

## 불포화레시틴을 이용한 나노리포솜의 개발에 관한 연구

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한불화장품 기술연구소

### Development of Nano-liposome with Unsaturated Lecithin

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**요약:** 지금까지 화장품 분야에서의 리포솜 제조를 위해 안정성 측면에서 주로 사용하였던 포화레시틴 대신 피부 친화력과 침투력이 우수한 불포화레시틴을 사용하여 일반적으로 알려진 피부 각질층 간극보다 작은 약 50 nm 이하의 크기를 갖는 나노리포솜을 제조하는 기술을 개발하고자 하였다. 나노리포솜의 안정성 확보를 위해 용매의 종류, pH 발란스, 균질화 압력 및 보조계면활성제와 안정화제의 종류 등을 변수로 하였고, 결과적으로 친유성 부분이 stearate 계열로 구성되는 보조계면활성제의 사용을 통해 안정성이 향상됨을 확인하였다. 하지만, 포화레시틴을 사용하여 제조한 리포솜의 경우에는 보조계면활성제의 사용이 오히려 안정성을 감소시키고, 적절한 양의 오일을 사용할 때 안정성이 일부 향상되는 것으로 나타났다. 이러한 결과들을 통해 나노 단위의 리포솜을 제조하기 위해서는 포화레시틴에 비해 불포화레시틴을 사용하는 것이 더욱 적절한 방법임을 확인할 수 있었다.

**Abstract:** In cosmetics, the saturated lecithin, one of the main surfactants to prepare liposome has been used for its stability but it has been substituting with unsaturated lecithin which has excellent skin affinity and penetration property. So we studied to prepare nano-liposome that size of particles were below than 50 nm by unsaturated lecithin. It was important that many factors including solvent such as propylene glycol, pH balance, homogenizing pressure, various cosurfactants and stabilizers to make stable nano-liposome. In our experimental conditions, cosurfactants with stearate class as lipophilic part were more suitable than others for our purpose. But in liposome by saturated lecithin, cosurfactants had negative effect and appropriate amount of oil should be used to be stable. These results indicated that unsaturated lecithin were more suitable than saturated lecithin to prepare nano-liposome.

**Keywords:** unsaturated lecithin, phosphatidylcholine, nano-liposome, stabilizing agent, long term stability

### 1. Introduction

Nowadays many products using nano-technology have been released and nano-technology would be more prospected in all kinds of industries especially cosmetic manufacturing and ingredient fields. Recently cosmetical nano-technology is called to nanosome technology such as liposome, niosome, cubosome, solid lipid nanoparticle (SLN), polymeric micelle, nano-emulsion and others for delivery or stabilizing system[1-6]. Among them, the liposomes are simple vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules such as phospholipids.

They are excellent formulations as a cosmetic active carriers, owing to their biocompatibility, skin penetration property and ability to entrap hydrophilic and lipophilic drugs[7]. In general, lecithin as a main surfactant to prepare liposome have been used animal (egg yolk) or vegetable (soy bean) origin. While the animal phospholipids basically contain saturated fatty acids, phospholipids from soy beans primarily contain unsaturated fatty acids (e.g. linoleic acid). This unsaturated lecithin makes them relatively fluid and deformable and this property is important for the penetration of the epidermal barrier. So we investigated to make nano-liposome using unsaturated lecithins which have high compatibility and penetration property smaller than intercellular space (below 50 nm).

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## 2. Materials and Methods

### 2.1. Materials

We used phosphatidylcholine as the main surfactant system and applied various cosurfactants such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, PEG 20 Glycerylstearate, PEG 45 Stearate and triethanolamine-4 phosphate/lecithin/vitis vinifera, etc. Also we employed various stabilizing agents and oils such as ceramide 3, cholesterol, triethanolamine, macadamia nut oil, caprylic capric triglyceride, mineral oil and cyclomethicone. Propylene glycol and ethyl alcohol were used as solvents to dissolve lecithin and methylparaben was employed as the preservatives.

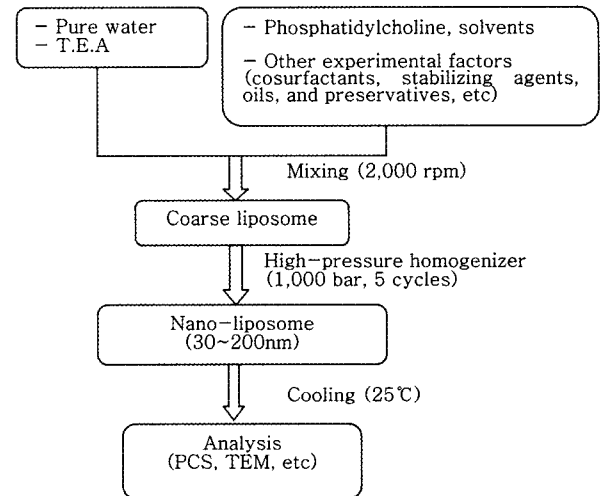
### 2.2. Methods

We prepared nano-liposome by general high-pressure homogenization methods[8]. Coarse lipid suspension was produced with homo mixer and then nano particles were prepared with high-pressure homogenizer at 1,000 bar and 5 cycles. After samples were cooled to 30°C, they were measured particle size distributions with circulation type laser particle size analyzer and photon correlation spectroscopy (PCS) for larger size (over 100 nm) and smaller size (below 100 nm), respectively[9]. Transmission electron microscopy (TEM) was employed to observe nano-shape of liposomes and long term stability was measured by visual observation and size analysis after 3 weeks and 6 weeks at 25°C, 42°C, and 50°C [10,11]. Preparation process was shown the Scheme 1.

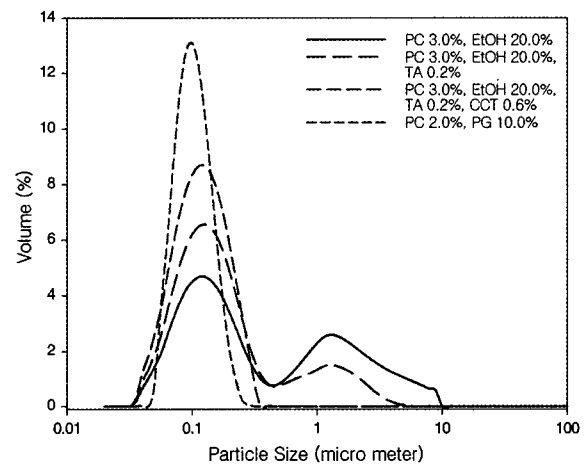
## 3. Results and Discussion

### 3.1. Experiments on Miscibility and Stability of Unsaturated Lecithin

To confirm miscibility with solvents, we used ethyl alcohol and propylene glycol and performed comparison experiments on many kinds of stabilizing agents such as triethanolamine, tocopheryl acetate and caprylic capric triglyceride, etc. As a results, we confirmed that liposomal stability could be increased a little by using of oil soluble components such as tocopheryl acetate and caprylic capric triglyceride. In addition propylene glycol was more effective solvent than ethyl alcohol because propylene glycol might increase the solubility of phosphatidylcholine (Figure 1). However, when only phos-



**Scheme 1.** A Schematic diagram of the preparation procedure.



**Figure 1.** The size distributions of unsaturated lecithin stabilizing experiment (PC: phosphatidylcholine, TA: tocopheryl acetate, CCT: caprylic capric triglyceride, PG: propylene glycol).

phatidylcholine was used without any modification, there were coalescence among the particles and formed larger particles around 1  $\mu\text{m}$  after preparation. Therefore, we attempted to stabilize liposomal vesicles by pH control to neutralize free fatty acids using triethanolamine. As a results, the coalescence was not happened with 2.0% phosphatidylcholine after 1 day, but was happened with 4.0% phosphatidylcholine. It seems that pH neutralization was not significant condition to stabilize unsaturated lecithins (Figure 2). So we used tricetareth-4 phosphate/lecithin/vitis vinifera as mixture

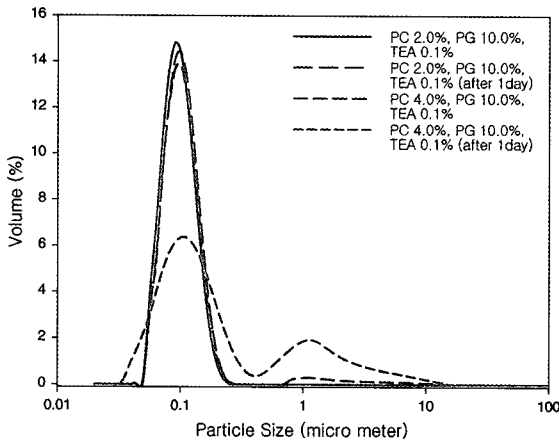


Figure 2. The size distributions of unsaturated lecithin stabilizing experiment (TEA: triethanolamine).

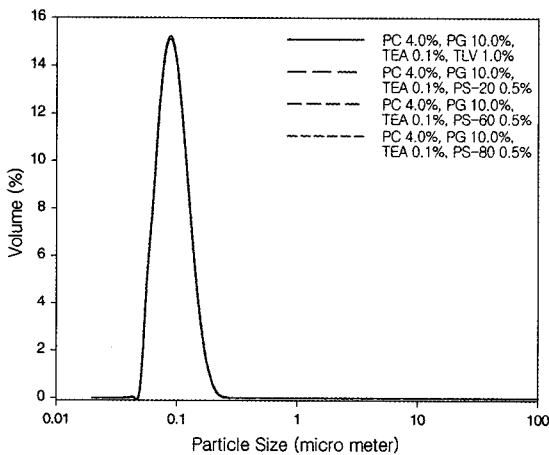


Figure 3. The size distributions of unsaturated lecithin stabilizing experiment (TLV: tricetareth-4 phosphate/lecithin/vitis vinifera, PS-20: polysorbate 20, PS-60: polysorbate 60, PS-80: polysorbate 80).

stabilizing agents and obtained satisfactory results. It seems that surfactant component in mixture composition was significant cause. Therefore, we used cosurfactants such as polysorbate 20, polysorbate 40 and polysorbate 80 and then confirmed so excellent stabilizing properties (Figure 3).

### 3.2. Experiments on Stability of Nano-liposome

To prepare the stable nano-liposome which particle size is below 50 nm, we performed comparing experiments on many factors such as cosurfactants, stabilizing agents, oils and saturated lecithin[12,13]. Experimental formulations were shown the Table 1.

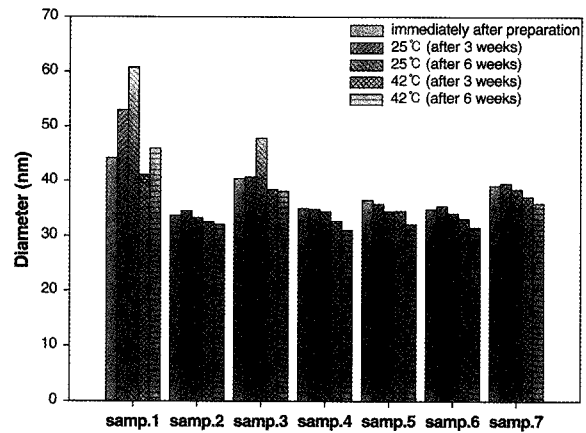


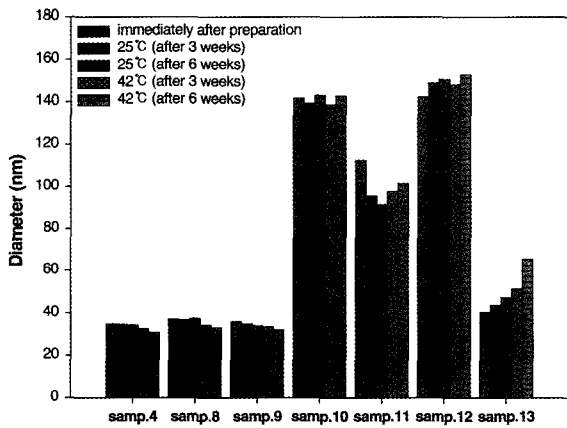
Figure 4. The size distributions of liposome stabilizing experiments on cosurfactants.

Liposomal stability was tested on various cosurfactants such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80 and PEG 20 glycerylstearate as same hydrophilic part and PEG 45 stearate as larger hydrophilic part. As a results, generally each cosurfactants were shown similar appearance except sample 1 and 3. Especially the case of sample 4 appeared relatively favorable stability and transparency after 6 weeks at 42°C. Even though not enough of a difference between each cosurfactants, we found that stearate class composed on the number of carbon 18 were more stable and molecular size of hydrophilic part was suitable to approximately 20 mol (Figure 4). As the stabilizing agents, we used ceramide 3 and cholesterol, but there was no difference from sample 4. It seems that these agents were not effective on this formulations but maybe could be effective on more complex formulations like industrial ones (Figure 5). Many kinds of oils such as plant origin (macadamia nut oil), polar type (caprylic capric triglyceride), non-polar type (mineral oil), silicone type (cyclomethicone) were tested to confirm interaction of oils with unsaturated lecithins. With the exception of cyclomethicone (sample 13), all of the samples were shown opaque appearance and large particle size. As a results, we supposed that application of oils were cause of conversion into nano-emulsions and polar oils formed relatively small particles because of more miscible compared with non-polar ones. The case of cyclomethicone maybe formed thin layer between lamellar membrane because of low molecular weight and low interfacial tension itself (Figure 5). Trans-

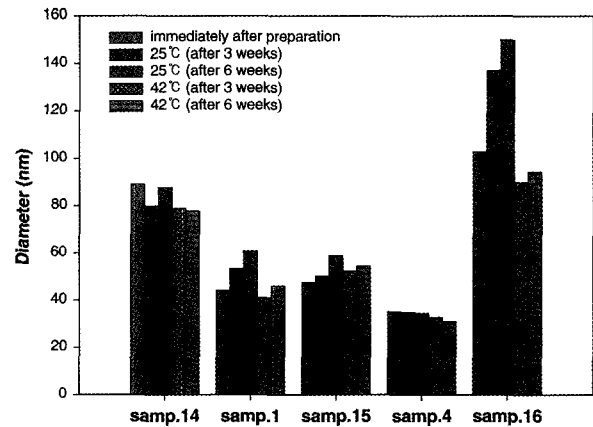
**Table 1.** The Formulations of Nano-liposome Stabilizing Experiments

	Sample (wt%)					
	No. 1 <sup>a</sup>	No. 2~7 <sup>b</sup>	No. 8~9 <sup>c</sup>	No. 10~13 <sup>d</sup>	No. 14 <sup>e</sup>	No. 15~16 <sup>f</sup>
Phosphatidylcholine	4.0	4.0	4.0	4.0	4.0	-
Hydrogenated lecithin	-	-	-	-	-	4.0
Propylene glycol	10.0	10.0	10.0	10.0	10.0	10.0
Triethanolamine	0.1	0.1	0.1	0.1	0.1	0.1
Methylparaben	0.2	0.2	0.2	0.2	0.2	0.2
Cosurfactant	-	0.5	-	-	0.5	(0.5)
Stabilizer	-	-	0.2	-	-	-
Oil	-	-	-	2.0	-	-
Pure water	q. s. to 100					

- <sup>a</sup> Control sample (without any experimental factors)
- <sup>b</sup> Experimental factors on various cosurfactants (sample 2: polysorbate 20, sample 3: polysorbate 40, sample 4: polysorbate 60, sample 5: polysorbate 80, sample 6: PEG 20 Glycerylstearate, sample 7: PEG 45 Stearate)
- <sup>c</sup> Experimental factors on various stabilizers (sample 8: ceramide 3, sample 9: cholesterol)
- <sup>d</sup> Experimental factors on various oils (sample 10: macadamia nut oil, sample 11: caprylic capric triglyceride, sample 12: mineral oil, sample 13: cyclomethicone)
- <sup>e</sup> Experimental factors on homogenization pressure (500 bar, 5 cycles, cosurfactant: polysorbate 60)
- <sup>f</sup> Experimental factors on hydrogenated lecithin (sample 15: without cosurfactant, sample 16: with cosurfactant as polysorbate 60)



**Figure 5.** The size distributions of liposome stabilizing experiments on stabilizers and oils.



**Figure 6.** The size distributions of liposome stabilizing experiments on saturated lecithin.

parency and particle size of sample 14 (homogenizing pressure was 500 bar) were worse than that of sample 4 (homogenizing pressure was 1,000 bar). Particle size of sample 14 was about twice bigger than sample 4. As a results, we confirmed that homogenizing pressure was one of the major factors for transparency and particle size control. In case of using saturated lecithin, particle size of sample 15 were smaller than sample 16 and transparency of sample 15 were better than sample 16. Both sample 15 and 16 were formed just a little precipitation, but sample 16 was more (Figure 6).

Sample 1, 11, 15 were tested for stability at 50°C after 4 weeks by measuring particle size. Particle size of liposomes with unsaturated lecithin were grown more bigger, but that of saturated lecithin did not change after 4 weeks at high temperature condition (50°C). It seems that fluidity of unsaturated lecithin was increased by high temperature because of double bond of unsaturated fatty acid chains. And the case of saturated lecithin had high transition temperature (higher than 50°C), so viscoelasticity of membrane was stronger than that of unsaturated lecithin. (Figure 7). Through

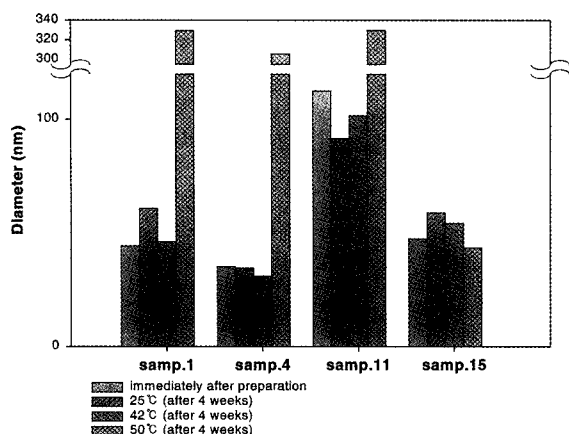


Figure 7. The size distributions of liposome stabilizing experiments at high temperature.

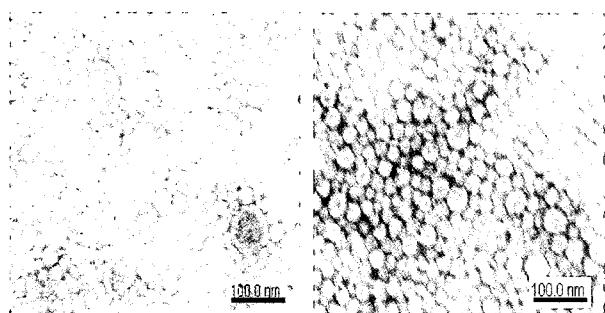


Figure 8. The TEM pictures of nano-liposome with unsaturated lecithin (left: sample 1, right: sample 4).

the TEM analysis, we confirmed that a number of multi-lamellar liposomes were formed in formulation without cosurfactants, but only mono-lamellar liposomes were formed in case of formulations using cosurfactants (Figure 8). The case of using saturated lecithin was observed some particles of shape like rice and we presumed that this appearance was caused by relatively low miscibility on solvents. Therefore, it maybe had more advantages to use unsaturated lecithin than saturated lecithin for liposome stability (Figure 9).

#### 4. Conclusions

In our study, we found that suitable solvents were necessary to prepare of nano-liposome using unsaturated lecithin. Aggregation was one of the major problems when we used ethyl alcohol as a solvent, but propylene glycol had good miscibility and stability on unsaturated lecithin. Also stability of liposome could be

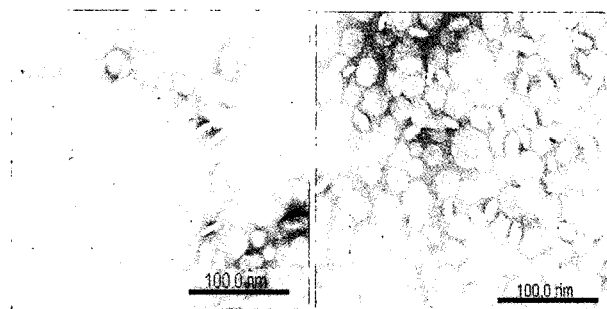


Figure 9. The TEM pictures of nano-liposome with saturated lecithin (left: sample 15, right: sample 16).

increased by addition of base material like triethanolamine for neutralization or addition of amphiphilic cosurfactants. It was suitable molecular size of hydrophilic part as approximately 20 mol of polyoxyethylene and stearate class as lipophilic part for stable liposomal preparation. To get smaller vesicular size and transparency, polar oil preferred to non-polar oil. Macadamia nut oil, it has large molecular weight, formed bigger vesicles than other oils. Because of the small molecular weight and low surface tension of silicone oils, cyclomethicone formed smallest vesicles. Liposomes with unsaturated lecithin were stabilized by cosurfactants, but in case of saturated lecithin was unstable. Because of the fluidity of liposomal lamellar membranes, cosurfactants had influence on liposomal stability. Lamellar membranes by unsaturated lecithin were more flexible than that of saturated lecithin, so cosurfactants decreased on excess flexibility and then stability was increased consequently. Through the TEM analysis, we confirmed that it had more advantages to use unsaturated lecithin than saturated lecithin for liposome stability. These results indicated that unsaturated lecithin is more effective than saturated lecithin to prepare nano-liposome.

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