The study on stabilization of Retinol-Nanoemulsion using Skin Lipid Matrix(SLM)

Joo Hyun Cho*, Choon Bong Lim, Hee Gil Chai, Sang Yong Eom, Jong Heon Kim, and Hong Geun Ji**

* Charmzone Co., Ltd., Korea; Email: cho1145@bcline.com

** H&A Pharma Chem, Korea

Keywords : Stabilization, Retinol, SLM, DC-liposome

Summary

In cosmetic area, retinol is prominent ingredient for anti-wrinkle but unstable against light, heat, oxygen and so on. Therefore the stabilization of retinol is required. Here, we capsulated doubly retinol in the SLM(Skin Lipid Matrix) that makes three dimensional lamellar structure similar to skin, after formation of primary liposome (retinol-nanoemulsion).

First, we make primary liposome from retinol / hydrogenated lecithin / polysorbate20 / caprylic & capric triglyceride / ethanol / and so on, and the mean diameter to 70 nm, using microfluidizer passed three times at 800 Bar, repeatedly. Then we produce DC-liposome (doubly capsulated-liposome) that was encapsulated primary liposome with SLM made of hydrogenated phosphatidyl choline / caprylic & capric triglyceride / 1,3-butylene glycol / ceramide3 / cholesterol /etc. We measured for color stability against light and heat with chromameter. As a result of this experiment, we observed DC-liposome was more than from 1.5 to 3 times as stable as general liposome. Livability of retinol has improved from 2 to 6 times when we analyzed it by HPLC. Also, penetration effect of DC-liposome has improved.

Introduction

A recent development in cosmetics has been the pursuit of high functionality. However, it is a common feature that the functional raw materials are unstable for light, heat and oxygen. Therefore, new technology of stabilization for functional raw material has been required. With this trend, we will take vitamin cosmetics and their stabilization method into account[1]~[2]. Vitamin A is the generic name for a class of nutritionally active, unsaturated hydrocarbons. It is present in animal system as A_1(retinol), A_2(3-dehydro-retinol) and in the plant system as carotenoid. Vitamin A_2 has about 40% of the effect of A_1 and both A_1 and A_2 exist in the form of ester of fatty acid. Retinol contains at least non-oxygenated β-ionone ring with an attached isoprenoid side chain. And retinol that contains all of the *trans* double bonds in the isoprenoid side chain is the most bioactive form of vitamin A(Fig. 1)[3]~[6]. Retinol is important in a wide variety of biological functions. These include

embryonic growth and development, vertebrate vision, immune reactions and epidermal differentiation. It is also a prime candidate for cancer chemoprevention. However, it comes into question that all Vitamin As decreased their activity by isomerization, photochemical and thermal oxidation. Such degradation reactions can be reduced the available vitamin activity of stored and processed foods. In general, conditions of high moisture, low pH and high temperature decrease the stability of retinol and its relatives. Retinol is a fat-soluble material that only occurs abundantly in fish, mammalian liver, milk fat and egg yolks. Due to its hydrophobic character, retinol is usually found in a complex with lipid droplets (milk fat globules) or micelles in foods. Such a condition which is expected to protect retinol from degradative reactions, can be used as multi lamellar liposomes in the lab[7]~[12].

Liposomes are spherical closed vesicles of phospholipid bilayers with an entrapped aqueous phase. The lipid layers are made up mainly of phospholipids which are amphophilic ; they have a non-polar region composed of two fatty acid and polar region composed of a phosphate group. In aqueous solution, they are arranged in bilayers, which form closed vesicles like artificial cells. The fatty acid tails, being non-polar, are located in the membranes' interior, and the polar heads turn outward in the bilayer. Liposomes are divided into two major classes based on the number of their lamellas. Multi Lamellar Vesicles(MLVs) consist of five or more lamellas and their size range from 100㎚ to more than 1㎛. Unilamellar are single bilayer structures, themselves subdivided into small (SUVs, < 100㎚) and large(LUVs, 100~1000㎚)[13]~[15].

In the cosmetic area, liposome is applied to stabilize the unstable materials in exterior condition (air, light, etc) and to maximize its efficacy and to increase skin absorption by using phospholipids, which have the great affinity for skin. Retinol has also been treated as an interesting molecule to be encapsulated in liposomes. The stability and delivery of liposome-incorporated retinol have been studied in several articles. However, the stability of retinol in liposome has not been sufficiently studied[16]~[18].

On this research, we made primary liposome firstly that is composed of retinol, lecithin, etc. And we made DC-liposome by encapsulating primary liposome in the SLM that makes three dimensional lamellar structure similar to skin. Then we measured for particle size and formation of liposomes by using laser light scattering system, freeze fracture-scanning electron microscopy and transmission electron microscopy. The color stability against light and heat was measured with chroma meter. we analyzed livability of retinol and penetration effect by HPLC. These results indicate that DC-liposome is more stable than general liposome and its penetration effect has improved because it was made use of skin familiar materials such as ceramide3, cholesterol, etc.

Materials and Methods

Materials

For this experiment, we used Retinol 50C(1.58 Million I.U./g) of BASF. Lipoid S100-3 and S75-3 of Lipoid company is used to make DC-liposome and primary liposome. All material in our experiment such as caprylic & capric triglyceride, ceramide3, cholesterol, ethanol, etc were used in

cosmetic grade. Finally, we used purified water that had passed through an anion-cation exchange resin column.

Equipment

The paticle size of liposomes is measured to study distribution of particles by using the laser light scattering system(Zetasizer 3000H, Malvern, UK). The formation of liposomes is observed by using freeze fracture-scanning electron microscopy (FF-SEM) and transmission electron microscopy (TEM). We used chromameter(Color JS 555, Color techno system, Japan) to evaluate the change in color of liposomes. We performed quantitative analysis of retinal by using HPLC(model510, Waters, USA) under the following conditions; detection by Ultravilolet spectrophotometer(325nm); C_{18} column(3.9 X 150 mm); 1.0 ml/min flow rate; methanol:water (90:10) mobile phase. Microfluidizer(M110F, Microfluidics, USA) is used for making all liposomes.

Method

1. Preparation of DC-liposome, identification of formed liposome and particle size measurement

A primary liposome was prepared from retinol / hydrogenated lecithin / polysorbate20 / caprylic & capric triglyceride, by subjecting the mixture of said components into a microfluidizer for three times in a consecutive manner, under pressure of 800 bar. Secondary liposome SLM consisting of hydrogenated phosphatidyl choline/ caprylic & capric triglyceride /1,3-butylene glycol /ceramide3 /cholesterol /ethanol was firstly prepared and then mixed with primary liposome, and then the resulting mixture was subjected to a microfluidizer to prepare DC-liposome. Particle size of obtained primary liposome and DC-liposome was measured by using a laser light scattering system (Zetasizer3000H, Malvern, UK) at room temperature. A TEM was used to determine the formation of the emulsion of the primary liposome. In addition, FF-SEM was used to determine the formation of lamella vesicle of the DC-liposome. Particle size and microphotographs are shown in Fig. 2, Fig. 3 respectively.

2. Heat or light induced color change in DC-liposome and general liposome

The change in color of each liposome which has been stored for a month under different conditions of 25℃+without light, 40℃ and light exposure was measured by using a chromameter. The result of color change is shown in Fig. 4.

3. Measurement of change in retinol content

Under different conditions of 25℃+without light, 40℃ and light exposure, a quantitative analysis on change in retinol content was carried out for one month. The variation of content of retinol is shown in Fig. 5.

4. Evaluation of the skin penetration by general liposome and DC-liposome

Two different types of cream were prepared; one containing the general liposome and the other containing DC-liposome, each in an amount of 2%. After applying the cream onto the skin of a hairless mouse, the retinol penetrated into the skin was extracted and then analyzed using HPLC. The data of skin penetration is shown in Fig. 6.

Results and Discussion

Identifying the formation of DC-liposome and primary liposome

In order to identify the formation of the primary liposome and DC-liposome as mentioned above, TEM and FF-SEM were respectively used. As shown in Fig. 2, the formation of primary liposome was confirmed. For the DC-liposome, a multi lamella structure was observed and the primary liposome was dual-encapsulated inside the 3D structure of the lamella sheet. Further, the particle of the primary liposome, which is formed inside the lamella was observed. These results suggest that, being located inside the multi lamella structure, the retinol compounds which is primarily nano-emulsified becomes dually stabilized.

Particle size distribution of DC-liposome and primary liposome

A particle size distribution of the primary liposome and the DC-liposome was measured with laser light scattering system, as shown in Fig. 3. For the primary liposome, the particle size distribution is concentrated in the region of 20 nm to 150 nm and its mean diameter was found to be about 70 nm. For the DC-liposome, the particle size distribution is concentrated in the region of 20 nm to 1000 nm and its mean diameter was found to be about 210 nm. Wider distribution of the particle size for the DC-liposome can be due to the fact that, in addition to the primary liposome particle size distribution, particles of MLV type liposome of SLM may also exist in the DC-liposome.

Color stability of DC-liposome and general liposome against heat and light

As shown in Fig. 4, color change of liposomes caused by heat or light was measured with the lapse of time. While the color of retinol was generally changed by heat or light, it was found that DC-liposome was more stable than the general liposome; i.e., 2 times more stable at light exposure, 1.5 times more stable at 40℃ and 3 times more stable at 25℃+without light. These results indicate that the DC-liposome was dually encapsulated inside the 3D structure of the lamella sheet. As a result, the retinol compounds are dually stabilized and their stabilization are significantly increased in the DC-liposome compared to general liposome.

Measurement of change in retinol content

Change in retinol content was followed for one month for the general liposome and DC-liposome as shown in Fig.5 (the liposomes were incubated under different conditions of 25℃+without light, 40℃ and light exposure, respectively). As shown in the following figure, it was found that the DC-liposome was 3.5 times more stable than the general liposome at light exposure, 6 times more stable at 40℃ and 2 times more stable at 25℃+without light. Thus, it appears that the DC-liposome is stabilized more than the general liposome thanks to the dual stabilization, and this is similar to the results obtained for the above-described color change measurement.

Skin penetration effect of general liposome and DC-liposome

As shown in Fig.6, the skin penetration of general liposome was about 0.118% and that of DC-liposome was about 0.189%, which indicates that the skin penetration of DC-liposome is superior to that of general liposome in an extent of about 60%. From such results, it appears that the DC-liposome improves skin penetration of retinol by having the constitution similar to that of skin, which comprises phospholipid, ceramide3, cholesterol, etc.

Conclusion

For industrial fields of cosmetics and pharmaceutics, a liposome has been widely studied and used as a vehicle to deliver bioactive materials. A liposome has been especially used to promote the absorption of the bioactive materials into skins or cells, etc.

In the present study, the general liposome was utilized to promote the absorption and bioavailability of the bioactive materials. With the experiments using a phospholipid, which is found in human body, the bioavailability was found to increase and this result indicates that the general liposome can effectively penetrate into the skins. However, a problem exists due to the fact that the general liposome has relatively low stability. To solve such problem, retinol was primarily nano-emulsified and dually encapsulated into the 3D structure of the lamella sheet. As a result, the primary liposome particles are located inside the multi lamella structure, with improved stability. In addition, the experimental results of the present study indicate that the DC-liposome penetrates into the skin as much as about 60% better than a general liposome. Such improvement can be due to the fact that the DC-liposome of the present study consists of skin constituents such as phospholipid, ceramide3, cholesterol, etc.

References

[1] Bernard Idson Vitamins and the Skin *Cosmetics & Toiletries* **108**, 79~94, 1993.

[2] H. B. Sauberlich Bicavailability of vitamins. *Prog. Food Nutr. Sci.* **9**, 1-33, 1985

[3] Willhite, C.C. Structure-activity relationships of retinoids in developmental toxicology. II.Influence of the polyene chain of the vitamin A molecule. *Toxicol. Appl. Pharmacol.* **83**, 563-575, 1986.

[4] C M. Lee *et al* Review of Animal Models in Carotenoid Research *J. Nutr.* **129**,2271-2277 1999.

[5] A Bondi *et al* Vitamin A and carotene in animal nutrition. *Prog. Food Nutr. Sci.* **8**, 165-191, 1994.

[6] M.I Dawson *et al* Chemistry and Biology of Synthetic Retinoids. CRC Press, Boca Raton, 1990.

[7] H Oikarinen *et al* Modulation of procollagen Gene Expression by Retinoids.*J. Clin. Invest* **75**, 1545~1553, 1985.

[8] A. Jarret *et al* The action of vitamin A on skin and mucous membranes *J.Appl.Cosmetol.* **7.**, 33~38, 1989.

[9] M Kennedy, *et al* Modulation of rabbit keratocyte production of collagen sulfated glycosaminoglycan and fibronectin by retinyl and retinoic acid. *Biochem Biophys Acta* **899**, 156, 1986.

[10] D Douer *et al* Treatment of newly diagnosed and relapsed acute promyelocytic leukemia with intravenous liposomal all-trans retinoic acid. *Blood* **97**, 73-80, 2001.

[11] G Fex *et al* Retinol transfer across and between phopholipid bilayer membranes *Biochim et Biophy Acta* **944**, 249~255 1988

[12] S.C Lee et al Stabilization of Retinol through Incorporation into Liposomes. *Journal of Biochem and Molecular Biology* **35**, 358-363, 2002.

[13] K. Rodgers Controlled release technology and delivery systems *Cosmetics & Toiletries* **114**, 53~60 1999

[14] C. J. Kirby Controlled delivery of functional food ingredients: Opportunities for liposomes in the food industry; in Liposome Technology II, Gregoriadis, G. (eds.) CRC Press, Boca Raton, 215-232, 1984

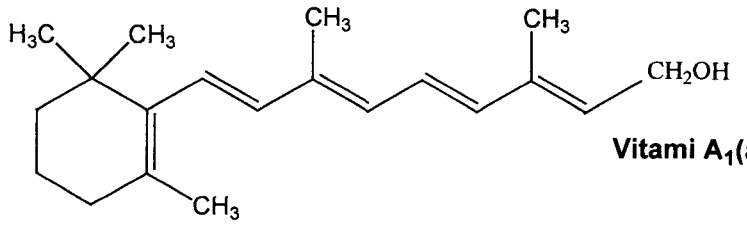
[15] H. H Kim *et al.* Novel liposome microencapsulation techniques for food applications trends. *Food Sci. Technol.* **2**, 55-61, 1991.

[16] T. Tsunoda *et al* Stability of all trans-retinol in cream *J. Soc. Cosmet. Chem* **46**, 191~198, 1995

[17] A.K Singh *et al* Liposome encapsulated vitamin A compounds exhibit greater stability and diminished toxicity. *Biophys. Chem.* **73**, 155-162, 1998.

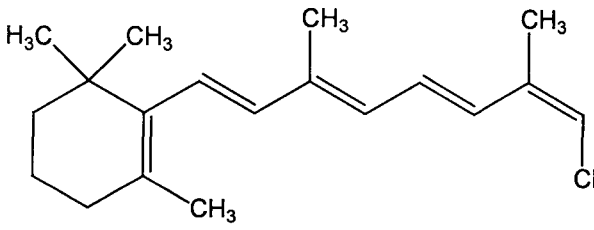
[18] A M. Young *et al* Photolysis of retinal in Liposomes and its protection with Tocopherol and Oxybenone *Photochem & Photobiol* **63**, 344~352 1996

Figure.1 Structures of Vitamin A

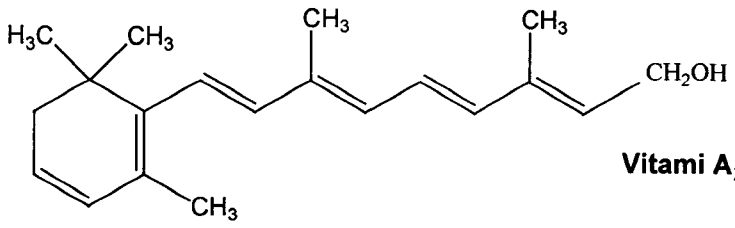


Vitami A_1(all trans type)

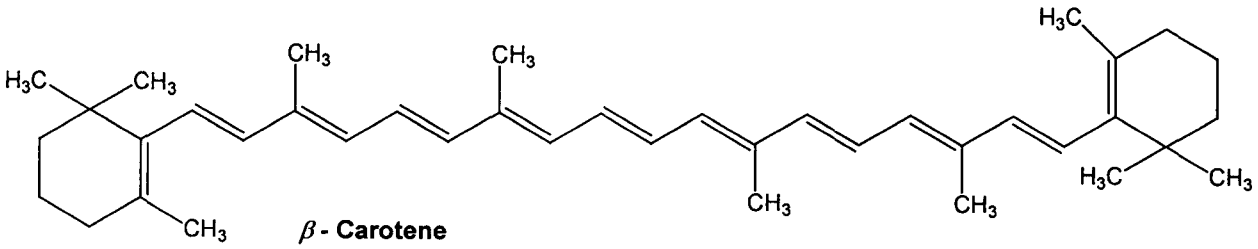
Thermal Isomerization



Vitamin A_1(13-cis-retinol)

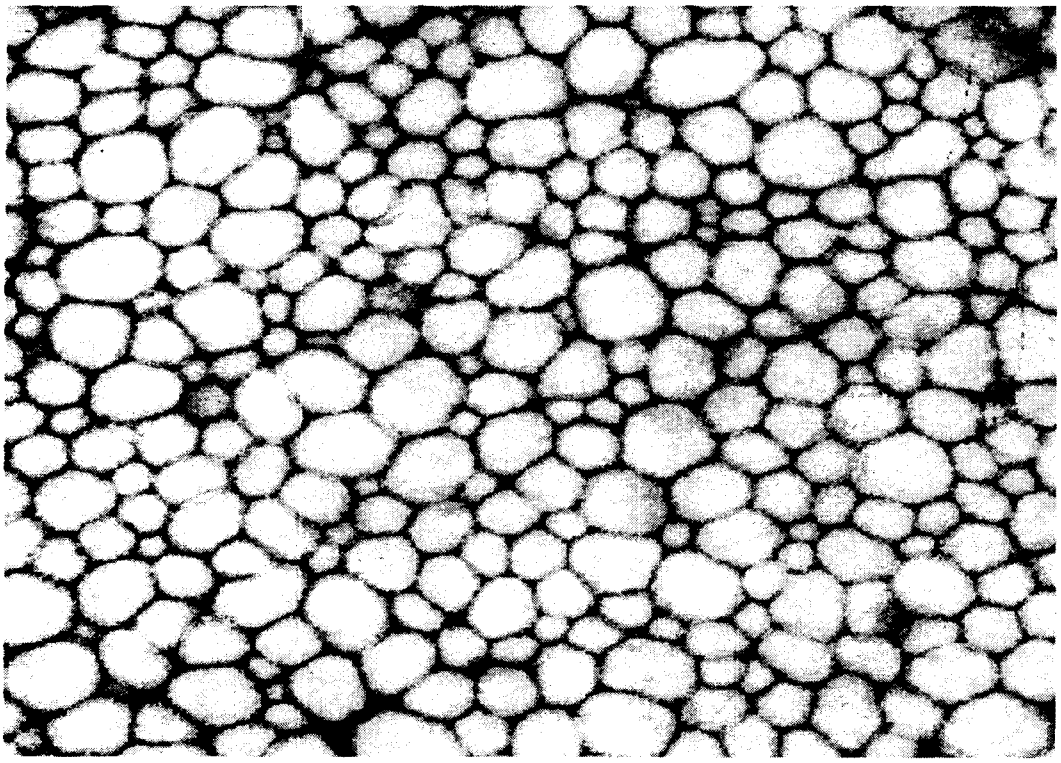


Vitami A_2(3-dehydro-retinol)

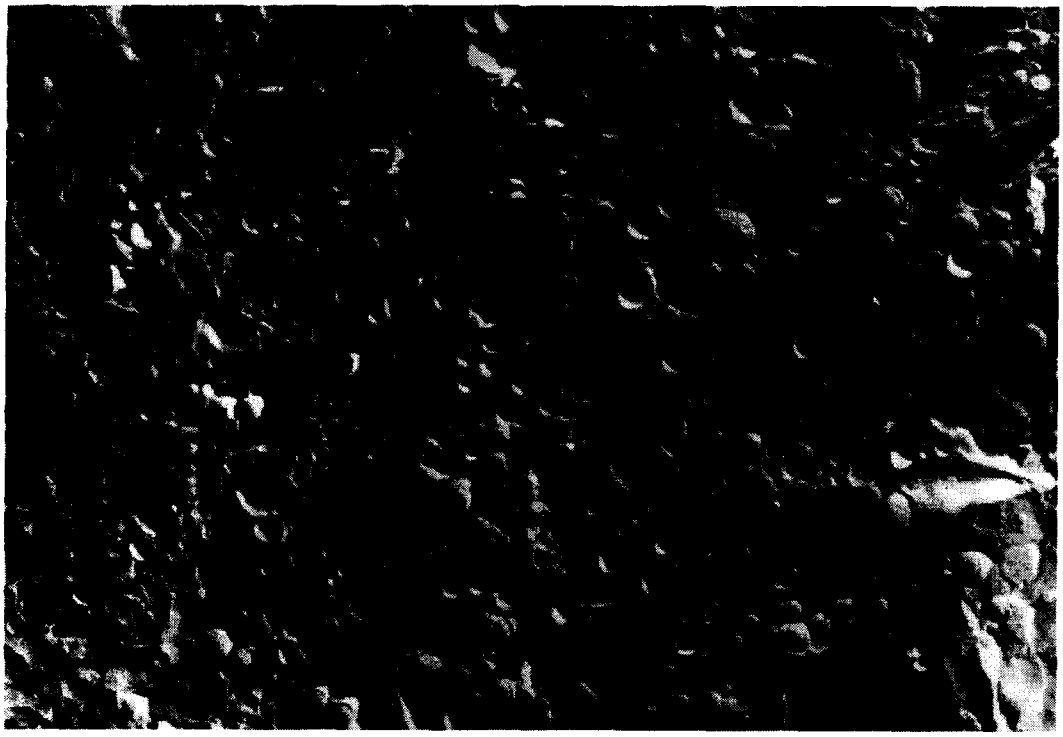


***β*- Carotene**

Figure.2 Microphotograhs of DC-liposome and primary liposome

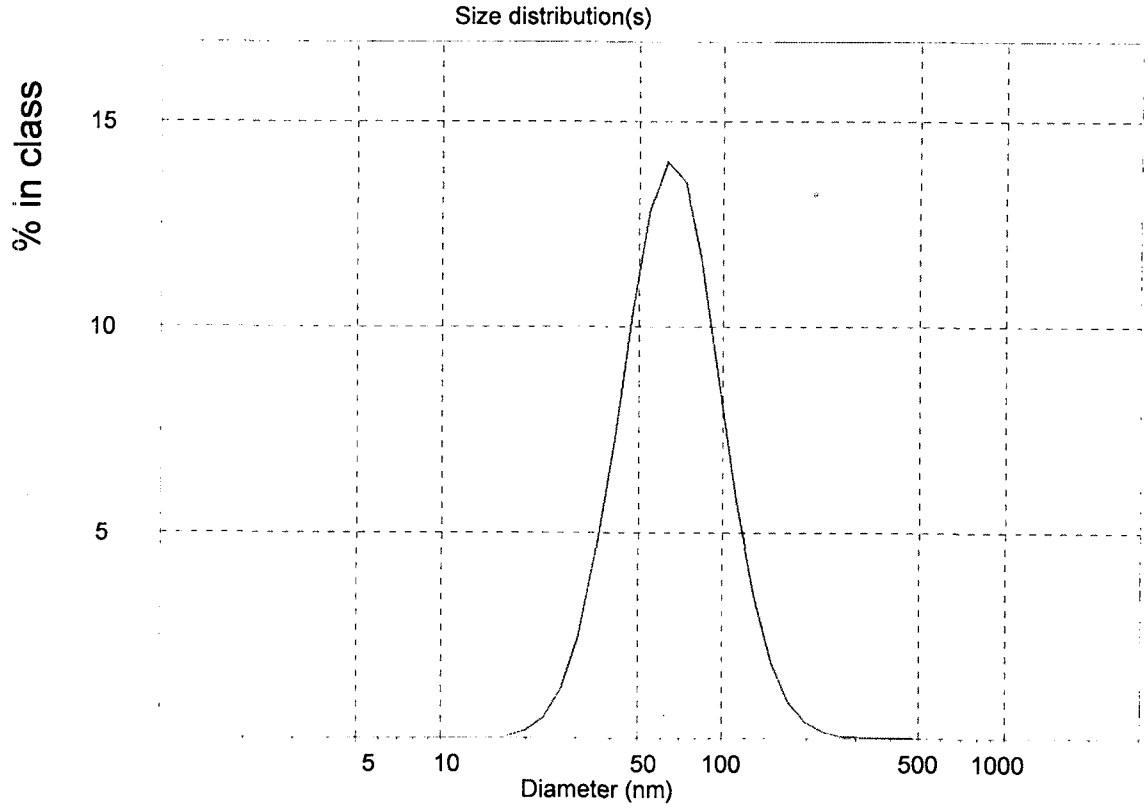


(a) Transmission electron microphotograh of primary liposome(Retinol-nanoemulsion)

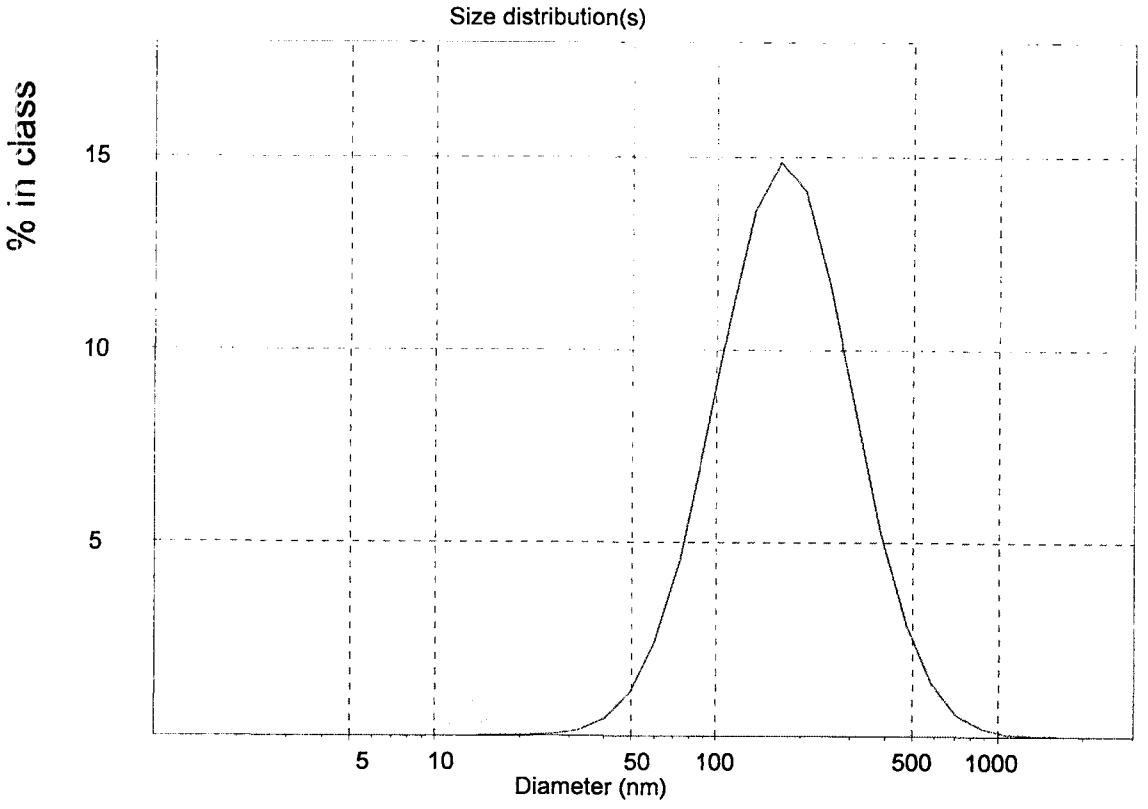


(b) Scanning electron microphotograh of DC-liposome, obtained after freeze-fracture

Figure.3 The distribution of particle size



(a) Primary liposome



(b) DC-liposome

Figure.4 Color stability of tested liposomes after one month storage at (a) Light exposure,(b) 40℃ and (c) 25℃, Without Light

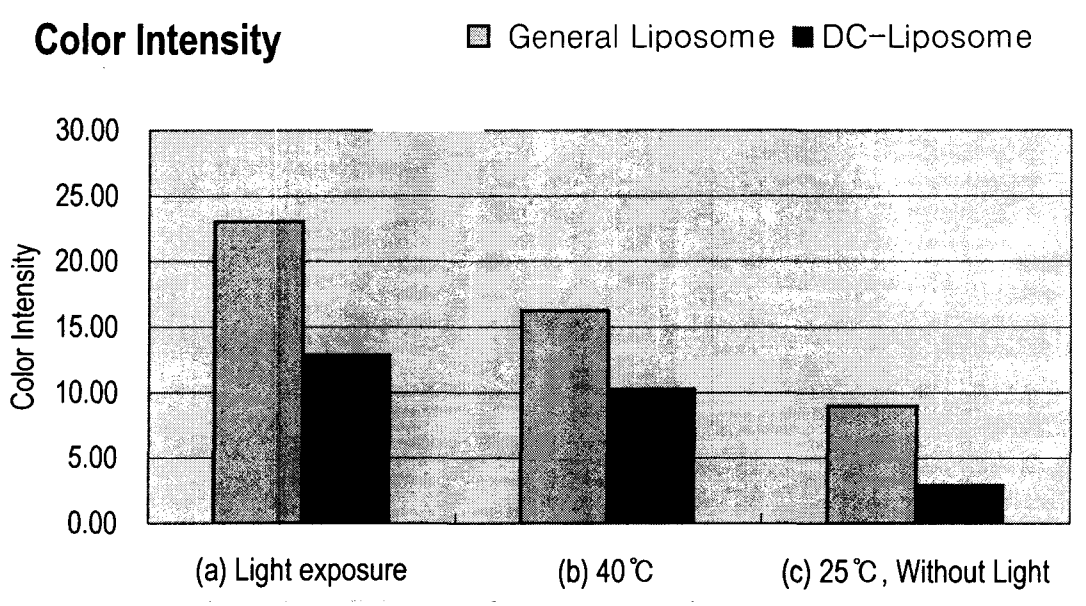
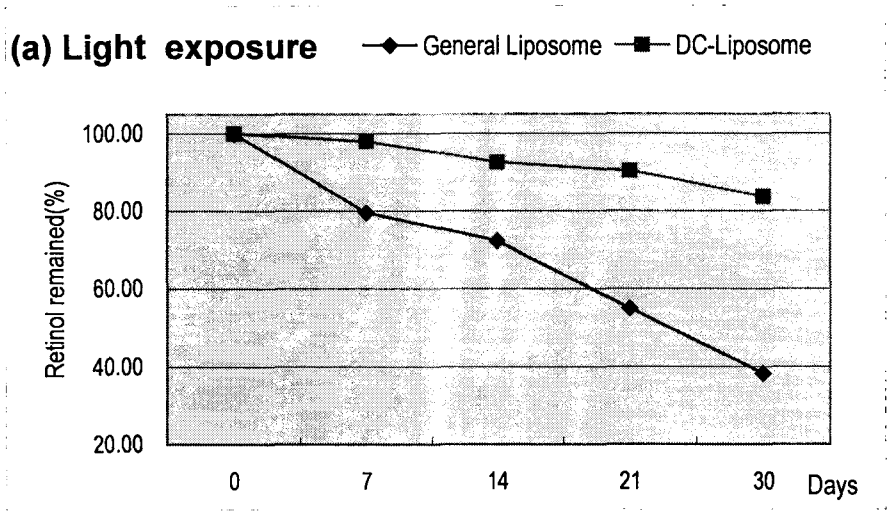
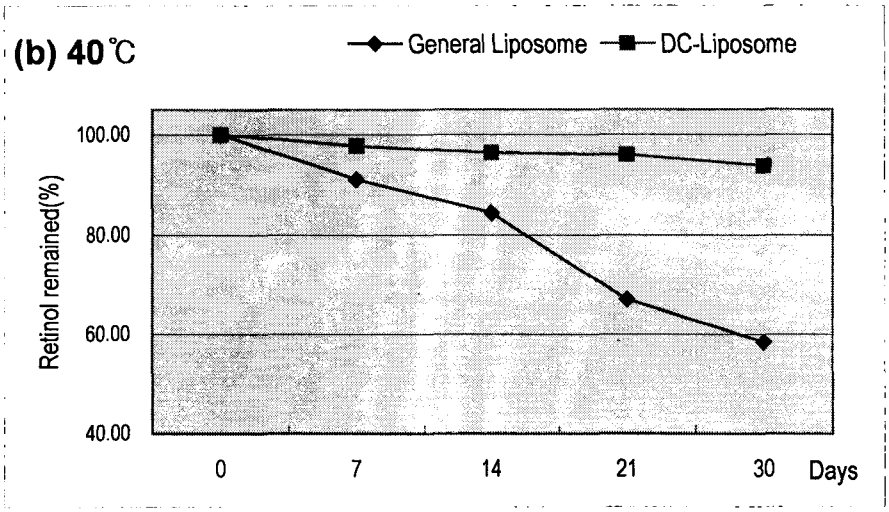


Figure.5 Change in Retinol content for one month of storage
at (a) Light exposure,(b) 40℃ and (c) 25℃, Without Light





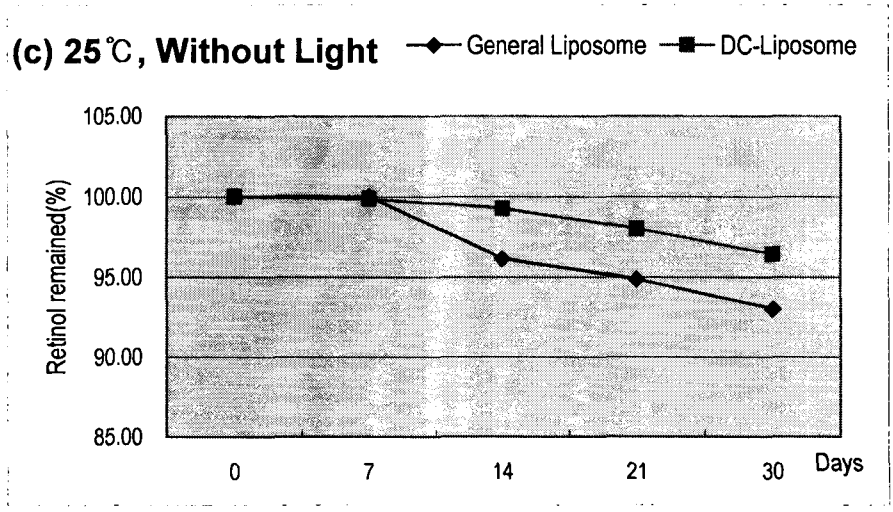


Figure.6 Skin penetration effect of liposomes