J. Soc. Cosmet. Scientists Korea
Vol. 30, No. 3, November 2004, 419-426

나노세라마이드의 캡슐화와 아토피 피부의 치료

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Nano Capsulization of Ceramide and the Efficacy of Atopy Skin

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요 약: 나노 세라마이드의 캡슐화(Nano-ceramide capsulation)는 세라마이드 III와 토코페릴리놀레이트를 나노 크기의 모노 베지클에 캡슐화시켜 피부각질층에 작용하도록 만든 기술이다. 그 제조에는 고압 마이크로플루다이저를 사용하였으며 조성은 다음과 같다. 모노 베지클의 막 강화제로서 0.5~5.0 wt%의 수소첨가레시친과 0.1~2.0 wt%의 리소레시친을 사용하였으며, 용제로서 5.0~10.0 wt%의 propylene glycol과 5.0~10.0 wt%의 ethanol을 사용하였다. 피부 보습기능과 아토피 치료를 위하여 활성성분인 세라마이드 III와 토코페릴리놀레이트를 사용하였으며 유화제는 함유하지 않았다. 나노 세라마이드 캡슐기술의 최적조건은 다음과 같다. 마이크로플루다이저의 통과압력은 1,000 bar, 통과횟수는 3회, 통과 온도는 60~70℃가 적당하였다. 또한, nano capsule의 pH는 5.8±0.5이었다. 평균입자크기는 63.1±7.34 nm로 물과 같은 투명한 성상을 보였으며, 제타포텐셜값은 -55.1±0.84 mV이었다. 임상실험 결과로서, 피부보습효과(*in-vivo*, n=8, *p-value*<0.05)는 비교시료보다 21.15% 개선되었으며, 치료 전보다는 36.31% 개선되었다. 더구나, 아토피 피부 효과는 아토피 피부 환자 10명에게서 양성반응을 보였다.

Abstract: The nano-ceramide capsulation is a technique that capsulates ceramide III and tocopheryl linoleate at the mono-vesicle to act on the horny layer in skin. In this technique, 0.5~5.0 wt% of hydrogenated lecithin and 0.01~2.00 wt% of lysolecithin are used as the membrane-strengthen agents of the mono-vesicle and 5.0~10.0 wt% of propylene glycol and 5.0~10.0 wt% of ethyl alcohol are used as solvents. Active ingredients such ceramide III and tocopheryl linoleate are utilized to enhance the moisturizing efficacy and treat atopy skin. These materials do not contain synthetic emulsifiers. The optimal conditions of nano-ceramide capsulation are such that particles pass Microfludizdizer 3 times at 1,000 bar and 60~70℃ and pH of nano capsules is 5.8±0.5. The average size of particles is 63.1±7.34 nm showing lucid state like water by the laser light scattering. A zeta potential value is -55.1±0.84 mV. Through clinical tests, the moisturizing effect (*in-vivo*, n=8, *p-value*<0.05) showed 21.15% of improvement comparison to comparison-samples and 36.31% of improvement compared to the state before treatment. Moreover, the effectiveness of atopy skin showed positive reaction from 10 volunteers.

Keywords: *nano, capsule, ceramide, atopy, emulsion*

1. Introduction

The epidermis tissue of skin does function that fill space between mainly cell and cell. Main ingredients of phospholipid have consisted of ceramide, free fatty acid, cholesterol, triglyceride and cholesterol sulfate. Ceramide in these exists most about whole 26%[1]. Ceramide was seperated first in brain tissue by Thudichum [2] in 1874 and existence of ceramide was reported skin's epidermis by Gray and Yardley in 1975[3]. Ceramide of epidermis exists by composition mixture state. There are 8 kinds of ceramide present[4], and the structural formula was proved[5]. This ceramide is very important to react protection function about moisture of epidermis. Specially, this is becoming basis of skin barrier function forming lamellar structure between cell and cell. Therefore, skin barrier function is not effectiveness if trouble happens to structure of skin and

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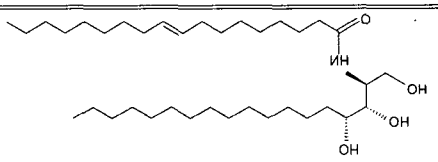
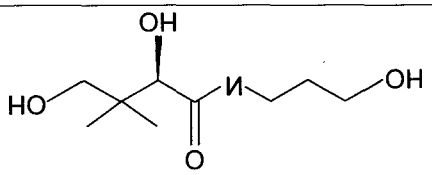
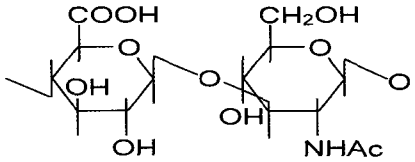
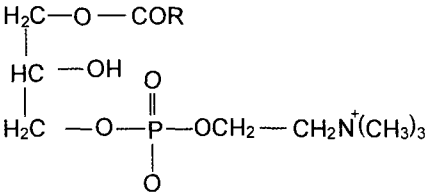
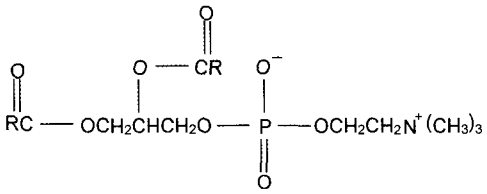
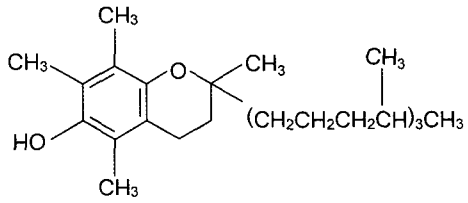
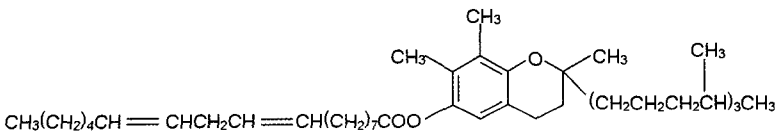
Table 1. The Chemical Structure of Ingredients Using Nano Capsule

Ingredients	Structure	Maker.
Ceramide ⅢB		Doosan Co. Biotech B.U., Korea
D-Panthenol		Roche Vitamins Ins., Switzerland
Hyaluronic acid		Bioland Ltd., Korea
Lysolecithin		Kyowa Hakko Kogyo Co. Ltd., Japan
Phosphatidyl choline		Lucas Meyer, Lipoid GmbH, Germany
Tocopherol		Cognis Co., Germany
Tocopheryl linoleate		Ennagram, France

basically skin's function is dropped. As a result, various skin trouble or skin disease is broken out or cutaneous symptoms can be deteriorated. On the other hand, tocopherol (vitamin E) was known that protect moisture loss of horny layer that is major cause of dry skin. Tocopheryl linoleate of them is consisted of esterificating bond tocopherol and linoleic acid. Role of this does function that prevents moisture loss, and strengthens essential free fatty acid and keeps skin's moisturization and softness. Tocopheryl linoleate was reported doing action such as hapten that cause immune reaction in somatology when used in skin's dermatitis adding to cosmetics in 1992[6]. And tocopheryl linoleate or acetate was proved that cause skin's dermatitis less when used adding to skin toner or cream[7]. If concentration of ceramide decreases by function of skin barrier,

skin is dried and itched[8]. That is, quantity of moisture loss because problem occurs in maintenance of moisturization through lipid membrane of skin and moisture content of horny layer is decreased. It is disease that these symptoms appear is atopic dermatitis[9].

Atopic dermatitis is reduced amount of skin nature of soil, and specially, was reported that amount of squalene and ceramide decreases[10,11]. Protecting horny layer by generally treatment method, and must use moisturizer that is effective in hydration of skin[12]. Therefore, this study studied about nano capsulation and effect to act more effectively to atopy skin. It established optimum condition that make mono vesicle using ceramide and tocopheryl linoleate by main ingredients. Manufacturing method of nano capsule used hydrogenated lecithin and unsaturated lecithin, lysolecithin, and added hyaluronic acid and D-panthenol by soluble in water humectant. Also, established optimum condition of nano capsule's composition by lecithin, and experimented about pH effect as well as measured moisturizing effect by clinical test, and reports an effective result about skin improvement effect of atopy to actuality atopy patient.

2. Experimental

2.1. Materials

Ceramide (Ceramide III B) was purchased from Doosan Co. Biotech B.U. (Korea). Other reagents were also purchased from commercial sources as follows: glycerin and ethanol (EtOH) from Duksan Pure Chemical, Co., Ltd. (Korea), propylene glycol (PG) from Shinyo Pure Chem. Co. (Japan), unsaturated lecithin (Emulmetik 930) from Lucas Meyer (Germany), saturated lecithin (Lipoid S 75-3) from Lipoid GmbH (Germany), lysolecithin from Kyowa Hakko Kogyo Co., Ltd. (Japan), tocopheryl linoleate (Vitenna L) from Ennagram (France), hyaluronic acid from Bioland Ltd. (Korea), tocopherol from Cognis Co. (Germany) and D-panthenol from Roche Vitamins Ins. (Switzerland). The making company and the structural formula were showed at Table 1. All the reagents were analytical grade and the water distilled twice was used throughout the study.

2.2. Instruments

Malvern system (Malvern PCS 4700, Malvern Instruments, U.K.) which is the light scattering device was used to measure the particle size of nano capsule. The wavelength of Malvern system was the 633 nm He-Ne laser (Siemens, LGK 7626). Results are the average value of ten measurements at a scattering angle of 90. The light scattering data were analyzed with the Malvern 4700 software. Zeta potential (Malvern, Zetasizer 2000, Malvern Instrument, U.K.) was used to look into the stability of the particle. Results are the average value of five measurements at the stationary level. The experiments were located in a constant temperature and humidity room for 20 min prior to moisture evaluation to minimize effect of atmospheric conditions on the measurement. Corneometer (CM820PC, Courage Khazka, Germany) was used to measure the moisture. Also pH meter (ORION model 420A) was used to measure the pH change. Spectrophotometer (HACH DR/4000U, 860 nm) was used to measure the turbidity, and the standard value was assigned using the twice distilled water. POLAX-2L of ATAGO (Japan) in analog mode was used to measure the refractive index.

2.3. Methods

2.3.1. Nano Capsulation Formula

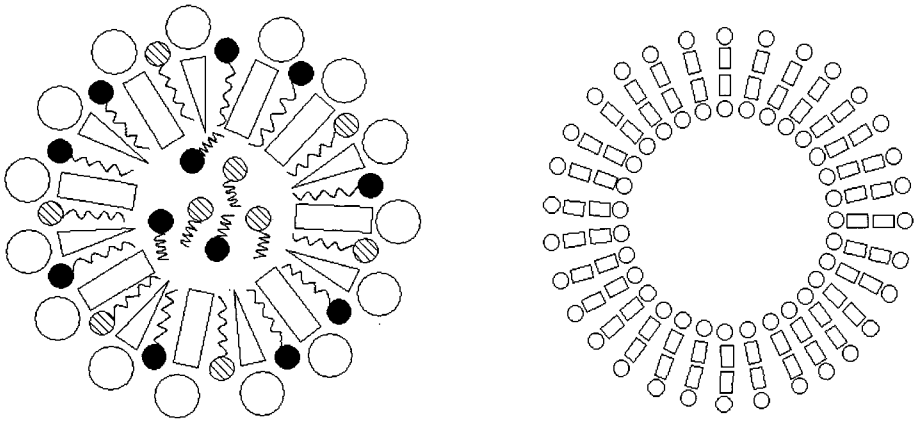
The ceramide (0.05 wt%) and the tocopheryl linoleate (0.05 wt%) was dissolved in a solvent of 10.0 wt% PG and 10.0 wt% EtOH and then the lecithin and the lysolecithin were enough dissolved. The next here was mixed glycerin, tocopherol and the ready-mixed substance. The ready-mixed substance was dissolved D-pathenol and hyaluronic acid in the water distilled twice. This mixed solution was left to obtain a homogeneous solution for 20 min. To prepare the nano capsule Microfluidizer (M-110Y, Microfluidcs Co., U.S.A) was used at a condition of 3 passes and 1000 bar at constant temperature of 60~70℃.

2.3.2. Moisture Measurement

The experiments were used 10 healthy 20's women in order to measure the skin moisture. The experiments were located in a constant temperature and humidity room for 20 min before moisture measurement to minimize the effect of the temperature and humidity. The measurements were made in the 1 cm^2 circle on center of both arms area. The moisture values were measured before and after applying the placebo and sample. The

Table 2. The Compositions of Placebo and Nano Capsule

No.	Placebo		No.	Sample	
	ingredients	content (wt%)		ingredients	content (wt%)
3	Saturated lecithin	0.50	6	Saturated lecithin	0.50
	Unsaturated lecithin	0.50		Unsaturated lecithin	0.50
	Lysolecithin	2.00		Lysolecithin	2.00
	PG	10.00		PG	10.00
	EtOH	10.00		EtOH	10.00
	Pure water	77.00		Glycerin	10.00
				Ceramide	0.05
				Tocopheryl linoleate	0.05
				Tocopherol	0.10
				Hyaluronic acid	5.00
				D-panthenol	3.00
				Pure water	58.80



(a) (b)

Figure 1. The schematic diagrams of nano capsule (a) and liposome (b).

applying concentration was 10 μL, and the moisture values obtained at 20 min. 1, 2 and 4 h after the application, the soft tissue polished the probe surface when measured the moisture. Each experimental results were used the average value. The formula of placebo and sample were showed in Table 2.

2.3.3. Measurement the Effect on Atopy Skin

The improvable effects on atopy skin were measured on the 10 volunteers. The sample was applied on the part of affected skin for the experiment twice a day in morning and evening after washing. The measurements for the affected part of atopy skin were made before applying the sample, and then after applying the sample were made using the seven-point scales with the naked eyes of an expert on the 28th days.

3. Results and Discussion

3.1. Nano Capsule Mechanism

The lecithin is composed of two hydrocarbon chains and a natural polar lipoid. The volume of hydrophobic is much bigger than that of the hydrophilic, hence, in general the lecithin forms vesicle that is made of double layer structure[13]. Among those vesicles the vesicles with phospholipid is defined as liposome[14,15]. Due to the special structures of the vesicle[16,17] and liposome[18] they have been widely applied in cosmetic and medicine industries. However, it has been reported that the stabilities of liposome are not good such as in

Table 3. The Composition and Physical Properties of the Sample 1, 2, and 3

Chemical Name	sample 1	sample 2	sample 3
Saturated lecithin	1.00	-	0.50
Unsaturated lecithin	-	1.00	0.50
Lysolecithin	1.00	1.00	2.00
PG	10.00	10.00	10.00
EtOH	10.00	10.00	10.00
Glycerin	10.00	10.00	10.00
Ceramide	0.05	0.05	0.05
Tocopheryl Linoleate	0.05	0.05	0.05
Tocopherol	0.10	0.10	0.10
D-Panthenol	3.00	3.00	3.00
Hyaluronic Acid	5.00	5.00	5.00
Pure water	59.80	59.80	58.80
Particle size (nm)	70.8	67.6	63.1
Zeta potential (mV)	-51.7	-50.4	-55.1
Turbidity (FAU)	43	35	28

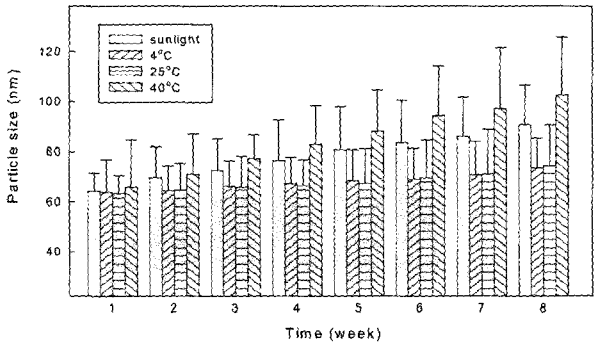


Figure 2. The particle size of the sample 3 (bar showed S.D, n=10).

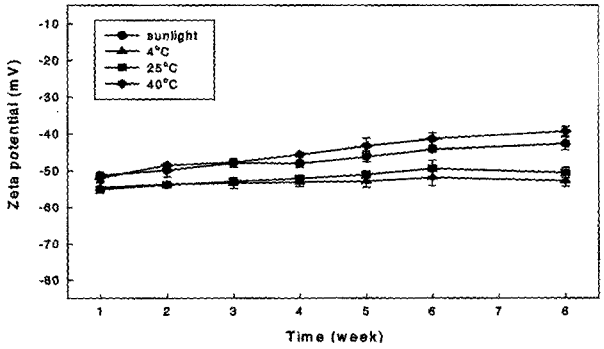


Figure 3. The zeta potential of nano capsule (sample 3) (Bar showed S.D, n=5).

common emulsions[19]. In recent to increase the stabilities of liposome some experiments on nano capsule with the nano sized technology are processing[20]. Figure 1 showed the structural form of the nano capsule. To enhance a mono-vesicle stability that was composed of the lecithin, the lysolecithin was added to act as a co-emulsifier finally the nano capsule was obtained. The lysolecithin located in the space of layer of lecithin enhances the stability of layer that was made of lecithin only hence the stable nano capsule could be obtained. Consequently, the nano capsule could penetrate into the stratum corneum of skin stably, so the ceramide an effective ingredient in the nano capsule enhanced the moisture of skin.

3.2. The Optimal Condition of Nano Capsule

The optimum condition of nano capsule was obtained through the changes of composition of the saturated lecithin, unsaturated lecithin and lysolecithin. Table 3 showed the particle size, zeta potential and turbidity for sample 1, 2 and 3, respectively. With the data in Table 3 the optimal condition of nano capsule was assigned as sample 3. To measure the stability of a nano capsule for the sample 3 the values of particle size and zeta potential were evaluated using Malvern system and Zetasizer 2000.

Figures 2 and 3 showed the variations size and stability of sample 3 at 4℃, 25℃, 40℃ and in sunlight for 8 weeks. In the case of 4℃ and 25℃, the particle size varied from 63.8 nm, 63.1 nm to 73.6 nm, 74.4 nm, respectively for 8 weeks so there was little changes in the particle size. However, in the case of sunlight and 40℃, after 8 weeks the particle size increased sharply to 90.4 nm and 102.6 nm, respectively. With respect to

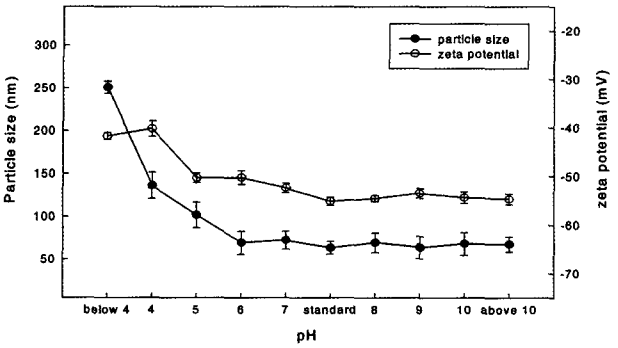


Figure 4. The particle size and zeta potential of the sample 3 according to the change of pH (particle size n=10, zeta potential, n=5).

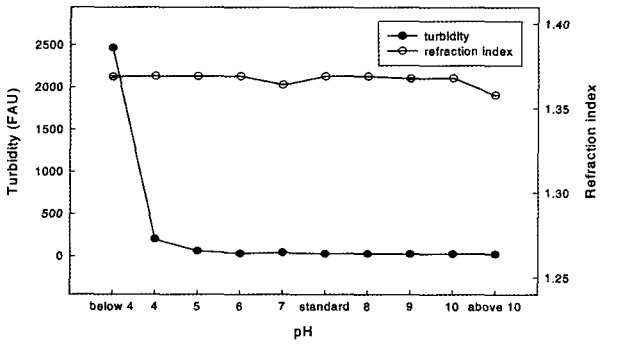


Figure 5. The turbidity and refractive index of the sample 3 according to the change of pH (turbidity n=1, refractive index, n=3).

the zeta potential very little changes showed at 4℃ and 25℃, respectively, and its values were a little low -42.6 mV and -39.3 mV for sunlight and 40℃, respectively for 8 weeks. Therefore, with these results it may be concluded that the optimal keeping condition is 4℃ and 25℃.

3.3. The Changes of the pH

The pH of nano capsule prepared was adjusted using the 0.001 N aqueous HCl and NaOH. Figure 4 showed the variation of the particle size and zeta potential as a function of pH. As can be seen, the particle size increased in the strong acid at and below pH 4, but there were little changes in any other pH. The value of zeta potential showed little change with varying the pH, hence the pH had no affect on the stability of nano capsule. In Figure 5 the turbidity and refractive index of nano capsule are revealed according to the change of pH. The turbidity, due to the larger size,

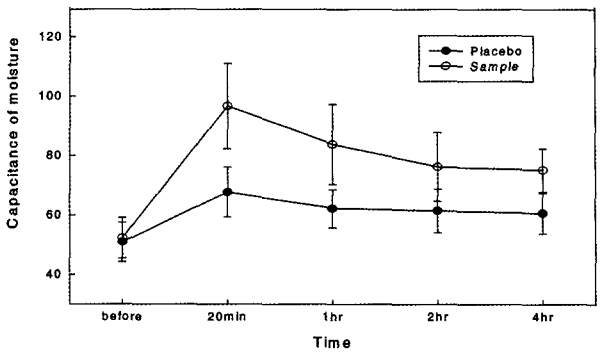


Figure 6. The variation of sample 3 according to the change time (* p-value>0.05, n=8).

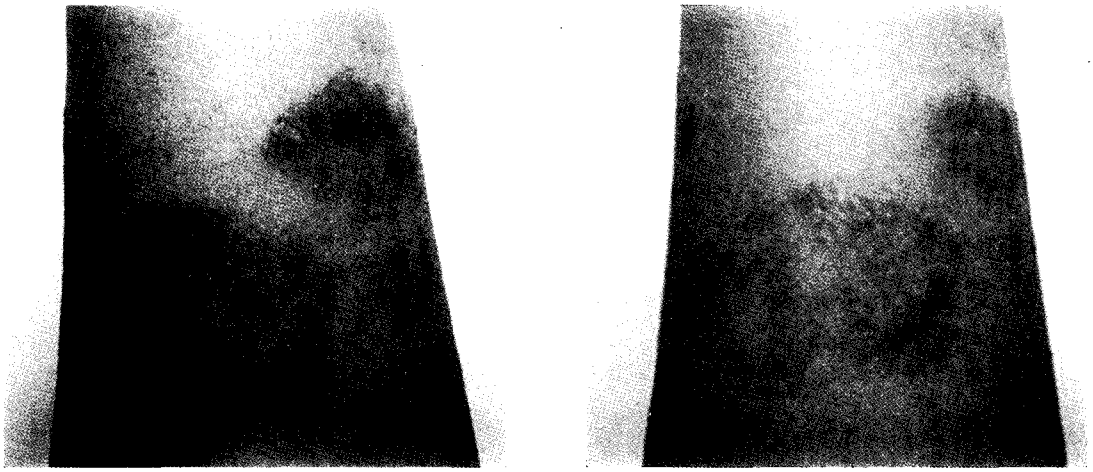
revealed a large in the strong acid value at and below pH 4 but showed almost clear in any other pH. The refractive index showed almost same value in the hall range of pH.

3.4. Moisture Effect

The moisturizing effects after were applied the placebo and sample on the both arm of 10 volunteers. Figure 6 showed the average values of the placebo and sample as a function of time. As shown in Figure 6, the moisture value before applying the placebo and the sample were 50.84±1.70, 52.24±1.95, respectively, but its value after applying increased, and the moisture increase were 34.24% for the placebo and 86.59% for the sample after 20 min, 15.16% for the placebo and 36.31% for the sample after 4 h, respectively. With these results, the moisture improvement of the placebo revealed the higher value of 21.15% than that of the sample. Therefore it can be explained that, by enclosing the effective ingredients in nano capsule, the penetration of the effective ingredient into the skin can be enhanced and it can be remained in the skin for a longer time.

3.5. Improvement Effect on an Atopy Skin

To investigate the moisture on the patients with atopic dermatitis were used *in-vivo* test. The moisture values on the affect part of patients with the atopic dermatitis were evaluated using the seven-point scales and then the affect parts were took the photographs. By using the seven-point scales the improvement effect of the sample revealed from 2.7 to 5.2 after 28th days compared to the applying before. And its effect could be confirmed with the photographs. Figure 7



Before After (28th days)

Figure 7. The photograph of atopy skin.

showed the picture of the affected part before and after applying the sample.

4. Conclusions

In the study of enclosing the effective ingredients such as ceramide and tocopheryl linoleate using the nano capsulation, the following conclusions were obtained;

1. The optimal concentrations of the nano capsule were 0.50 wt% for saturated lecithin, 0.50 wt% for unsaturated lecithin and 2.00 wt% for lysolecithin, respectively.

2. At below pH 4 the particle size increased and the turbidity revealed a cloudy, and at any other pH 4 the particle size and the turbidity did not show any changes. The variation of pH did not affect the refractive index and the stability.

3. The moisturizing effect of the sample showed a value of 21.15% compared to the placebo. In other words, the effective ingredients such as ceramide and tocopheryl linoleate showed the moisture improvement.

4. By using the seven-point scales, the capacitance of moisture increased from 2.7 to hence the improvement effect on the atopy skin through the nano capsule could be confirmed.

Acknowledgment

This work was supported by the Soongsil University Research Fund.

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