in Fig. 8, chitosan nanoparticles were completely reconstituted into aqueous solution without aid of cryoprotectants and any aggregates. However, retinol powder was practically in soluble in water as shown in Fig. 8.

Since retinol is known to be photo-sensitive and labile drug, the stability of retinol is one of the most important factors during encapsulation process and storage by drug formulation. To evaluate the drug stability during encapsulation process, retinol was extracted from the lyophilized chitosan nanoparticles encapsulating retinol using water/EtOH solvent mixtures and then analyzed using HPLC system. As shown in Fig. 9, original retinol appeared at 4.3 min of retention time. The extracted retinol showed completely similar retention time in the HPLC results. These results indicated that retinol was stably encapsulated into the chitosan nanoparticles and maintained their peculiar properties. As shown in Fig. 9, extracted retinol concentration was evaluated by HPLC against initial drug feeding ratio. Peaks of extracted retinol in HPLC chromatogram were increased according to the increase of feeding amount of drug at same retention time. The extracted concentration of retinol as drug contents and loading efficiency was summarized in Table 2.

Table 2
Drug contents and loading efficiency of retinol-encapsulated chitosan nanoparticles

*Chitosan/retinol weight ratio (mg/mg)	Drug contents (%, w/w)		Loading efficiency
	Theoretical	Experimental	
50/2	3.9	2.4	63.0
50/5	9.1	6.1	67.5
50/10	16.7	10.7	64.2
50/20	28.6	21.0	76.3

* Glucosamine/retinol ratio was calculated as follows: M of glucosamine unit in the chitosan/M of retinol.

Encapsulation efficiency was more than 60% at all formulations. These results indicated that retinol was stably and efficiently encapsulated into the chitosan nanoparticles through ion complex.

4. Conclusion

Retinol-encapsulated chitosan nanoparticles were prepared for application of cosmetic and pharmaceutical applications. Retinol-encapsulated chitosan nanoparticle has a spherical shape and its particle sizes were around 50–200 nm according to the drug contents. Particle size was increased according to the increase of drug contents. Solubility of retinol is able to increase by encapsulation into chitosan nanoparticles by more than 1600-fold. It was suggested that retinol was encapsulated into chitosan nanoparticles by ion complex at results of FT-IR spectra. Specific peak of chitosan at 1590 cm^{-1} was divided to semi-doublet due to the electrostatic interaction between amine group of chitosan and hydroxyl group of retinol. At ^1H NMR spectra, specific peaks of retinol disappeared when retinol-encapsulated chitosan nanoparticles were reconstituted into D_2O while specific peaks both of retinol and chitosan was appeared at D_2O/DMSO (1/4, v/v) mixture. XRD patterns also showed that crystal peaks of retinol were disappeared by encapsulation into chitosan nanoparticles. Retinol-encapsulated nanoparticle were completely reconstituted into aqueous solution as same as original aqueous solution and zeta potential of reconstituted chitosan nanoparticles was similar to their original solution. At HPLC results, extracted retinol showed similar retention time and retinol concentration was almost corresponded to the feeding amount of drug. This study showed successive encapsulation and reconstitution process of retinol-encapsulated chitosan nanoparticles.

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