J. Soc. Cosmet. Scientists Korea
Vol. 31, No. 2, June 2005, 141-146

7-디하이드로콜레스테롤을 함유한 키토산 코팅 처리 Solid Lipid Nano-particle의 개발에 관한 연구

이 근 수† · 김 태 훈 · 이 천 일 · 표 형 배 · 최 태 부*

한불화장품㈜ 기술 연구소, *건국대학교 생물화학공학부
(2005년 5월 16일 접수, 2005년 6월 10일 채택)

Development of Chitosan Coated Solid Lipid Nano-particles Containing 7-Dehydrocholesterol

Geun-Soo Lee†, Tae-Hoon Kim, Chun-Il Lee, Hyeong-Bae Pyo, and Tae-Boo Choe*

Hanbul Cosmetics Co., R&D center, 72-7, Yongsung-ri, Umsung-gun, Chungbuk 369-830, Korea
*Section of Chemical and Biological Engineering, Konkuk University
(Received May 16, 2005; Accepted June 10, 2005)

요 약: 불안정한 생리활성물질들은 외부 환경에 의해 빠르게 분해된다. 그러므로 이러한 물질들을 안정화시키기 위한 캡슐화 기술은 매우 중요하다. 비타민 D_3의 전구체인 7-디하이드로콜레스테롤(7-DHC)은 일반인의 표피 각화세포에서 열충격 단백질(Heat Shock Protein)의 발현을 단백질과 mRNA의 수준에서 증가시키는 것으로 알려졌다. 하지만 7-DHC의 국소용 피부 제제로의 이용은 낮은 용해도와 화학적 불안정성 때문에 이용이 제한되었다. 본 연구에서 7-DHC는 나노에멀젼(NE), 고형 지질 나노 입자(SLN) 그리고 키토산이 코팅된 고형 지질 나노 입자(CASLN)에 봉입하였다. NE와 SLN은 지질의 용융점 이상의 온도에서 고압의 호모제나이져를 통과시켜 제조하였다. CASLN은 SLN 분산액에 키토산을 용액을 첨가하여 제조하였으며 양(+)의 제타전위를 나타내었다. NE, SLN, CASLN 속에서 7-DHC의 안정도를 각각의 온도조건에서 시간의 경과에 따라 확인하였다. 열분석과 X선 회절 분석은 지질의 결정화 정도를 확인하기 위해서 수행하였다. 그 결과, CASLN은 기존의 SLN보다 불안정한 7-DHC를 효과적으로 봉입함으로서 안정성을 개선시켰다.

Abstract: Unstable cosmetic active ingredients could rapidly break down in chemical and photochemical process. Therefore, it has become a very important issue to encapsulate active ingredient for the stabilization. 7-Dehydrocholesterol (7-DHC), a precursor of vitamin D_3, has been shown to increase levels of protein and mRNA for heat shock protein in normal human epidermal keratinocytes. However, topical dermal application of 7-DHC is restricted due to its poor solubility and chemical unstability. In this study, 7-DHC was incorporated into nano-emulsion (NE), solid lipid nano-particle (SLN), and chitosan coated solid lipid nano-particle (CASLN), respectively. In order to prepare NE and SLN dispersion, high-pressure homogenization at temperature above the melting point of lipid was used. Hydrogenated lecithin and polysorbate 60 were used as stabilizer for NE and SLN. CASLN was prepared by high speed homogenizing after adding chitosan solution to the SLN dispersion and showed positively charged particle properties. Decomposition rate of 7-DHC in NE, SLN and CASLN was studied as a function of time at different temperature. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies were performed to characterize state of lipid modification. It appeared that CASLN is the most effective to stabilize 7-DHC and may be used for a useful topical dermal delivery system.

Keywords: *7-dehydrocholesterol (7-DHC), chitosan coated solid lipid nanoparticle (CASLN), hydrogenated lecithin, career, long term stability*

† 주 저자 (e-mail: aria@hanbul.co.kr)

1. Introduction

Nowadays many products using nano-technology have been released and nano-technology would be more prospected in cosmetic manufacturing and ingredient field [1]. Unstable cosmetic active ingredients could be degraded rapidly by chemical and photochemical process. Therefore, it has become a very important issue to encapsulate active ingredient for the stabilization. Solid lipid nano-particles (SLN), developed at the beginning of the 1990s, have attracted increasing attention as an alternative carrier system to traditional colloidal systems, such as emulsions, liposomes and polymeric micro- and nano-paticles. The small particle size ensures close contact to the stratum corneum and should increase the amount of encapsulated agent penetrating into the viable skin. The solid matrix of SLN allows the sustained and controlled release of actives and may stabilize the encapsulated actives against the chemical degradation [2-5]. Furthermore, occlusive properties as a result of film formation of the skin was reported [6]. For cosmetic applications of SLN, 7-dehydrocholesterol (7-DHC) was employed as a model ingredient. 7-DHC may induce heat shock proteins by directly activating heat shock protein synthesis [7]. Safe methods of inducing heat shock proteins would be valuable in the development of biologically active sun-protection and anti-aging products.

In this study, we prepared SLN loaded with 7-DHC as a means to improve its poor water solubility and chemical unstability. And chitosan coated solid lipid nano-particle (CASLN), positively charged SLN, was prepared by using the interactions between negatively charged SLN and cationic chitosan polymer. Decomposition rate of 7-DHC, size and zeta-potential variations of SLN as a function of chitosan concentration were investigated.

2. Material and Methods

2.1. Materials

7-Dehydrocholesterol (MMP, USA), cetyl palmitate (used as matrix lipid, Kokyu Alcohol, Japan), hydrogenated lecithin (Lipoid, Germany), polysorbate 60 (Uniqema, USA), chitosan (≥ 85% deacetylation, atrium, Canada), 1,3-butylene glycol, and cetyl octanoate were used and were cosmetic-grade. All other chemicals used were regent- or cosmetic-grade.

2.2. Measurement of Zeta-Potential

Zeta potential was measured by the Zeta-Sizer 3000 HS (Melvern, U.K), after diluting the sample in a buffer solution at pH 4.85.

2.3. HPLC Analysis

An HPLC system equipped with a photodiode-array detector (Waters 996), Waters 2695, was used to analyze 7-DHC. Chromatographic conditions for 7-DHC were as follows; a reversed-phase HPLC column (Waters symmetry C18, 4.6×250 mm), flow rate at 1.0 mL/min, detection at 282 nm, column oven temperature at 20℃. The mobile phase consist of methanol (HPLC grade).

2.4. Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD)

To characterize state of lipid modification, DSC (Shimadtu DSC 60, Japan) and XRD (Scintag XDS 2000, USA) analysis of bulk raw cetyl palmitate (CP) and lyophilized SLN were performed. DSC scan were recorded at heating rate 1℃/min, from 20℃ to 90℃. And XRD scanned 3° to 40°, 2θ at a step size of 0.02°.

2.5. Average Particle Size and Morphology

Particle size analysis was performed by laser diffraction particle size analyzer (Hydro MS 2000, Malvern, UK). To determine the size of CASLN, 10 g of the CASLN was suspended in 90 g water and treated for 10 min in an ultrasonic bath.

2.6. Preparation of SLN, CASLN and NE Loaded with 7-DHC

For the preparation of solid lipid nano-particle (SLN), the lipid phase was melted at 80℃ and 0.7% of 7-DHC was added to the melt lipid. The hot lipid phase was dispersed in a surfactant solution and a coarse emulsion was formed using a Robomics (TK robo, Japan). The coarse emulsion was passed through a high-pressure homogeniser (APV Gaulin 2000, Denmark). Three cycles at 550 bar and 70℃ were carried out. For the chitosan coated solid lipid nano-particle (CASLN), chitosan solution was added to the dispersed SLN.

And nano-emulsion (NE) were prepared in exactly

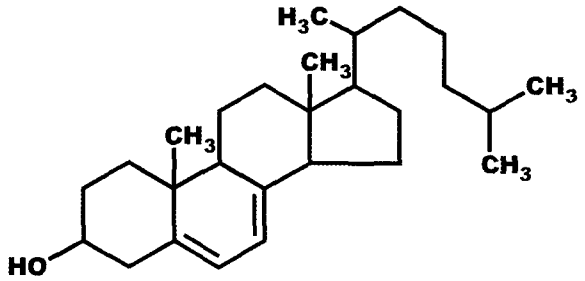

Figure 1. Chemical structure of 7-dehydrocholesterol (7-DHC).

the same manner as the SLN dispersion only replacing the solid lipid cetyl palmite (CP) by the cetyl octanoate. The formulas and preparation method were showed in Table 1 and Figure 2.

3. Results and Discussion

3.1. Average Particle Size and Zeta Potential Variation

The average particle size of CASLN was about 140 nm (Figure 3). In the SLN, using the zeta potential analysis, we confirmed coating aspect of cation (chitosan) on the lipid surface arranged with negative portion (hydrogenated lecithin). Variation of charge in the membrane surface was controlled by concentration of chitosan. As shown in Figure 4, a negatively-charged SLN (-7.45 mV) is obtained, when no chitosan is present. When adding 0.1% chitosan, the zeta potential of SLN become +5.44 mV. At higher chitosan concentrations (0.2% and 0.3%), the zeta potential is about +11 mV.

Table 1. Formulas of Emulsion Containing 7-dehyydrocholestrol

Components	Content (wt%)		
	NE	SLN	CASLN
Butylenes glycol	10.0	10.0	10.0
Hydrogenated lecithin	3.5	3.5	3.5
Cetyl palmitate	-	8.0	8.0
Preservative	0.20	0.20	0.20
7-Dehydrocholesterol	0.70	0.70	0.70
Cetyl octanoate	8.0	-	-
Polysorbate 60	0.50	0.50	0.50
Chitosan	-	-	0.10 ~ 0.30
Water		q.s to 100	

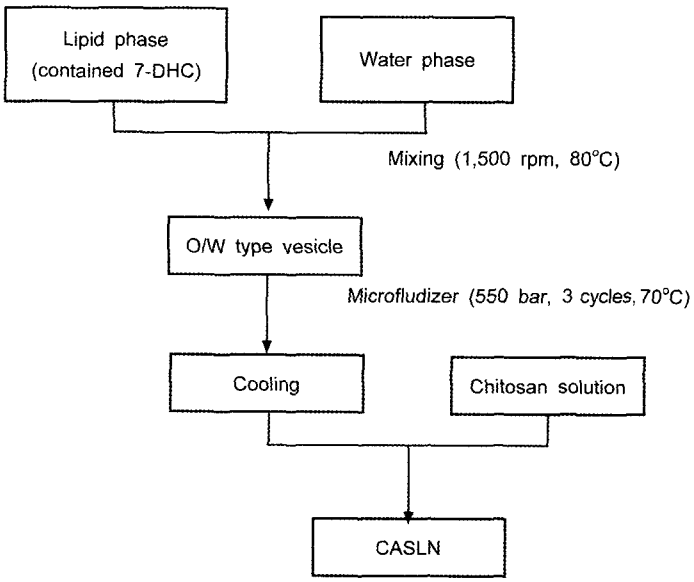

Figure 2. Manufacturing process of CASLN containing 7-DHC.

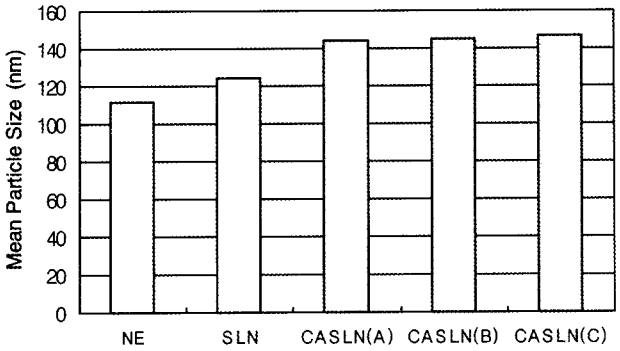

Figure 3. Average particle size of NE, SLN, CASLN. NE: nano-emulsion, SLN: solid lipid nano-particle, CASLN(A): 0.1 wt% chitosan coated solid lipid nano-particle, CASLN (B): 0.2 wt% chitosan coated solid lipid nano-particle, CASLN(C): 0.3 wt% chitosan coated solid lipid nano-particle.

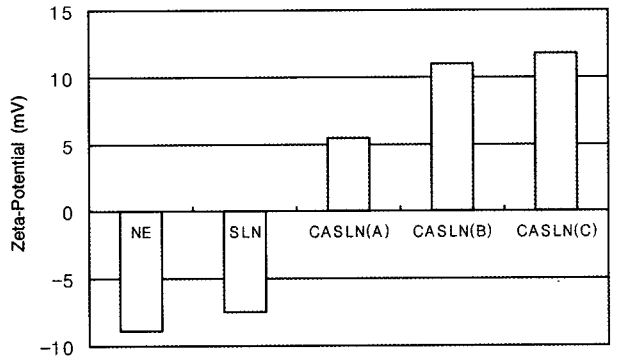

Figure 4. Zeta-potential variation as a function chitosan of concentration. NE: nano-emulsion, SLN: solid lipid nano-particle, CASLN(A): 0.1 wt% chitosan coated solid lipid nano-particle, CASLN(B): 0.2 wt% chitosan coated solid lipid nano-particle, CASLN(C): 0.3 wt% chitosan coated solid lipid nano-particle.

These results clearly indicate that the cation portion of chitosan was coated to the lipid (cetyl palmitate) surface. That reason is as follows. It was decided to first form the emulsion by using hydrogenated lecithin. The lipid emulsion was expected to be negatively charged, it was assumed that the positive charge of chitosan molecules would be capable of interacting with the absorbed negative charge of lecithin at the lipid-water interface. The change in zeta potential from negative to positive value was also observed by Magdassi *et al* [8]. When chitosan is added *in suit*, electropositively charged portion was formed on the surface of the lipid.

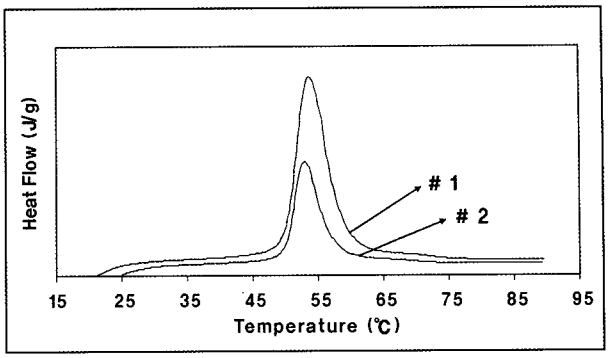

Figure 5. DSC scan of lyophilized SLN powder heating from 20℃ to 90℃ at a rate of 1℃/min. #1: bulk matrix material (cetyl palmitate), #2: lyophilized SLN.

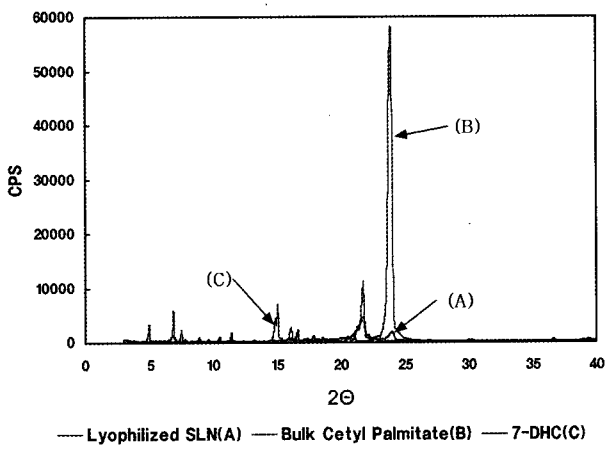

Figure 6. X-ray diffraction patterns of lyophilized SLN, bulk cetyl palmitate, pure 7-DHC.

3.2. DSC and XRD Investigations

The result of DSC measurement was shown in Figure 5. The higher melting enthalpy values should suggest higher ordered lattice arrangement and *vice versa*. Therefore, the lipid within SLN should be in a less ordered arrangement compared to the bulk cetyl palmitate corresponding to the DSC analysis. Therefore, the melting point of cetyl palmitate is dependant on the 7-DHC in the carrier.

The scattering pattern of nano-particles is demonstrated in Figure 6. The diffraction pattern of the bulk cetyl palmitate showed remarkable difference from that of the SLN with weak intensity. And there were no characteristic peaks for 7-DHC in lyophilized SLN. These results indicate that lyophilized SLN was less ordered crystalline state and 7-DHC was not in crystalline form in SLN.

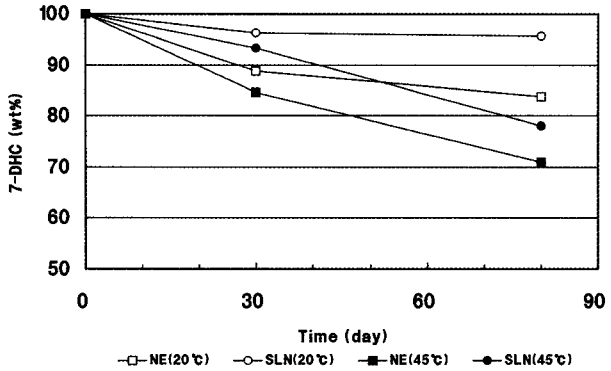

Figure 7. Stability of 7-DHC in NE and SLN at different temperature. NE: nano-emulsion, SLN: solid lipid nano-particle.

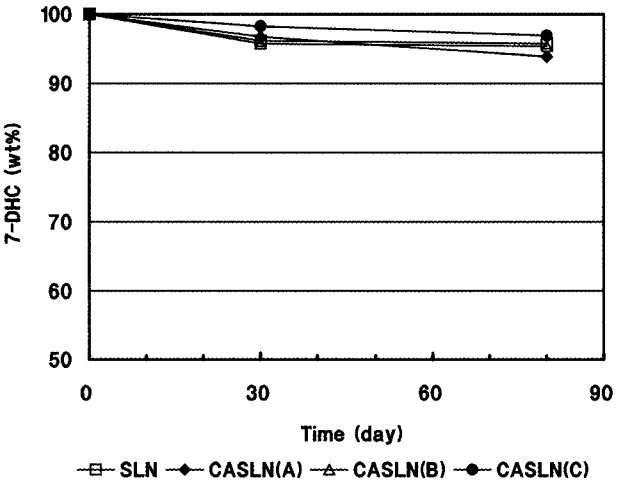

Figure 8. Stability of 7-DHC in CASLN at 20℃ for 80 days. SLN: solid lipid nano-particle, CASLN(A): 0.1 wt% chitosan coated solid lipid nano-particle, CASLN(B): 0.2 wt% chitosan coated solid lipid nano-particle, CASLN(C): 0.3 wt% chitosan coated solid lipid nano-particle.

3.3. Stability of 7-DHC Loaded in Different Formulation

7-DHC is important in a wide variety of biological functions. However, this active material degraded with ease by the outer condition. In this study, 7-DHC was incorporated into CASLN and the kinetics of 7-DHC degradation investigated. The NE, SLN and CASLN containing 7-DHC were stored different temperatures (20℃, 45℃). The time course of the 7-DHC degradation was monitored for 30 to 80 days. Chemical stability of 7-DHC in different conditions are shown Figure 7, 8 and 9. Comparing NE and SLN, 7-DHC

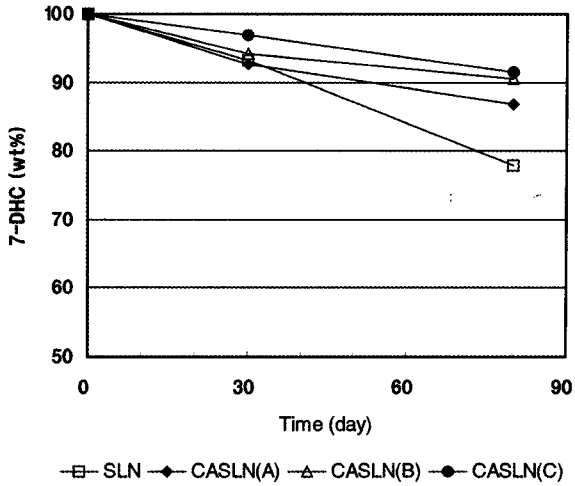

Figure 9. Stability of 7-DHC in CASLN at 45℃ for 80 days. SLN: solid lipid nano-particle, CASLN(A): 0.1 wt% chitosan coated solid lipid nano-particle, CASLN(B): 0.2 wt% chitosan coated solid lipid nano-particle, CASLN(C): 0.3 wt% chitosan coated solid lipid nano-particle.

was more stable in SLN. And the remaining wt% of 7-DHC at 45℃ for 80 days was about 80% in the CASLN, respectively. Therefore, 7-DHC was more effectively stabilized in CASLN (chitosan coated SLN) than normal SLN.

4. Conclusions

In our study, the model ingredient 7-DHC had been successfully incorporated in SLN and CASLN. CASLN had positive zeta-potential and increased mean particle size compared to SLN as a result of interactions negatively charged SLN with cationic chitosan polymer. Both DSC and XRD results suggested that the majority of SLN was less ordered crystal packing, and this was favorable for increasing the drug loading capacity. And the percentages of remaining 7-DHC in CASLN was about 80% at 45℃ after 80 days. It appeared that CASLN was the most effective to stabilize 7-DHC and may be used for a novel topical delivery system. Finally, CASLN may be used for a potential encapsulation method of unstable active ingredients.

References

1. M. Magill, Microencapsulation of the personal care

industry, *Cosmet. Toiletr.*, **105**(6), 59 (1990).
2. V. Jenning, M. Schafer-Korting, and S. Gohla, Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properies, *J. Control. Release*, **66**, 115 (2000).
3. S.-J. Lim and C.-K. Kim, Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid, *Int. J. Pharm.*, **243**, 135 (2002).
4. V. Jenning and S. Gohla, Comparison of wax and glyceride solid lipid nano particles, *Int J. Pharm.*, **196**, 219 (2000).
5. V. Jenning and S. Gohla, Encapsulation of retinoids in solid lipid nanoparticles (SLN), *J. Microencapsulation*, **18**(2), 149 (2001).
6. S. A. Wissing, A. Lippacher, and R. H. Muller, Investigations on the occlusive properties of solid lipid nanoparticles (SLN), *J. Cosmet. Sci.*, **52**, 313 (2001).
7. T. Mammone, N. Muizzuddin, E. Goyarts, D. Gan, P. Giacomoni, K. Marenus, and D. Maes, Normal human epidermal keratinocytes treated with 7-dehydrocholesterol express increased levels of heat shock protein, *J. Cosmet. Sci.*, **55**, 149 (2004).
8. S. Magdassi, U. Bach, and K. Y. Mumcuoglu, Formation of positively charged microcapsules based on chitosan-lecithin interactions, *J. Microencapsulation*, **14**(2), 189 (1997).