PROCEEDINGS OF THE 20TH IFSCC CONGRESS

RETINOL STABILIZATION BY PSEUDO-LIPOSOME AND LAMELLAR LIQUID CRYSTAL

Seung-Ji Lee, Byoung-Kee Jo, Young-Jin Lee, Chang-Suk Ryu, Beom-Jun Kim, and Chang-Hyun Suk

R&D Center, Coreana Cosmetics Co. Ltd., Cheonan, Korea

SUMMARY

It is well known that all-*trans*-retinol is not only very unstable in heat, light, air, and water, but also skin-irritant despite a good anti-wrinkle effect. Therefore, it is very difficult to stabilize retinol and make the safe retinol containing cosmetics by using a certain concentration of retinol with real effect. In order to dissolve these problems and apply retinol for skin care cream, firstly retinol is to be encapsulated in the vesicle called Lipospher (pseudo-liposome) which is made by homogenizing under high pressure the mixtures of lecithin, retinol, caprylic/capric triglyceride, and hydroalcoholic solution; and then this retinol containing Liposphere is to be intercalated in lamellar liquid crystal layer which is prepared by emulsifying in an optimal ratio the mixtures composed of non-ionic emulsifier (cetearyl glucoside, sorbitan stearate & sucrose cocoate etc), cetearyl alcohol, stearic acid, cholesterol, and ceramide. In addition, the stability of the retinol containing oil in water cream by adding the polymeric emulsifier such as acrylate /C10-30 alkyl alkylate crosspolymer is to be ensured even at 55 C. Retinol containing oil in water cream prepared through above procedure could be very stable at 45 C for at least 50 days. The structure identification of lamellar liquid crystal was determined using polarized light microscope and electron microscope.

Conclusively, we could make the very stable retinol containing oil in water cream by triple procedure, that is, encapsulation of retinol in Liposphere, intercalation of retinol in lamellar liquid crystal layer, and assurance of the high temperature stability of cream even at 55 C

INTRODUCTION

Recently, retinoids led to be highlighted as a high efficient component through the booming of high-performance cosmetics, especially anti-wrinkle agent. Among the retionoids, retinol is an endogenous compound naturally occurring in human body and essential for production, differentiation, and multiplication of epithelial tissues (1). Moreover, retinol is regarded as a desirable cosmetic substance because of its lower stimulus compared to retinoic acid.

However, retinol is not only very unstable in heat, light, oxygen, and water, but also skin irritant despite of good anti-wrinkle effect. Many studies on the stability of retinol have been carried out and reported, but retinol could not have been used commonly for cosmetics up to now (2-4).

Therefore, at this experiment in order to dissolve these problems and apply retinol for skin care cream, firstly retionl is to be encapsulated in the vesicle called Liposphere. And then Liposphere is to be intercalated in lamellar liquid crystal layer prepared by emulsifying the mixtures of non-ionic emulsifier, stearic acid, cetearyl alcohol, cholesterol, and ceramide. Additionally retinol containing oil in water cream adding polymeric emulsifier is to be stabilized even at 50 C.

Conclusively, this retinol containing oil in water cream is not only very stable, but also very safe by time-releasing (5).

EXPERIMENTAL

Materials

All-*trans*-retinol (3150 units/mg), hydrogenated lecithin obtained from soybean, cetearyl glulcoside, sorbitan stearate & sucrose cocoate, POE (20) sorbitan monostearate, sorbitan stearate, acrylate/C10-30 alkyl acrylate crosspolymer, cholesterol, ceramide, cetearyl alcohol, steraric acid, caprylic/capric triglyceride

Lipospher preparation **(6-7)**

Lipospher was prepared by homogenizing at 1200 bar using a microfluidizer, the premixtures of oil phase composed of hydrogenated lecithin, caprylic/capric triglyceride, and all-*trans*-retinol and hydroalcoholic solutions mixed in a specific ratio of water and alcohol. In order to obtain homogenous product, the premixtures were to be passed through the interaction chamber several times. The Lipospher prepared by this technique is very fine vesicle(mean size 118nm) that all-trans-retinol is enclosed by the phospholipid monolayer, whereas liposome is composed of one or more bilayer membrane filled with hydrophilic core (Figure 1).

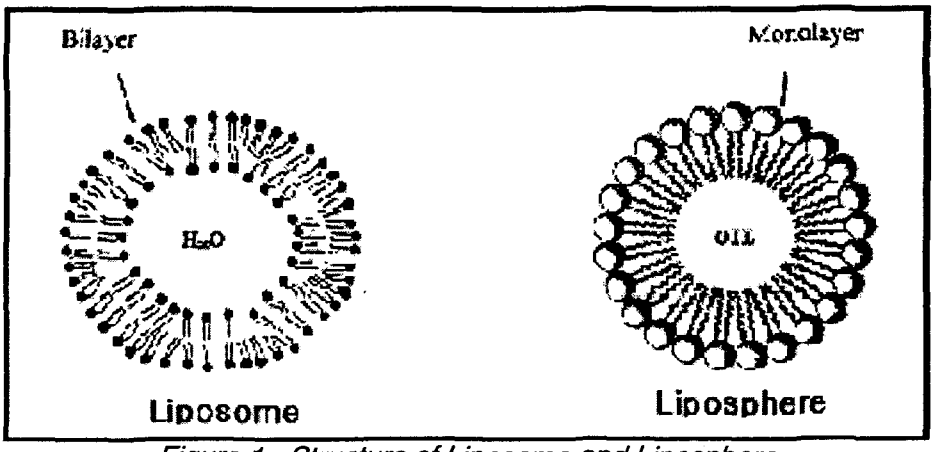

Figure 1. Structure of Liposome and Lipospher

Cosmetic cream preparation

Cosmetic cream formulas are shown in Table 1. The cosmetic creams are oil in water emulsions prepared by adding and homogenizing the oil phase to the aqueous phase, both preheated to 75 C except retinol added at 40 C. The moderate homogenization must be performed to mix retinol homogeneously after adding it.

Storage test

30ml of Lipospher (contained 2.0% retinol) and cosmetic cream (contained 0.2% retinol) respectively in 50ml transparent glass bottle sealed with a cap were stored under various temperature conditions.

Cream A	(wt .%)	Cream B	(wt. %)
Sorbitan Stearate & Sucrose	2.20	Sorbitan Stearate & Surcrose	1.50
Cocoate	1.00	Cocoate	0.60
Cetearyl Glucoside	0.40	Cetearyl Glucoside	2.50
Ceramide	0.20	Glyceryl Stearate	1.50
Cholesterol	0.30	Cetearyl Alcohol	13.50
Stearic Acid	4.00	Caprylic/Capric Triglyceride	5.00
Cetearyl Alcohol	16.00	Glycerin	0.20
Caprylic/Capric Triglyceride	5.00	Preservative	10.00
Glycerin	0.20	Liposphere	0.05
Preservative	10.00	BHT	65.15
Liposphere	0.05	Water	
BHT	60.65		
Water			
Cream C	**(wt. %)**	**Cream b**	**(wt. %)**
Sorbitan Stearate	1.00	Sorbitan Stearate & Sucrose	1.50
POE (20) Sorbitan Monostearate	1.50	Cocoate	0.60
Microcrystalline Wax	1.00	Cetearyl Glucoside	2.50
Caprylic/Capric Triglyceride	10.00	Glyceryl Stearate	1.50
Mineral Oil	6.00	Cetearyl Alcohol	13.50
Glycerin	5.00	Caprylic/Capric Triglyceride	5.00
Xanthan Gum	0.30	Glycerin	0.15
Preservative	0.20	Acrylate/C10-30 Alkyl Acrylate Crosspolymer	0.20
Liposphere	10.00	Preservative	10.00
BHT	0.05	Liposphere	0.08
Water	64.95	Potassium Hydroxide	0.05
		BHT	64.92
		Water	

Table 1. Cosmetic cream formulas

Measurements

Determination of all-trans-retinol (8,9)

We weighed about 0.05g of each sample into a 1.5-ml eppendorf tube, added 1ml of water/n-butanol (1:1), and then dissolved using vortex mixer. The prepared sample was centrifuged for 2 min using table centrifuge. 400µl of methanol was added into the upper layer (n-butanol layer) of centrifuged sample solution, and then mixed. The sample solution was analyzed by HPLC at 325nm.

Identification of lamellar liquid crystalline phase(10-16)

The presence of the liquid crystalline phase in cosmetic cream was proved by observing its birefringence using polarizing microscope (Leica DMRBE) and electron microscope (JEOL 1200EX). When using electron microscope the samples were frozen at -200 C in liquid nitrogen. Freeze fracturing was carried out using a Reichert-Jung cryofract at 150 C under vacuum.

RESULTS AND DISCUSSION

Influence on retinol stability of Liposphere

In order to compare the retinol stability in Liposphere with that in caprylic/capric triglyceride without Liposphere, the percent remaining of the original amount of retinol was examined after five days storage at

50 C. This result shows that the retinol in Liposphere is more stable than in the only capralic /capric triglyceride as seen in Table 2.

all-*trans*-retinol in Liposphere	98.93%
all-*trans*-retinol in carprylic/capric triglyceride	38.52%

Table 2. Remaining % of all-trans-retinol(2.0%) after five days storage at 45 C

Influence of the molecular structure on retinol stability

In order to investigate the retinol stability according to the difference of molecular structure, we prepared oil in water creams distinguished by the relative formation quantity of the liquid crystal (Table 1) and examined the formation quantity of the liquid crystal of each cream under a polarizing microscope and electron microscope (Figure 2, 3).

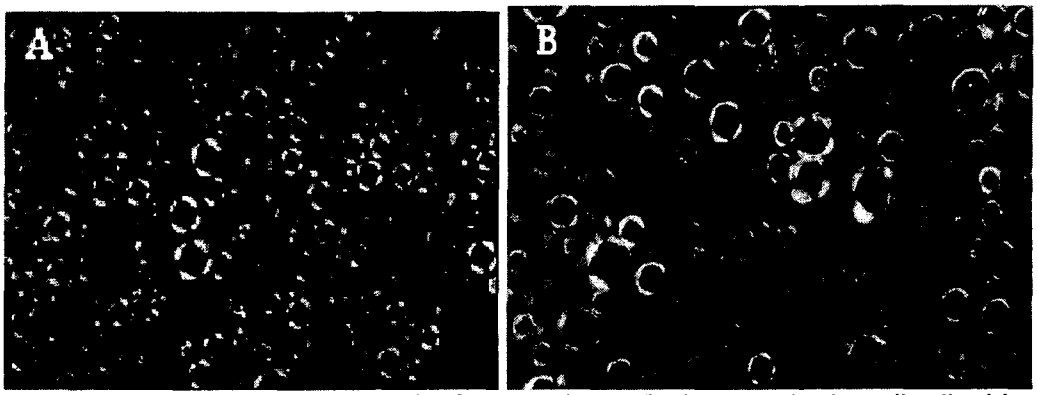

Figure 2. Polarizing photomicrograph of cream A and B showing the lamellar liquid crystal (A) : Picture of cream A (B): Picture of cream B

Figure 3. Electron Micrograph (enlarged 19200 times) of cream A under freeze fracturing showing the lamellar liquid crystal and some membrane like structures on their surface

At this result, it is observed that cream A and B form the liquid crystal, moreover the formation quantity of the liquid crystal of cream A is much greater than that of cream B as seen in the polarizing photomicrographs, but cream C does not form the liquid crystal.

The liquid crystal forms a rigid network at interface of oil /water and within the continuous phase composed of lipid phase, aqueous phase, and liquid crystal in the state of three phases. This network forms a rheological barrier against coalescence. The emulsion made up of the liquid crystal is extremely stable, therefore the retinol stability under this condition could be kept very well despite of storing in the high temperature like 45 C, whereas the ordinary cream without liquid crystal is very difficult to keep retinol stable (Table 3). From this result, it can be deduced that the retinol stability is proportional to the formation quantity of the liquid crystal.

	After 1 week storage	After 2 weeks storage	After 3 weeks storage
Cream A	99.52%	99.25%	97.73%
Cream B	97.15%	94.44%	90.06%
Cream C	95.11%	89.56%	82.90%

Table 3. Remaining % of all-trans-retinol (0.2%) of cream A, B and C after three weeks storage at 45 C

On the other hand, the retinol stability is extremely dependent on the temperature . So the retinol is generally stable in the lower temprature, but unstable in the higher temperature.

Figure 4 shows that the comparision of the discoloration level beteween cream A and B and C at 45 C can be distinguished very easily, but cannot be distinguished at 4 C. At these results, it could be clarified that the less stable the retinol in cream was, the severer the discoloration degree occured. In this context the retinol stability of cream A is much better than that of cream B and C at 45 C, but the retinol stabilities of all creams are almost same at 4 C without regard to the kind of cream.

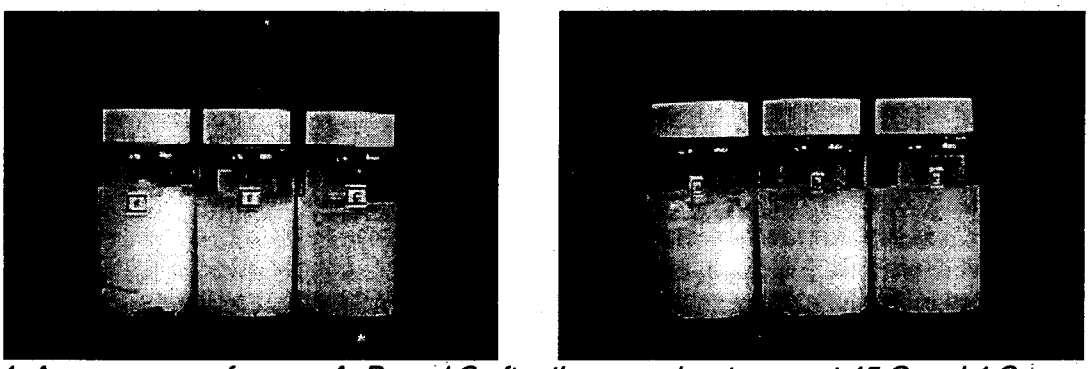

Figure 4. Appearances of cream A, B, and C after three weeks storage at 45 C and 4 C respectively
A, B and C : Photographs of cream A, B, and C at 45 C
a, b, and c : Photographs of cream a, b, and c at 4 C

Influence of the high temperature stability on the retinol stability

The emulsion is generally less stable at the higher temperature than at the lower. Accordingly, in order to improve the high temperature stability of emulsion, the carboxy vinylpolymer is usually used in most emulsions.

For this reason, the acrylate/C10-30 alkyl acrylate crosspolymer acting as a polymeric emulsifier was used to improve the high temperature stability of cream B at this experiment and cream b was prepared (see Table 1), and then the stabilities of cream B and b were checked after three days storage at 45 C and 55 C respectively (Table 4), and also the remaining amount of all-trans-retinol of cream B and b was examined after three weeks storage at 45 C (Table 5).

This test result shows that the high temperature stability of cream b is better than that of cream B, consequently the retinol stability of cream b is maintained better than that of cream B (Table 4,5)

As a result, it is can be deduced that the better the high temperature stability of cream was ,the more stable the stability of retinol was maintained.

	45 C	55 C
Cream B	Stable	Separated into 2 layers with aqueous phase and oil
Cream b	Stable	Stable

Table 4. Stability of cream B and b after three days storage at 45 C and 55 C respectively

	45 C	55 C
Cream B	90.06%	80.23%
Cream b	93.29%	86.11%

Table 5. Remaining % of all-trans-retinol (0.2%) of cream B and b after three weeks storage at 45 C

REFERENCES

1. Y. Koizumi, Fragrance Journal, 20, 26 (1992)
2. C. Kwasaki and M. Hida, Vitamins, 15, 383 (1958)
3. T. Tabata, Vitamins, 18, 164 (1961)
4. S. Hayashi and Y. Nishii, Vitamins, 28, 269 (1971)
5. Friberg, S., J. Soc. Cosmet. Chem., 30, 309 (1979)
6. E. Hoff, H. P. Nissen, and H. Mintel, SOFW, 120, 530 (1994)
7. F. Zulli and F. Suter, SOFW, 123, 880 (1997)
8. B. Nowakowsky, Analytical Method M 96/0058/01e from BASF Co., Oct. 1996
9. T. Tsunoda and K. Takabayashi, J. Soc, Cosmet. Chem., 46, 191 (1995)
10. T. Suzuki and H. Tsutsumi, J. Jap. Oil Chem. Soc., 36, 40 (1987)
11. T. Suzuki , H. Tsutsumi, and A. Ishida, 12th Congress IFSCC, Paris, 1, 117 (1982)
12. T. Suzuki , H. Takei, and S. Yamazaki, J. Colloid Interface Sci., 129, 491 (1989)
13. Friberg, S. and Larsson, K., Liquid crystal and emulsion, Advances in liquid crystals, Brown, G.M., Ed., Academic Press, London, 1976, 2, 173-195
14. Mandani, K. and Friberg, S., Prog Colloid Polymer Sci. 65, 164 (1978)
15. Junginger, H., Akkermans, A. A. A. D., and Heering, W., J. Soc. Cosmet. Chem., 35, 45 (1984)
16. Friberg, S., Food Emulsions Friberg, S., Ed., Marcel Dekker, New York, 1976, Chap. 1.