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A Method of Manufacturing Liposome Vesicles in a Supercritical State and Stabilizing them by Encapsulating Pure Retinol inside Them

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1. Introduction

With the recent aging society, the cosmetics market having actual effects is expanding. [1] and [2]. In particular, products having an effect on whitening or wrinkle improvement are in the spotlight, and various products having a high moisturizing effect and a pore care effect as a treatment for severe dry skin are expanding [3]. Adenosine, kinetin, retinol, and retinol derivatives are known as representative raw materials that are effective in improving wrinkles in Korea [4]. This ingredient is known to be a drug that is vulnerable to temperature and light, making it difficult to secure the stability of the formulation of cosmetics due to severe color change and its titer falling [5]. Supercritical technology is partially used to extract active ingredients from fruits, leaves, and stems of plants [6]. In recent years, it is also usefully applied to a method of manufacturing decaffeinated coffee by removing caffeine in the process of roasting coffee at a specific critical point. In order to solve this difficulty, liposome encapsulation technology is being applied as a method of preventing the inflow of light or oxygen through the capsule method [7]. Methods of enhancing stability by mixing antioxidants and stabilizers with nanoemulsions developed by this liposome manufacturing method have been mainly reported [8] to [10]. Another method is to use a special method, such as packing inside a semi-solid liquid crystal droplet to ensure stability, but there is no fundamental solution [11]. In general, in order to make such a special formulation, it is inevitable that the titer of retinol has already fallen in the process of heating and stirring the constituent materials by heating and completing the final product [12]. In order to solve this problem, the nano liposome inclusion body is designed to be possible without heating. We report a manufacturing method of forming a liposome in pure retinol in a supercritical state, encapsulating it, and encapsulating it in a nano liposome by passing it through a high-pressure microfluidizer at cold temperatures, and the results of research on the skin transdermal absorption effect using this.

2. Materials and methods

2.1. Materials

Pure retinol used in this study was pure retinol (Sigma-Aldrich, Germany) obtained from nature. As mixed surfactants, phosphatidylcholine (Sigma-Aldrich, Germany), phosphatidylinositol (Sigma-Aldrich, Germany), sucrose dystearate (Biobeautech, Korea), and potassium phosphate (Daejeong, Korea) were used as they were. As a wall membrane enhancer, glutathione (Lightpharm Tech, Korea), carosine

(Eco I&T, Korea) was used as a water-soluble stabilizer for polyquaternium-51 (Hans Korea), and carosine (Eco I&T, Korea). As a water-soluble stabilizer, tocotrienol, tocopherol, and its derivatives (Sigma-Aldrich, Germany) were used.

2.2. Preparing method of SC-nano-liposome

The method for preparing nanoliposomes in the supercritical state is described in Table 1 together with the control group. A control group was made by a general preparation method, and general liposomes and supercritical liposomes were prepared by using a microfluidizer. The mixed surfactant and the cationic thickener were injected at a range of -10 to 30 ° C, and carbon dioxide was dissolved by injecting and stirring at 30 to 500 bars to produce liposome vesicles. A stabilizer and pure retinol were encapsulated in these multiple lamellar vesicles to form a huge liposome. This was passed through a high-pressure microfluidizer equipped with cooling water to prepare a nanoparticle liposome [13].

Table 1. Preparation Prescriptions for Supercritical Liposomes Enclosed with Pure Retinol: Comparison of the Stability of Pure Retinol Enclosed with Control, General Liposomes, and Supercritical Liposomes

Phase	Ingredient Name	Control Wt%	Gene-Liposome Wt%	SC-Liposome Wt%	Remarks
A	Glycerin	2.00	2.00	2.00	Moisturizer
	Dipropylene Glycol	3.00	3.00	3.00	Moisturizer
	1,2-Hexanediol	2.00	2.00	2.00	Preservative
	Ethylhexylglycerin	0.10	0.10	0.10	Preservative
	Polyquernium-51	5.00	5.00	5.00	Conditionor
	Water	73.00	73.00	73.00	Solvent
B	Glyceryl Stearate/PEG-100 Stearate	5.00	-	-	Emulsifier
	Hygrogenated Lecithin	-	5.00	-	Emulsifier
	Phosphatidyl Choline / Phosphatidyl Inositol / Sucrose Distearate /	-	-	5	Emulsifier
	Photasium Phosphate	0.10	0.10	0.10	Additive
	Capric/caprylic triglyceride	5.00	5.00	5.00	Emollient
	Pure retinol	1.00	1.00	1.00	Additive
C	Carnosine	0.50	0.50	0.50	Additive
	Glutathione	0.50	0.50	0.50	Additive
	Water	5.00	5.00	5.00	Solvent
D	Tocopherol	1.00	1.00	1.00	Additives
	Tocotrienols	1.00	1.00	1.00	
Total		**100.00**	**100.00**	**100.00**	**100.00**

2.3. Percutaneous absorption *in-vitro* evaluation method

The transdermal absorption experiment was conducted under sink conditions using a Franz diffusion cell (effective area: 0.64 cm2, aqueous phase volume: 5.2 mL). Using a Franz Diffusion Cell, a 1% polysolve-20 (tween-20, Uniqema Co. LTD) solution was used for the aqueous solution. A membrane is mounted on the upper layer, a portion filling the sample in the hole and the lower end are a tube that fills the hole with water, a stirring bar below which the temperature is controlled, and the separated artificial skin can be placed under the portion filling the sample and transdermal absorption can be measured [14]. This method is the method most commonly used as a delivery system in the pharmaceutical field as it is. As for the membrane, a commercialized artificial skin was purchased and used without any special treatment. A skin permeation experiment was carried out by applying 1 g of each sample to the skin layer. The artificial skin was mounted on a diffusion device, and during the experiment, the aqueous phase was stirred at 600 rpm using a magnetic stirrer. Each sample was collected at regular time intervals for 24 hours, and the permeability of the transdermal cortex was measured by HPLC [15].

3. Results

3.1. Characteristics of nano-liposome at supercritical state

It is common for general liposomes to be dissolved in a highly toxic chloroform solvent using phospholipids or dissolved in polyol at high temperatures (90~105 ° C) to make liposomes through a swelling reaction. However, since the manufacturing method for forming liposomes in a supercritical state has a characteristic that it does not have to be heated, it was judged as an optimal method for encapsulating a thermodynamically unstable raw material and completed through this study. The characteristics of making nanoliposomes in a supercritical state are as follows. First, it can stabilize unstable components such as amino acids, polyphenolic antioxidants, ascorbic acid, and peptides. Second, it is safe for the skin because it does not use highly toxic organic solvents. Third, it is fresh because it can be sealed without a heating process. Fourth, energy can be saved. However, as a disadvantage, decompression facilities are expensive. Fig. 1 is a schematic diagram that mechanically explains the path of pure retinol to the dermal layer through transdermal absorption of supercritical nanoliposomes. It is predicted that it penetrates into the skin through these pathways and stimulates cytokines through various enzyme reactions in the skin to exert various effects.

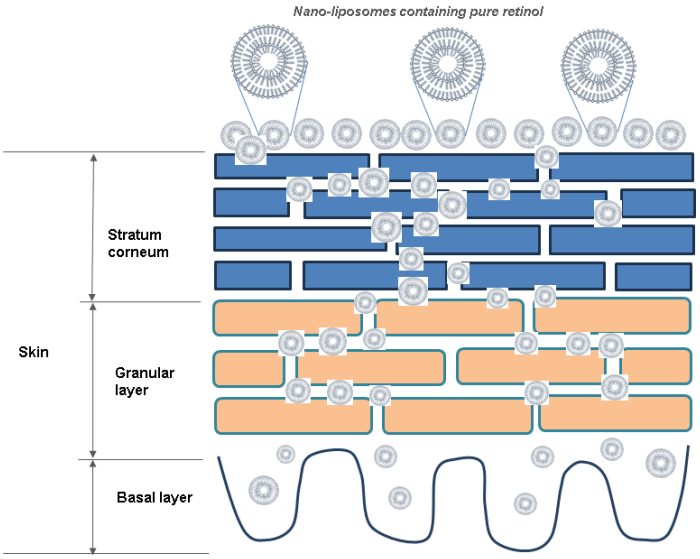

Fig. 1. A schematic diagram of Nano-liposome explaining the skin absorption pathway.

If pure retinol is kept stable and absorbed into the skin, it is expected that it will exert various activities and effects. It is important to keep the titer of retinol as stable as possible in the process of encapsulating the liposome.

3.2 Encapsulation pure retinol in nano-liposome vesicles at supercritical state

The photograph obtained by sealing and stabilizing retinol in the supercritical state is shown in Fig. 2. In Fig. 2, (a) is prepared by the general emulsification method, (b) is a general liposome, and (c) is a photograph of encapsulation of retinol in the supercritical state. The appearance of the nanoliposome was a yellow gel, and the particle diameter distribution was 170 nm to 280 nm. The pH was 7.6, and the zeta potential was -38.2 mV, showing excellent dispersibility. The stability test of the general emulsification method and the general liposome and the supercritical liposome was evaluated, and the efficiency according to the encapsulation method during the manufacturing process was compared and evaluated.

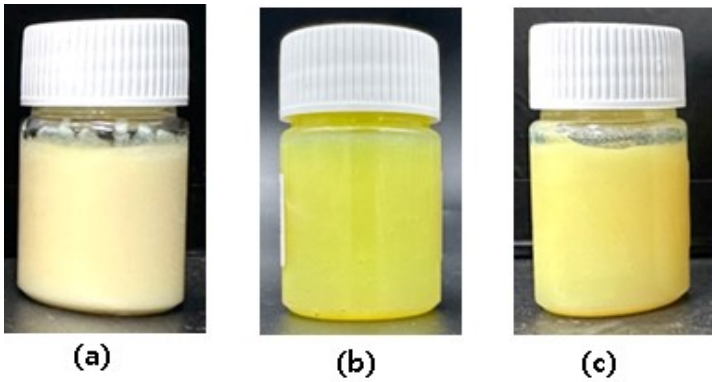

Fig. 2. The pictures of supercritical Nano-liposome containing pure retinol.

3.3 Structural analysis of nano-liposome

Fig. 3 shows the results of electron microscope analysis of the sample sealed and stabilized retinol in the supercritical state. As shown in Fig. 3, as a result of observing the liposome enclosed in the supercritical state with Cryo-TEM, it was confirmed that a multilayer lamellar structure was formed. It was considered that pure retinol and a stabilizer could be maintained stably because pure retinol and a stabilizer were mounted inside the vesicle of the nanoliposome.

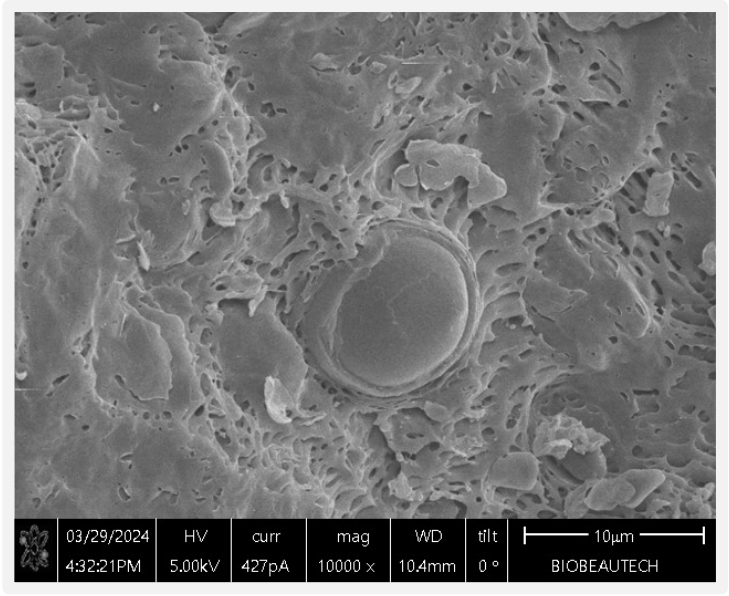

Fig. 3. Cryo-TEM electron micrograph of nano-liposomes containing pure retinol; confirming that multiple lamellar layers were formed using supercrtical state.

In addition, the results of measuring the particle size distribution of the sample of pure retinol enclosed by the supercritical method are shown in Fig. 4. As shown in the graph, it was found to be in the range of 170 nm to 280 nm. From this result, it was judged that it was a condition for good transdermal absorption, and the transdermal absorption power was evaluated.

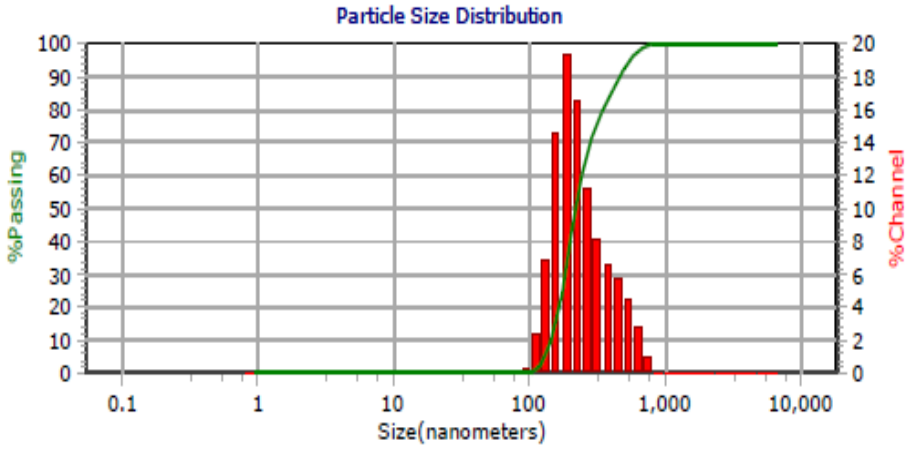

Fig.4. Cryo-TEM electron micrograph of nano-liposomes containing pure retinol; confirming that multiple lamellar layers were formed using supercrtical state.

3.4 the stability of pure retinol

After 8 weeks, the stability of retinol was reduced by 78.99% in the case of control. On the other hand, as a result of quantitative analysis of the stability of pure retinol encapsulated in a supercritical state by HPLC, the initial concentration was 3,096 IU/g, maintained at 98.7% at 3,056 IU/g after 4 weeks, and after 8 weeks, 97.1% was quantitatively analyzed at 3,006 IU/g, showing excellent stability. On the other hand, in general liposomes, 66.8% decreased after 8 weeks. This phenomenon was judged to be the effect of sealing retinol by applying heat during the process of preparing liposomes.

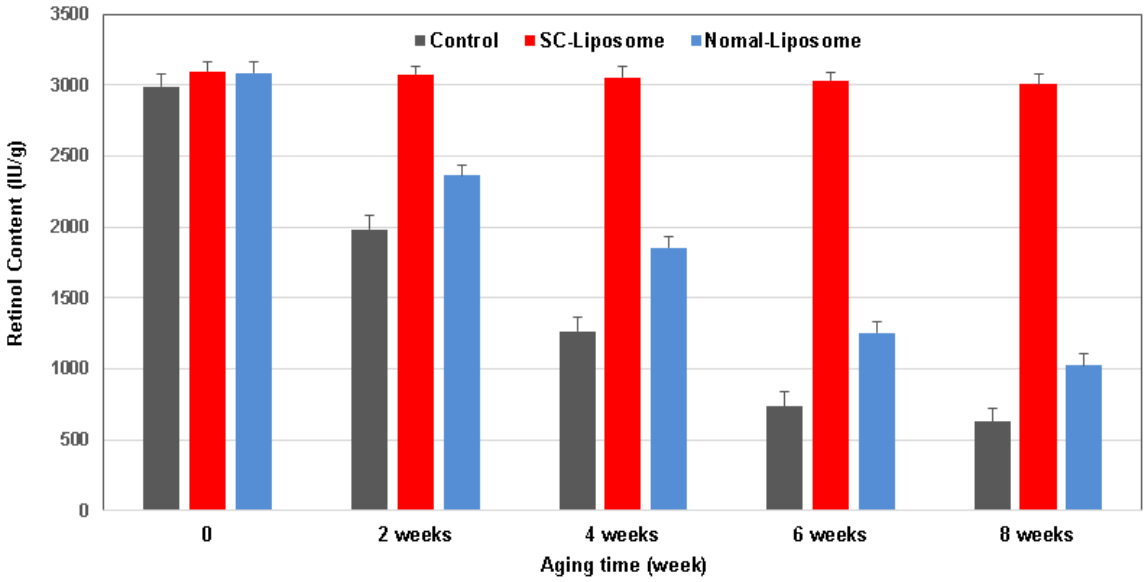

Fig. 5. Stability of supercritical nano-liposomes containing pure retinol measuring by HPLC determination for longterm storage.

If a mass production system is established by introducing a method of stabilizing retinol in a supercritical state without a heating process, it is expected that retinol cosmetics that are safe to store for a long time can be widely used.

3.5 Percutaneous absorption measurement

Fig. 6 shows the results of testing and evaluating the amount of transdermal absorption by in-vitro measurement using artificial skin and Franz Cell. As shown in Fig. 6, it was confirmed that 25 IU/g penetrated even after 24 hours for control, and 226 IU/g was transdermal absorption for general liposomes. In the case of the sample encapsulated with liposomes in the supercritical state, it was confirmed that about 52 IU/g penetrated after 2 hours, 186 IU/g after 6 hours, and 286 IU/g after 24 hours.

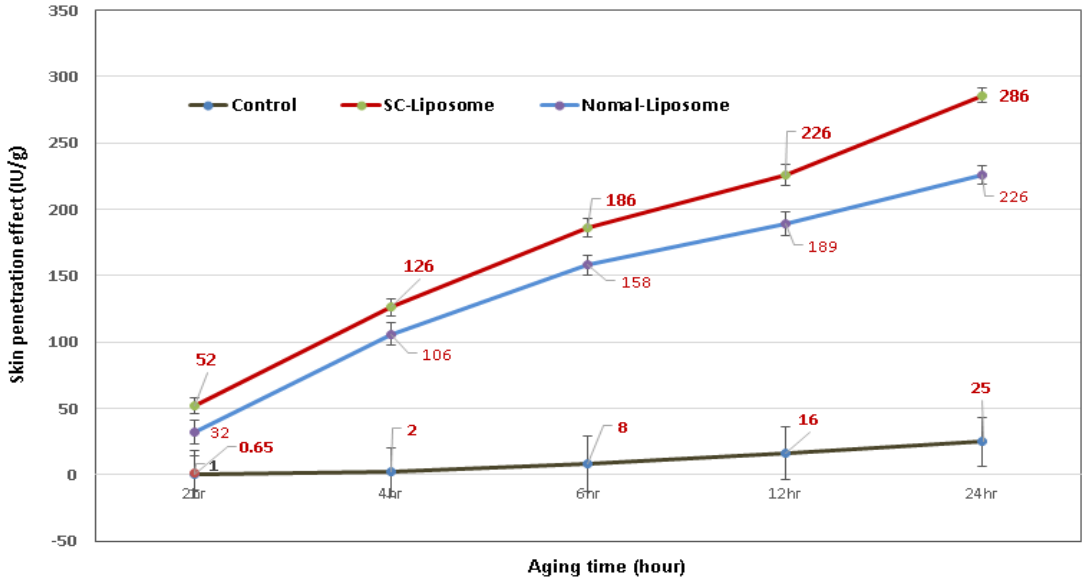

Fig. 6. Skin penetrating activity of supercritical nano-liposomes containing pure retinol measuring by HPLC determination using French cell *in-vitro* method.

These results may induce an increase in the amount of transdermal absorption according to the retinol encapsulation technology of the supercritical method, but it can be considered that the amount of transdermal absorption differs more depending on the enclosed particle size. The larger the particles, the less transdermal absorption amount, and the smaller the particles, the higher the transdermal permeation amount. Based on these results, it is expected that it will be widely applied in the development of cosmetics containing pure retinol.

4. Discussion

In a situation where methods of increasing stability by mixing antioxidants and stabilizers with nanoemulsions developed by this liposome manufacturing method have been mainly reported, the method of increasing stability by forming liposomes in a supercritical state is considered to have high technical value. Another method is to use semi-solid liquid crystal emulsions, but it was considered that retinol could not be fundamentally stabilized with a technology that applies heat to form lamellar liquid crystals according to the critical micelle concentration and phase transition temperature. It was considered that a more stable emulsifier could be obtained only by using a combination of antioxidants and stabilizers while fundamentally blocking this problem because retinol has a disadvantage in the heating process and decomposing it in light. In this study, in order to solve this problem, nano liposome inclusion was designed to be possible without heating. The manufacturing method of pure retinol forming a liposome in a supercritical state, encapsulating it in it, passing it through a high-pressure microfluidizer at

cold temperatures, and encapsulating it in nano liposomes, and using this, stabilizing it with research on the effect of percutaneous absorption of the skin.

5. Conclusion

The results of a clinical study on transdermal absorption by forming liposomes in a supercritical state and sealing active ingredients without heating pure retinol, which is vulnerable to temperature and light, are as follows. First: pure retinol was enclosed in a huge liposome using a mixed surfactant to form liposomes in the supercritical state. Second; it was able to pass through a high-pressure microfluidizer and turn it into a nanoliposome. Third, as a result of evaluating the stability according to the change over time, it was found that the stability was excellent in the order of control, general liposomes, and supercritical liposomes. Fourth, as for the amount of transdermal absorption, the amount of transdermal absorption decreased as the particle size increased, and as the particle decreased, the transdermal absorption strategy increased. In particular, the non-heating process showed excellent stability in the liposome sample enclosed in the supercritical state, and it was concluded that there was a differentiated technical value as the amount of transdermal absorption increased. Based on these results, it is expected that it will be widely applied to the development of cosmetics containing unstable pure retinol.

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Keywords: supercritical, pure retinol, liposomes, stability, transdermal absorption

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