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A Triply Stabilized System to Improve Retinol Stability

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Key words

retinol, stability, skin penetration, triply stabilized system, porous silica, non-phospholipid vesicle, skin lipid matrix

Abstract

The stability and skin penetration of retinol can be increased by triply stabilizing it. This means adhering it to porous silica encapsulated in non-phospholipid vesicles that are distributed among the lamella of a matrix of skin lipids.

Retinol is an especially powerful ingredient to reduce wrinkles, but its stability depends on environmental factors such as solvent, temperature, oxygen and light. In this article we describe how its stability and skin penetration can be improved by using a triply stabilized system (TSS).

Retinol has a wide variety of biological functions such as immune reactions, epidermal differentiation, vision in vertebrates, and stimulating embryonic growth and development. It is also a prime candidate for cancer prevention.[1-4]

Vitamin A is the generic name for a class of nutritionally active and unsaturated hydrocarbons. It is present in the animal kingdom as vitamin A_1 (retinol) and vitamin A_2 (3-dehydro-retinol), and in the vegetable kingdom as carotenoid (Figure 1).

Vitamin A_2 has approximately 40% of the effect of vitamin A_1, and both A_1 and A_2 exist in the ester form of fatty acid. Retinol contains at least one non-oxygenated beta-ionone ring with an attached isoprenoid side chain. Retinol that contains all trans double bonds in the isoprenoid side chain is the most bioactive form, however its efficaciousness declines over time because vitamin activity is decreased by isomerization, photochemical oxidation and thermal oxidation. Such degradation reactions can also reduce vitamin activity of stored and processed foods. In general, the stability of retinol and its relatives is slightly reduced in conditions of high humidity, low pH and high temperature.[5-6]

Retinol is a fat-soluble material and abundant in fish and mammalian liver, milk fat and egg yolks. Due to its hydrophobic character, it is usually found in a complex with lipid droplets or micelles. Therefore if liposome technology could be applied, we could expect to protect retinol from reactions that degrade it.[7-9]

Liposomes are spherical closed vesicles of phospholipid bilayers with an entrapped aqueous phase. The lipid layers are mainly made up of phospholipids that have amphophilic character. In aqueous solution, they are arranged in bilayers, which form closed vesicles like artificial cells.

In the cosmetic area, liposomes are used to stabilize unstable materials against exterior conditions, maximize their efficacy and enhance skin absorption because of the phospholipid's great affinity for skin. The stability and delivery of liposome-incorporated retinol have been studied in several articles. However, the stability of retinol in liposomes has not been sufficiently studied.[10-11] (Editor's note: The accompanying Editor's Note lists some other techniques that have been suggested for improving the stability of retinol in cosmetic formulations.)

The Triply Stabilized System

In this study reported here, the stability and skin penetration of retinol are improved by a triply stabilized system (TSS). According to this system:

1. A porous silica is prepared and retinol is adsorbed in the pores.

2. The porous silica and any remaining retinol are encapsulated in solid lipid nanoparticles (SLN) to improve retinol's stability against light and heat.

3. The SLN are then organized into a multi-lamellar matrix of skin lipids (SLM) to improve retinol's skin penetration effect (Figure 2).

Preparation of the TSS

Adsorbing retinol into silica pores: Porous silica was prepared using the sol-gel method (see sidebar). One of the most important reasons for using porous silica is to give thermal and chemical stability that are not easily

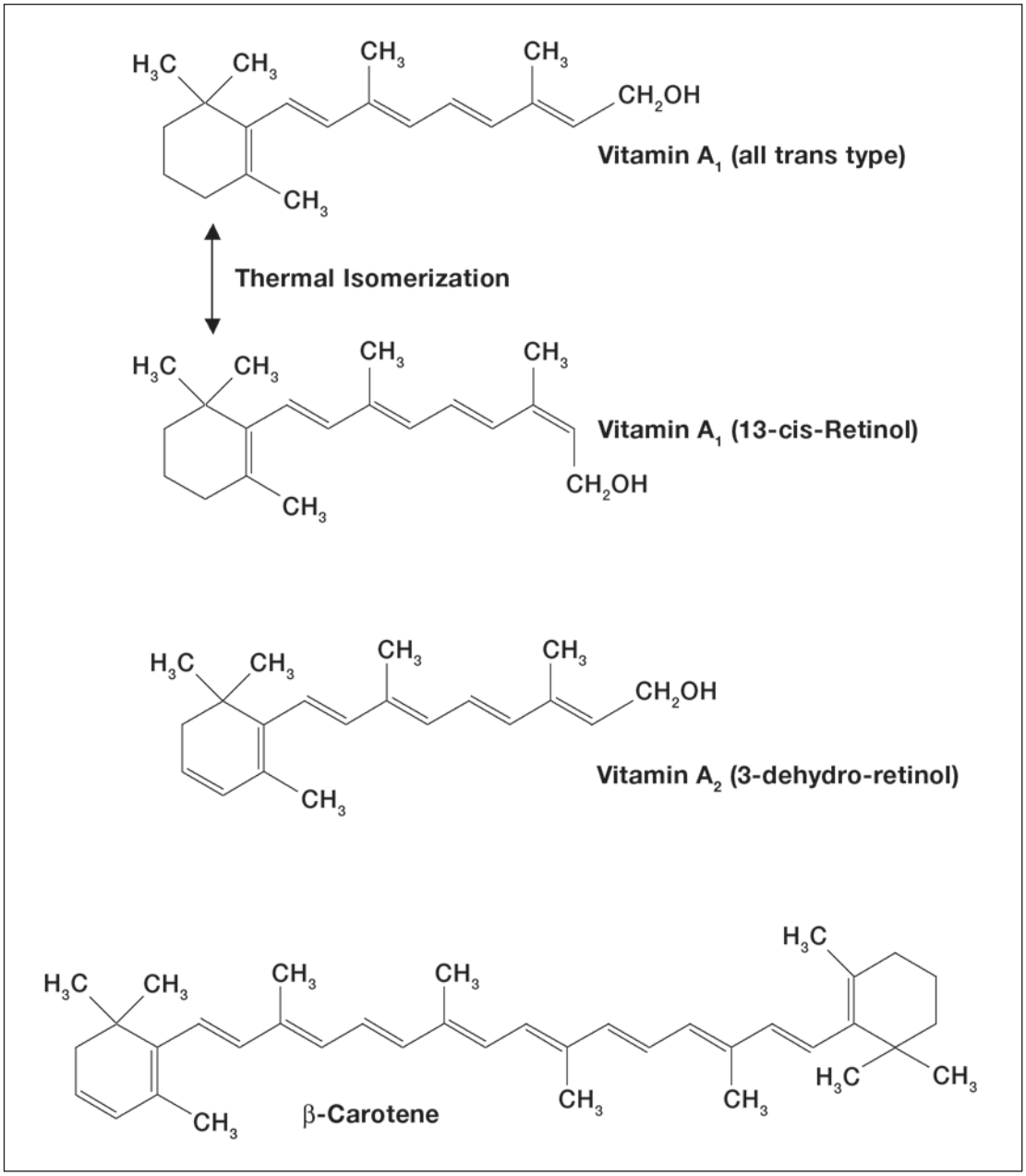

Figure 1. Structures of vitamin A

obtained from organic materials. Then retinol was entrapped into the porous silica by dispersion to give thermal and chemical stability and induce the diffusion of the active ingredient during long periods, giving an extended-release property.

Creating the SLN liposome system: Using a system of non-phospholipid vesicles (NPV) and a high-pressure homogenizer[a], the free retinol and the retinol that was adsorbed in silica pores were encapsulated to give double stabilized retinol. We call this system the Solid Lipid Nanoparticles (SLN) liposome system because it contains solid particles in its core. We also refer to this as the "primary liposome." Primary liposomes are non-phospholipid vesicles that contain free retinol and retinol entrapped in silica. Primary liposomes are prepared by steps 1 and 2 of the TSS. In contrast, we will also refer to a general liposome system of non-phospholipid vesicles that contain only retinol liquid in their core.

Synthesizing Skin Lipid Matrix and TSS: The Skin Lipid Matrix (SLM) is a high viscous compound consisting of 20% hydrogenated phosphatidyl choline, 20% caprylic (and) capric triglyceride, 10% propylene glycol, and 50% ceramide 3 and other ingredients. We mixed and heated all the ingredients together until they dissolved. Then we used high-pressure homogenizer to get the SLM. This matrix

[a] *High-pressure Homogenizer, Model M110F, Microfluidics, Newton, Massachusetts, USA*

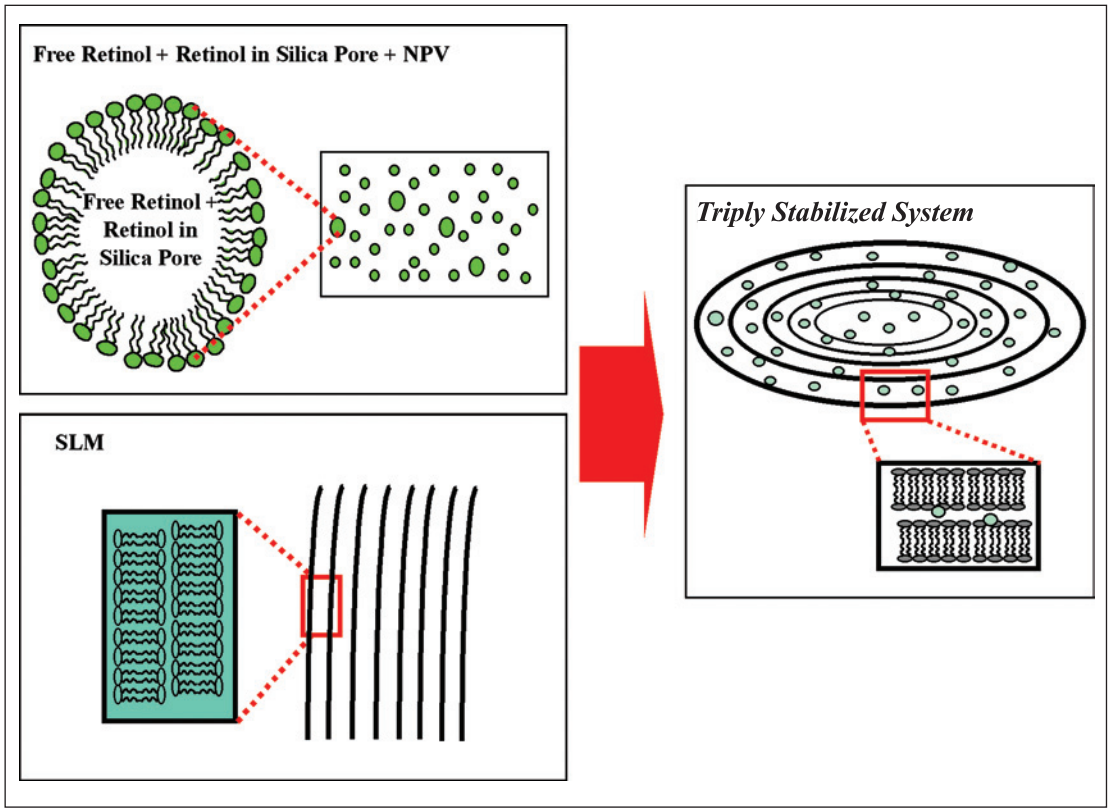

Figure 2. Schematic diagram of the Triply Stabilized System

Editor's Note: Some techniques suggested for improving the stability of retinol in cosmetic formulations

The Retinol 50 C used in the Triply Stabilized System from H&A PharmaChem is one of two retinol active ingredients available from BASF. In this note the editors report on some alternative approaches to stabilizing retinol in cosmetic formulations.

RetiSTAR Stabilized Retinol from BASF is an oily dispersion containing retinol, tocopherol and sodium ascorbate in caprylic/capric triglycerides. The ratio and absolute concentration of these three ingredients is reportedly well balanced to achieve maximum stability (at least 12 months) of retinol in cosmetic emulsions without the need for manufacturing or packaging under inert gas. (RetiSTAR is a registered trademark of BASF Aktiengesellschaft, Ludwigshafen, Germany.)

A Liu et al patent (US Pat 5,976,555) assigned to Johnson & Johnson from 1996 describes topical O/W emulsions containing retinol and other retinoids and possessing good physical and chemical stability. The compositions contain an emulsifier system, a co-emulsifier, an oil phase, and the retinoid. A stabilizing system may contain a chelating agent or antioxidants or both. The compositions are claimed to retain at least about 70% of the retinoids after 13 weeks' storage at 40°C.

A search through the archives of *Cosmetics & Toiletries* magazine disclosed the following recent articles touching on retinol and its stability:

- Don Orth et al, Stability and skin persistence of topical products, *Cosmet Toil* 113(10) 51-63 (1998)
- Song et al, Polyethoxylated retinamide as an anti-wrinkle agent, *Cosmet Toil* 114(6) 53-58 (1999)
- H Zi, Retinyl palmitate at 5% in a cream: Its stability, efficacy and effect, *Cosmet Toil* 114(3) 61-70 (1999)
- A Jentzsch, Defending against photoaging: A new perspective for retinol, *Cosmet Toil* 117(10) 58 (2002)

also contains membranes in the form of vesicles and multilamellar sheets and can be used as an emulsifier.

After obtaining the SLM, we slowly stirred the primary liposome and the SLM with the homogenizer to make the TSS. In other words, we combined primary liposomes (steps 1 and 2) and SLM (step 3) to get the TSS.

As with the SLN, the SLM also plays an important role in stability. So two materials, in this case, act simultaneously to give stability to retinol.

Materials and Measurement Equipment

Materials: In this experiment, we used retinol[b] and two liposomes[c] from commercial sources. This retinol – a viscous yellow oil that crystallizes at low temperatures – is approximately 50% solution of retinol in polysorbate 20, stabilized with BHT and BHA. Other materials were also from commercial sources. We used purified water that had been passed through an anion-cation exchange resin column. The TSS was prepared according to the steps described earlier.

Analysis equipment: We used transmission electron microscopy (TEM) and freeze-fracture scanning electron microscopy (FF-SEM) to observe liposome formation. The particle sizes of liposomes were measured by using a laser light scattering system[d] and the evaluation of color change was performed using a chromameter[e]. We performed quantitative analysis of retinol by using HPLC[f] under the following conditions: UV spectrophotometer (325 nm) detector; C18 column (3.9 X 150 mm); 1.0 ml/min flow rate; methanol:water (90:10) solution as a mobile phase.

Method

Color stability against light and heat: The color stability after one month was measured for each liposome, for the general liposome and the TSS, under storage conditions of 25°C without light, 40°C under normal lighting conditions, and at room temperature under normal lighting conditions.

Survivability of retinol: Under the conditions of 25°C without light, 40°C and light exposure for one month, we carried out quantitative analysis on variation of retinol content.

Skin penetration effect: We prepared two different types of creams; one contained 2% of general liposome and the other contained 2% of the TSS. After applying the creams to the skin of a hairless mouse, we examined the retinol penetration into the skin after 12 hours. The experiment was carried out in Frantz-type diffusion cells. Also HPLC was used for quantitative analysis of extracted retinol.

Results and Discussion

Identifying formation of liposomes: We confirmed the primary liposome, SLM and TSS by using TEM and FF-SEM. As shown in Figure 3, primary liposomes had a spherical shape, as expected. SLM had multi-lamella sheets. And the TSS had a mixture of primary liposome and SLM.

Particle size distribution: A laser light scattering system was used to measure particle size distribution. The sizes of primary liposomes ranged from 20 through 150 nm, and the mean size was 70 nm (Figure 4). Particles distributed in the TSS were 20 through 1,000 nm and the mean size was 210 nm. Broad distribution of TSS is attributed to mixing of primary liposomes and SLM.

Color stability against light and heat: In the case of exposure to light, the TSS was approximately twice as stable as the general liposome (Figure 5). For 40°C and 25°C without light, it was more stable by factors of 1.5 and almost 3, respectively. Considered as a whole, the TSS was more stable compared to the general liposome. We believe the reason for stability in our system is that retinol is triply stabilized; that is, it is stabilized in porous silica, NPV and SLM.

Survivability of retinol: Figure 6a shows the content change under light exposure. In the general liposome system, after 30 days, retinol content remaining was about 35%. In our system, its content was about 80%. Under storage at 40°C (Figure 6b), retinol content in the general liposome system was decreased to about 55% and about

(Cont. on Pg. 69)

The Sol-Gel Method

The sol-gel method – a simple and cheap process – involves the phase transformation of colloidal suspension (sol) into a continuous liquid phase (gel) by gelation of the sol to form a network and this gel is going to be densified by a thermal annealing. The precursors for preparing these suspensions consist of a metal or metalloid elements surrounded by various reactive ligands. Metal alkoxides are mainly used because they easily ;react with water. The preferred metal alkoxides are alkoxysilanes such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS). Three reactions are generally used to describe the sol-gel method: hydrolysis, alcohol condensation and water condensation.

[b] *Retinol 50C (1.58 Million I.U./g), BASF, Ludwigshafen, Germany.*
[c] *Lipoid S 100-3 and Lipoid S 75-3, Lipoid GmbH, Ludwigshafen, Germany*
[d] *Zetasizer 3000H, Malvern Instruments Ltd, Malvern, Worcestershire, UK*
[e] *Color JS 555, Color Techno System Corporation, Tokyo, Japan*
[f] *Model 510, Waters Corporation, Milford, Massachusetts, USA*

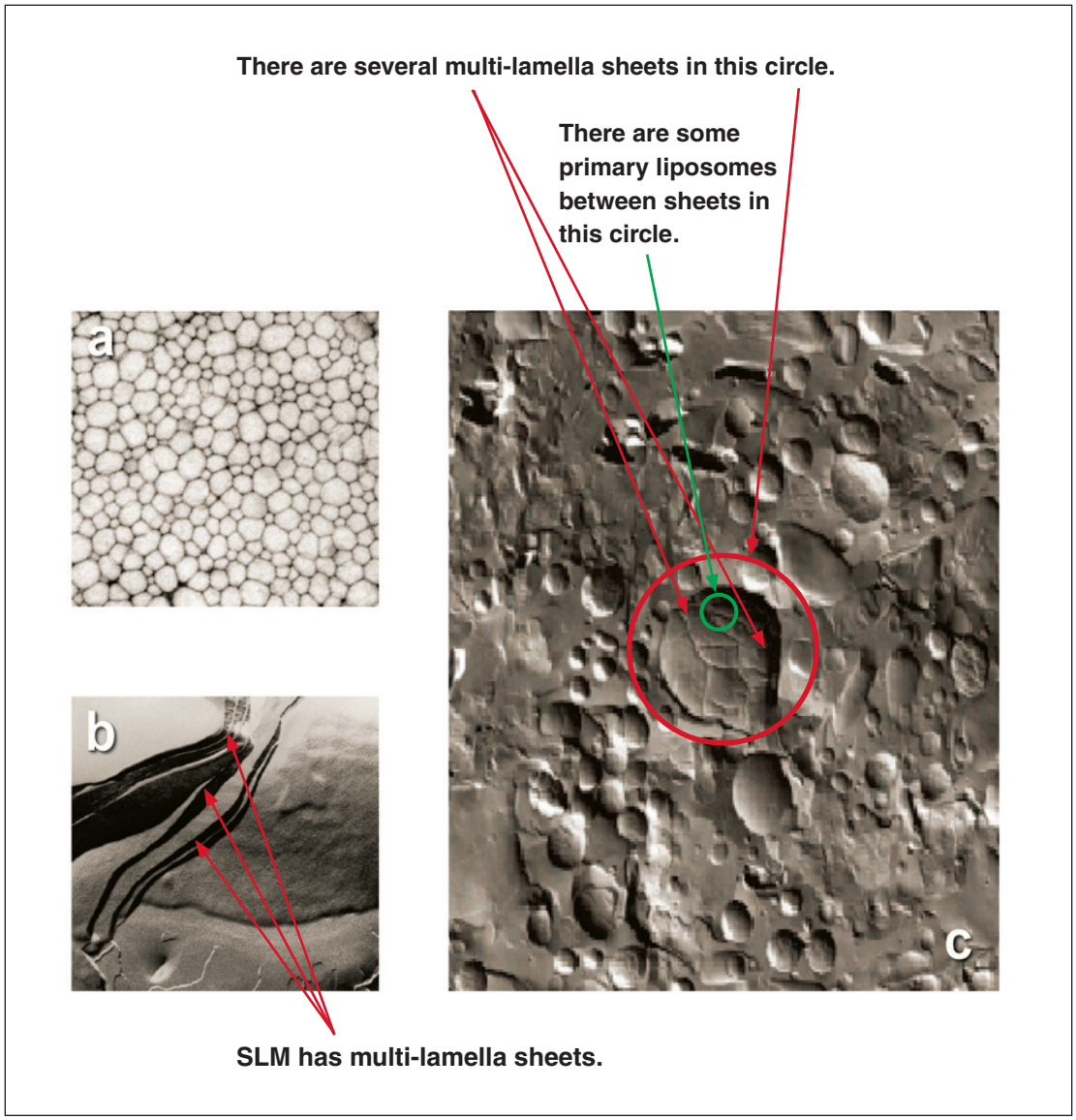

Figure 3. Images of stages of the Triply Stabilized System
a = TEM image of primary liposomes
b = FF-SEM image of SLM showing multi-lamella sheets
c = FF-SEM image of the TSS showing some primary liposomes between sheets

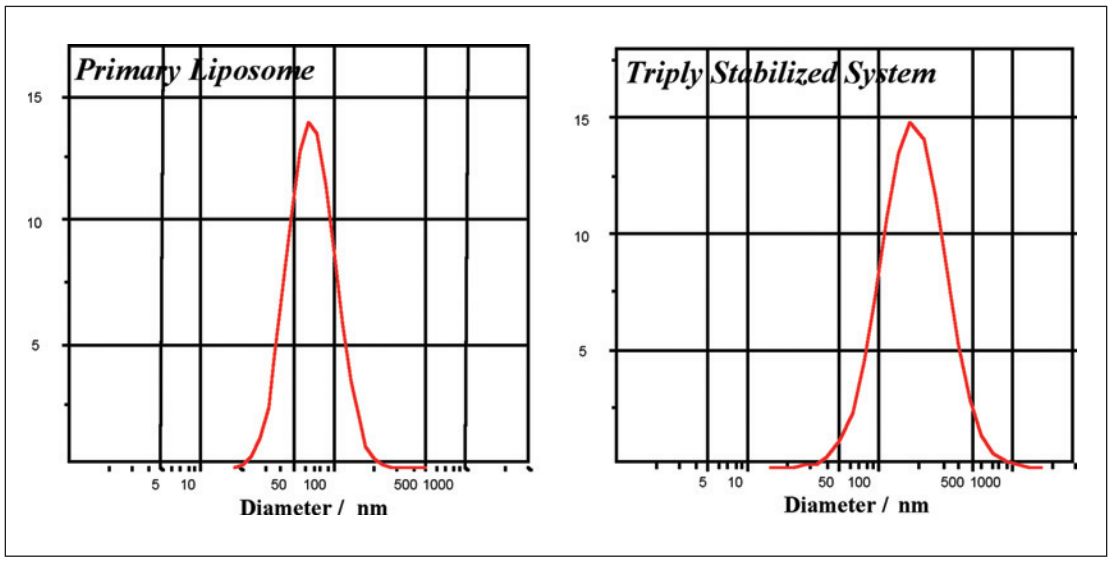

Figure 4. Particle size distribution

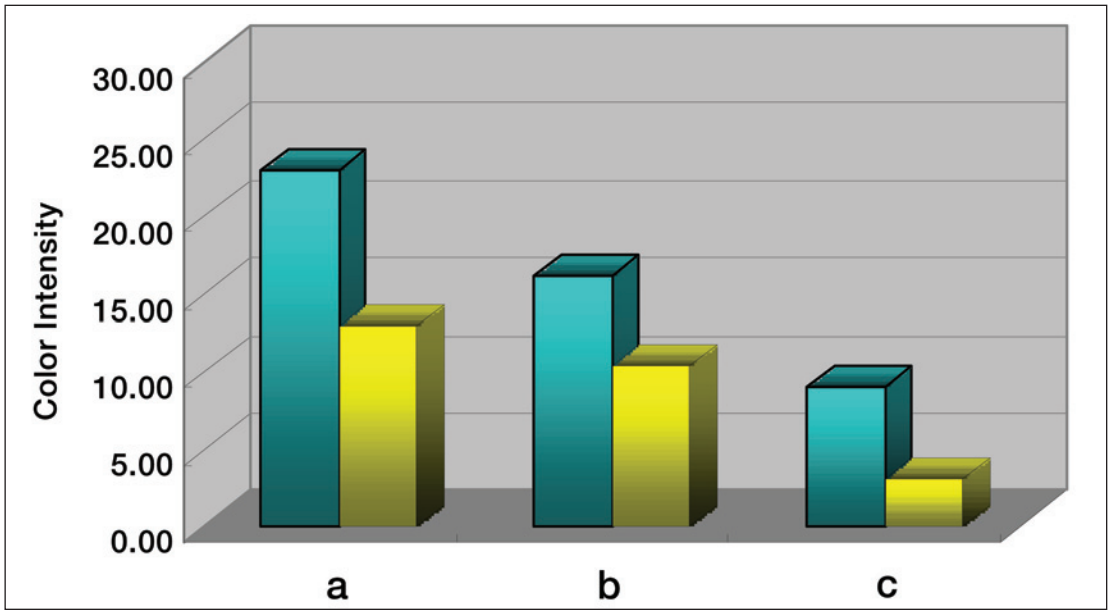

Figure 5. Color stability against light and heat for general liposome (blue) and TSS (yellow)
a = Normal lighting and room temperature
b = Normal lighting and 40°C
c = No light and 25°C

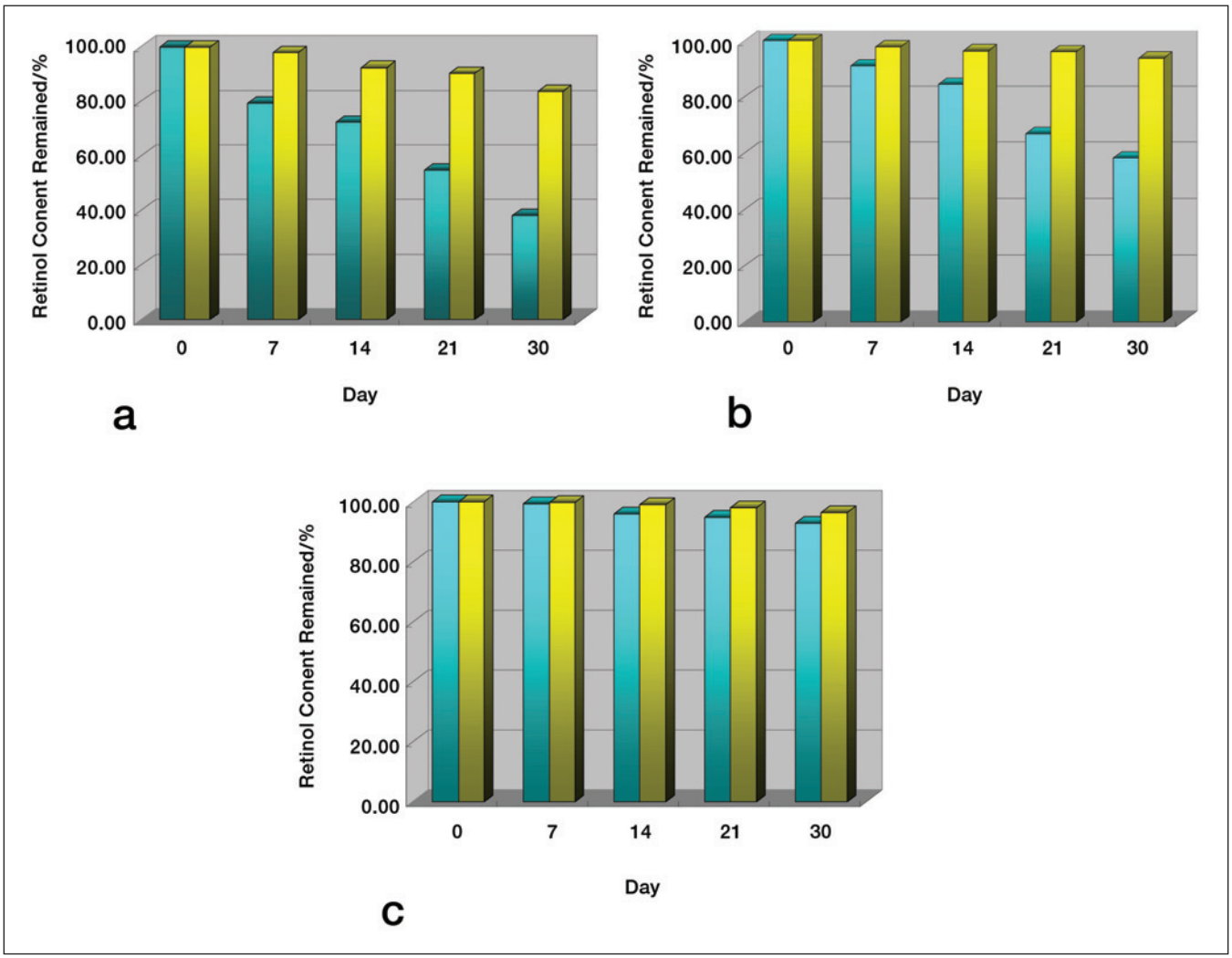

Figure 6. Change in retinol content at Day 0, 7, 14, 21, and 30 for general liposome (blue) and TSS (yellow)
a = Normal lighting and room temperature
b = Normal lighting and 40°C
c = No light and 25°C

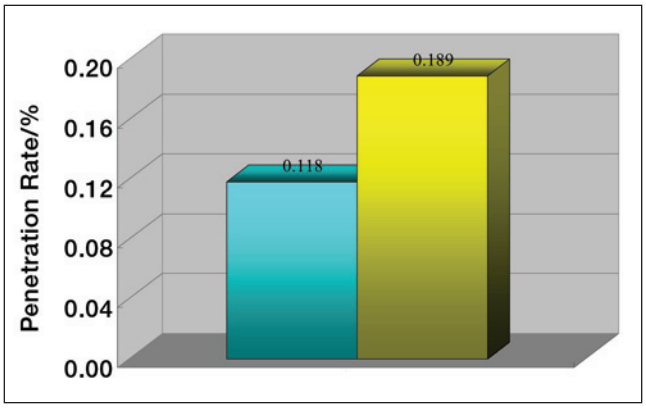

Figure 7. Skin penetration rate (%) of general liposome (blue) (0.118%) and TSS (yellow) (0.189%)

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90% in the TSS. When stored at 25°C without light exposure (Figure 6c), the general liposome system retained about 90% of its retinol, while the TSS stored similarly retained about 95% of its retinol. The enhanced survivability is again attributed to the triple stabilizing actions of the TSS.

Skin penetration effect: We prepared two different types of cream containing 2% of general liposome and 2% of TSS. We had already determined the initial concentration of retinol by HPLC analysis.

We applied the creams onto the skin of a hairless mouse. The retinol that had penetrated into the skin was extracted after 12 hours. Then we calculated the penetration ratio in percent as [(the concentration of retinol penetrated into the skin after 12 hours) / (the initial concentration of retinol)] x 100.

Figure 7 shows that the amount of retinol that penetrated the mouse skin after 12 hours was 60% greater from TSS than from the general liposome. If we consider only the particle size of the liposome, and if we assume the general liposome is the same size as the primary liposome because they both have the same composition except for the presence of porous silica, we would expect the general liposome to have a better penetration rate than TSS (which has a larger particle size). But we discovered that with TSS, the penetrating materials are actually primary liposomes, not the whole TSS, and these primary liposomes are similar to general liposomes in size. That is, our SLM had multi-lamellar structure. When TSS is in contact with skin – especially the stratum corneum – only the primary liposomes in the outermost layer of TSS are released and pass into the skin.

Because the lamellar layers of SLM are composed of ingredients that are similar to skin components, they have good skin affinity. Therefore, layers will be fused with skin. Primary liposomes

in a new outermost layer are released and pass through. This process repeats many times. So unlike the retinol in a general liposome that has only one or just a few bylayers, retinol in the TSS is not released all at once. That means retinol is slowly and steadily released from the outermost lamellar layer to the inmost lamella. Therefore, penetration efficiency is enhanced due to SLM. This fact is confirmed by Figure 7, but we think we need more data (such as retinol concentration penetrated into the skin vs. time) to more fully explain this phenomenon.

Conclusions

The skin adsorption and bio-availability of retinol can be improved by putting the retinol in a general liposome (a non-phospholipid vesicle), but the general liposome is relatively unstable.[12] In order to improve its stability, we used TSS, a system that triply encapsulated the retinol. TSS was prepared by combining primary liposomes (a non-phospholipid vesicle containing both free retinol and retinol adsorbed in the pores of silica) and a matrix of skin lipids. Primary liposome was 20 through 150 nm in size; its mean diameter was 70 nm. In the TSS, particle size was 20 through 1,000 nm; mean diameter was 210 nm.

Our system was more stable than general liposomes. According to chromameter data, the color stability of the TSS was 1.5 to 3 times greater than the color stability of general liposome systems. We also confirmed through HPLC analysis that retinol in our system was more long lasting. The TSS also improved skin penetration of retinol. Finally, The TSS delivers these benefits at low cost (25-80% cheaper than other retinol products commercially available in the Korean market) and with a high content (approximately 4.5%) of pure retinol.

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