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# Long-term Retinol Decomposition Profile and Emulsion Chassis & Package Impact to Destabilization

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abstract

Objective: Retinol (vitamin A) is known as safe and best anti-aging functional ingredient for cosmetic application. However retinol is unstable under environmental condition – temperature, light, components and aged time etc. Therefore, there have been many efforts to stabilize retinol against oxidation by applying the liposomes utilizing many kinds of applicable ingredients. However, most of the studies were limited to a period within 1-3 months with some typical anti-oxidants and there was rarely a study conducted over 12 months, although cosmetics were often used much longer. In this study we chose triply encapsulated retinol with polycaprolactone, lecithin and silica, and applied to 5 bio-mimetic cosmetic oil-in-water emulsions to find solutions against the decomposition of retinol.

## Introduction

Retinol (vitamin A) is one of most effective components in skincare application and essential nutrients in body and promoting differentiation of skin cells. It is also one of the most bioactive forms for the retinoids when applied to the skin and stimulates biosynthesis of collagen and elastin, reducing wrinkles and increasing the skin elasticity. Therefore retinol has been intensively investigated to study the physiology and function on skin by many authors. Retinol stored on skin was converted to all-trans retinaldehyde by retinol dehydrogenase, and all-trans retinaldehyde was again converted to the biologically active form of all-trans retinoic acid which binded to RAR receptor by retinal dehydrogenase through 2 step metabolism [1, 2]. And this metabolized retinol acted as one of major anti-aging controllers by inhibiting the expression of matrix metalloproteinase in the process of photo-damaging and chronological aging [3]. And it also appeared, that retinol thickened the epidermis by proliferating keratinocyte via stimulating c-Jun transcription factor and also contributed to improving the dermis layer by proliferating extracellular matrix primarily consisting of collagen, elastin and fibroblast via βTGF/CTGF pathways [4]. Trans retinoic acid enhanced the growth response of epidermal keratinocytes to epidermal growth factor and β-transforming growth factor [5]. And according to *Voorhees et al* [6], 0.25 % retinol showed almost the equal efficacy to 0.025 % retinoic acid and to 0.6 % retinyl palmitate which was one of the retinol derivatives. (**Fig. 1**)

In addition, retinol has many other functions on skin by maintaining the bright skin through preventing skin darkening or roughness. Despite retinol's excellent effects of wrinkle improvement, it is easily oxidized and decomposed by air, oxygen, moisture, thermal heat and light, which makes it difficult to apply it in cosmetics causing its potentials such as skin irritation when decomposed in some cases [7].

Retinol has double bonds in 4 sites of its alkyl chains in structure. Therefore it is easily decomposed by external attacks causing it not to properly work as an efficacious component to skin and body. Therefore many investigations have been published on what affects the stability and how to enhance the stabilization further. According to these publications, temperature, the ratio of oil to water content in emulsion, pH, air and oxygen have been key factors to induce the decomposition faster [8]. Low temperature was desired, and high oil content was also preferred. A peroxide level in surfactants consisting of key ingredients for emulsion needed to be avoided for retinol formulation. There are some other experimental reports on chemical stabilizers as well. BHT (butylated hydroxyl toluene) and BHA (butylated hydroxyl anisole) close to 0.1 % appeared to equally effective anti-oxidant to prevent an oxidation of double bonds in retinol (vit. A), and

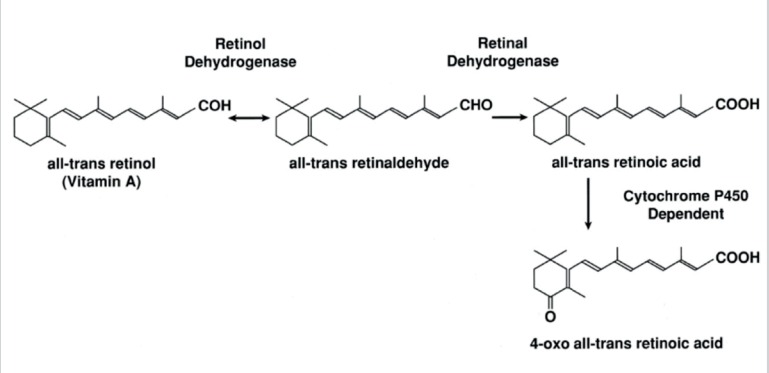



Fig. 1

BMDBM(butyl methoxy dibenzoylmethane) known as UV filter for cosmetics also demonstrated its good performances against oxidation of retinol [9, 10]. In addition, Chong-kook *Kim's at al* [11] investigated the stabilization of all-trans retinol for 72 hours by loading lipophilic anti-oxidants in solid lipid nano-particles (SLN) consisted of egg PC and Tween 80, and concluded that 77:33 ratio between egg PC and Tween 80 was the optimum to control the particle size in SLN, and BHT-BHA combined option worked better than other lipophilic anti-oxidants like tocopherol and vitamin C. Besides a study by stabilizing anti-oxidants, there are some efforts by applying new chassis like nano-lipid carriers, oil-in-water emulsion, water-in-oil emulsion or O/W/O (oil-in-water-in-oil) multiple emulsions. These studies demonstrated that higher viscous cream-type chassis stabilized the retinol better than lotion-type emulsion and polymer-based hydrogel [12,13,14] and retinol stability in nano-carriers relied on its ratio between surfactant and PC (phosphatidylcholine) which affected the membrane robustness of nano particles [15, 16, 17]. However, all of these studies were conducted during a limited period of 2-3 days or a few weeks at the laboratory, using the retinol as pure ingredient. But the retinol usually applied for cosmetics have utilized the encapsulated ingredient in advance by several nano-lipid techniques such as microfluidizer and then added into cosmetic chassis to satisfy consumer's long-term-use regimen habit over 12M. Therefore we aimed to investigate the long-term stability profile of silica-coated triple encapsulated retinol in actual 5 cosmetic chassis and studied the decomposition impact by temperature, key structuring component and type of package.

## Materials and Methods

### Materials

Triple encapsulated nano-particle retinol with lecithin, polycaprolactone and silica was purchased from ACT Co LTD and this ingredient was kept in a hard stainless bottle against oxidation. Hydrogenated lecithin for study was purchased from SRM Co LTD, lipoid Co LTD and Nippon fine chemicals. These lecithins were chosen because their phosphatidylcholine content is more than 70 % to provide emulsifying performance and stabilization. As Ceramides were chosen Hydroxypropyl Bispalmitamide MEA from Macrocare Co LTD and palmitamide MEA from Nippon fine chemicals which were known to help forming the lamellar crystals in cosmetic emulsion, and cholesterol (Vegapure 95 FF) was purchased from BASF. As regards silica-coated nano-particle retinol, it was known as consisting of shell and core structure containing 3.5 % retinol (116,000 IU/g), and shell was mostly occupied with lecithin coated with silica, while core was aggregated with biocompatible polycaprolactone (patent KR 10-2011-0068855).

## Methods

### Preparation of Cosmetic Emulsion

Total 5 kinds of oil-in-water formulation batches (**Tab. 1**) were prepared to monitor the impact of each key element. LC-036 ~038 chassis were differentiated according to lecithin, cho-

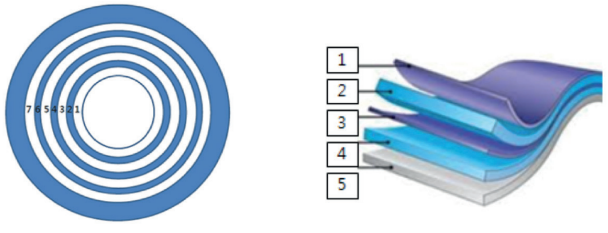
| Trade name | Ingredient | LC-036 | LC-037 | LC-038 | LC-039 | LC-040 |
|---|---|---|---|---|---|---|
| Soya SPL75 | Hydrognated lecithin | 0.5 | | | | |
| Emulmetik 950 | | | 0.5 | | | |
| Phytocompo PP | | | | 0.5 | | |
| PC-104 | Phyto-ceramide | 0.5 | | | | |
| PMEA | | | 0.5 | | | |
| Vegapure 95 FF | Phyto-cholesterol | 0.5 | 0.5 | 0.5 | | |
| Oilwax LC | Cetyl Palmitate, Sorbitan Palmitate, Sorbitan Olivate | 3 | 3 | 3 | | |
| Arlacel 165 | Glyceryl stearate (and) PEG-100 stearate | | | | 0.5 | 0.5 |
| Biophilic H | Hydrogenated Lecithin/C12-16 Alcohols/Palmitic Acid | | | | 1 | 1 |
| GMS105 | Glyceryl stearate | | | | 1 | 1 |
| Montanov 68 | Cetearyl Alcohol/Cetearyl Glucoside | | | | 2 | |
| - | Glycerin | 10 | 10 | 10 | 10 | 10 |
| | Cetearyl alcohol | 2.8 | 2.8 | 2.8 | 2 | |
| | Behenyl alcohol (70 %) | 1.4 | 1.4 | 1.4 | 1.4 | |
| | Medium chain triglyceride | 10 | 10 | 10 | 10 | 10 |
| | Retinol (3.5 %) | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| | preservative | 1 | 1 | 1 | 1 | 1 |
| | qs water | qs | qs | qs | Qs | qs |
| chassis | | C/O/W | C/O/W | C/O/W | O/W | hydrogel |

**Tab. 1** Formulation on cosmetic chassis

lesterol and ceramide, while LC-039 was typical oil-in-water emulsion consisted of fatty alcohols and LC-040 was a simple hydrogel emulsion without fatty alcohols. For LC-036~038, 3 different commercial materials were separately applied to see any potential difference in contribution. All lecithins applied were ones containing phosphatidylcholine (PC) over 70 % and hydrogenated types to assure the emulsifying performance and to avoid potential oxidation in oil-in-water emulsion during aging. The fabrication of emulsion utilized Primix homogenizer and all the formulations were made in a same manufacturing process by raising the temperature over 80 °C and cooled down to 40 °C and 30 °C. Homogenization was given per each step to help forming the homogeneous texture in emulsion. Silica-coated encapsulated retinol was added on 40 °C just before the emulsion formed the structuring by internal aggregation of fatty amphiphiles. And to assure the homogeneous distribution of retinol, the emulsion was continuously agitated till 30 °C room temperature and it was re-homogenized again just before draining out. And it was finally filled into AL-laminated multiple layer tube package.

## Package Component Impact to Retinol Stability

Retinol is easy to be oxidized when faced up to air, oxygen and light. Therefore to prevent these kinds of oxidation, the formulated batches were filled into specially designed multi-layer aluminum (AL)-laminated polyethylene(PE) tube reinforced to air-to-barrier performances when formulation was completed. To confirm the impact of type of components, 2 types of 5 and 7 layer AL-laminated PE tubes and 3 layer PE tube without AL-laminated were applied. 7 layer laminated package was consisted of LLDPE (linear low density polyethylene)/Copolymer adhesive/AL/Copolymer adhesive/PET/Copolymer adhesive/LLDPE with 500 µm thick, and 5 layer AL-laminated tube package was consisted of PET/Copolymer adhesive/AL/Copolymer adhesive/LLDPE with 400 µm thick, while 3 layer PE tube consisted of LLDPE/Copolymer adhesive/LLDPE with 420 µm thick. (**Fig. 2**)




**[7 layer AL-laminated tube]**
LLDPE/Copolymer adhesive/ AL/Copolymer adhesive/PET/ Copolymer adhesive/LLDPE

**[5 layer AL-laminated tube]**
PET/Copolymer adhesive/AL/ Copolymer adhesive/LLDPE

**Fig. 2** Package components to fill in bulk emulsion.

## Analysis by Polar Microscopic View

Polar microscope is a very useful tool to observe the birefringent structure in colloids. This method allows one to distinguish the refractive structure of emulsions and to determine the shape and size of their droplets as well as liquid crystal features. Each emulsion after fabrication at the lab was put into each conditioned room for designed testing and the specimen under 25c was pulled out for observation after 1M aged. The microscope was Carl Zeiss-made Axio Scope A1 model equipped with polar lens EC-Plan Neofluar 40 times as well as optical. The observation was primarily performed with 400 times magnification with high clarity camera Axio Cam MRc.

## Analysis by SAXS and WAXS

Transmission Small and wide angle x-ray scattering (TR-SAXS and WAXS) measurement was performed at the PLS-II 9A U-SAXS beamline of PAL. The X-rays coming from the in-vacuum undulator (IVU) were monochromated using a double crystal monochromator and focused both horizontally and vertically (FWHM 300 µm (H) x 30 (V) µm at sample position) using K-B type mirrors. Scattering data were recorded with a 2D CCD detector (Rayonix SX165, USA). The sample-to-detector distance is 6.5m and 0.2m for TR-SAXS and TR-WAXS, respectively.

## Retinol Concentration Assay

For analysis of retinol decomposition in each chassis, each specimen under each conditioned room was pulled out and 0.1g of specimen was solubilized into 10 ml water by applying sonication. After that, the solution was additionally diluted with 1:1 ratio of ethanol and isopropanol to 50 ml. This solution was mixed for 20 min and homogenized by sonication for 10mn. To prevent a bias from retinol distribution in filled tube in package, the first 5g was discarded before preparing the assaying specimen. The measurement was performed 3 times per each sample. For preparation of standard curve, 50,000IU (15mg) of standard retinol was first solubilized into 1:1 ratio of 100 ml ethanol and isopropanol solution, and 5ml of this solution was again diluted with 100 ml for 2,500 IU standard solution.

## HPLC Method Analysis

HPLC was carried out on Agilent HPLC 1290LC separations module (Agilent Corp.) equipped with UV detector. The HPLC method was applied conforming KFDA (Korea food & drug association) guideline except for initial sample preparation step because high viscous emulsion was not easily solubilized in the solvent. The column used Water symmetry RP C18 filled with silica. The detailed specification was 150 mm* 3.9 mm * 5 µm. The mobile phase was 90 % methanol and 10 % distilled water. The flow rate and injection volume was set to 1 ml/min and 10µL respectively under 40c and run time was 7 min. UV absorbance detection was set at 325nm.

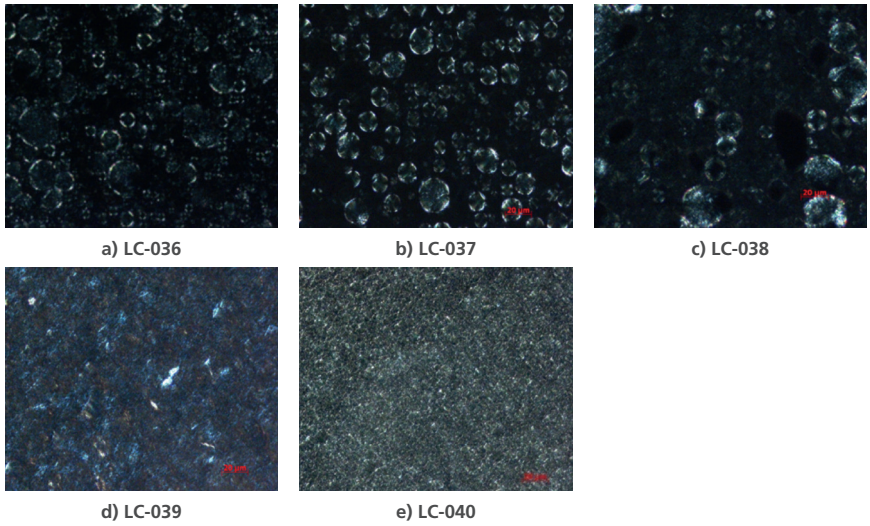



**Fig. 3** Polar microscopic observation on each cosmetic chassis. LC-036~038 exhibited the birefringent maltese cross views, while LC-039~040 did not. The homogeneity and distribution order of maltese crosses was LC-038 > LC-037 > LC-038.

The calibration curve was linear with r2=0.99 and in the concentration range of 50~5,000IU/L with 5 data point.

### Measures by UV Vis Absorption Spectrophotometer

UV Visible spectrophotometer was applied to check the absorption profile to retinol from 200nm to 500nm. The model was Optizen POP made by Mecasys Co LTD. The cell utilized the precision type (Hellma, Germany) made of quartz Suprasil with 10 ml content. The surface mode was applied on 2 nm reading.

### Statistical Analysis

Statistical analysis was carried out using single factor one-way ANOVA. All experiments were performed in triplicate as much as possible to prevent any variation and to determine significant difference. A 0.05 level of probability ($P < 0.05$) was taken as the level of significance.

## Results and Discussion

### Microscopic View Analysis

5 emulsion chassis were largely distinguished with 3 different structures. LC-036 and LC-037 two chassis containing lecithin, ceramide and cholesterol exhibited small and big birefringent lamellar crystals with about 10-20 µm particles. And LC-038 containing both lecithin and cholesterol also represented big lamellar crystals, but it was represented like deformed chunks and the distribution was much more inhomogeneous than LC-036 and LC-037. LC-039 without lecithin, ceramide and cholesterol but containing fatty amphiphiles provided some random crystals on top of waxy structure and never showed any birefringent crystals. And LC-040 without lecithin, ceramide, cholesterol and fatty amphiphiles just showed typical polymer hydrogel structure without any crystal features. (**Fig. 3**)

### SAXS & WAXS Analysis

X-ray scattering technique was applied to investigate the polymorphic changes by applying silica-coated retinol, lecithin, ceramide and cholesterol. The molecules comprising of long chain fatty compound such as long chain hydrocarbon of fatty acid have been known to possess polymorphism [18]. And many other investigators have applied this technique to understand the drug release mechanism as well [19, 20]. In SAXS analysis, d-spacing distances were observed from 14.48 nm to 34nm in the broad range according to formulation. Specifically LC-036~038 containing lecithin represented longer d-spacing from 24 nm, 30 nm, 34nm than LC-039~040 not containing lecithin for which they just showed 14nm and 22nm, indicating that lecithin in emulsion induced forming the lamellar structure and the bigger distance in layer-by-layer. This bigger polymorphic structural change contributed in re-encapsulating the silica-coated encapsulated retinol and in stabilizing to result in better stability for these emulsion chassis. And In WAXS analysis, LC-036~LC-038 exhibited the typical two peaks at 0.41 nm and 0.37 nm, indicating that these chassis consisted of orthorhombic phases. On the contrary, LC-039~040 chassis provided one peak at 0.41 nm which was typical of hexagonal liquid phase for emulsion. To summarize, it could be deduced that the polymorphism induced by lecithin influenced the stability of silica-coated retinol in the lamellar crystal chassis by re-encapsulating the retinol into layer-by-layer. (**Fig. 4**)

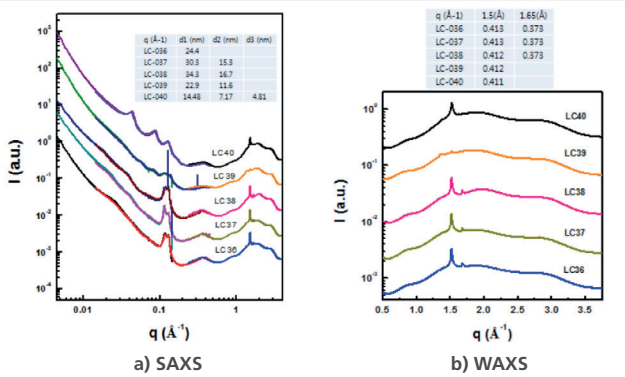



**Fig. 4** Small and wide angle x-ray scattering technique revealed d-spacing in lamellar alkyl hydrocarbon chains got bigger and polymorphic transition from hexagonal to orthorhombic when lecithin was applied. This transition in structure stabilized the retinol against decomposition.

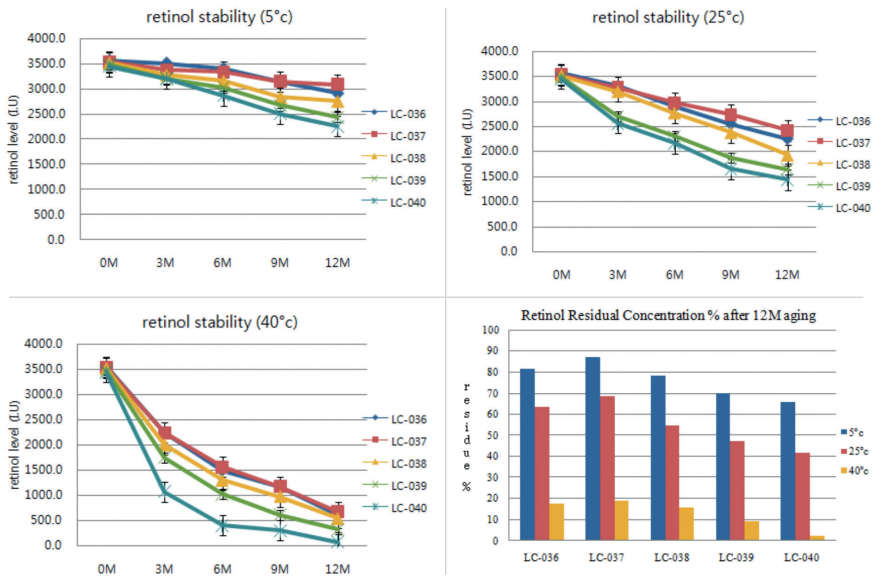



**Fig. 5** HPLC technique to measure remaining concentration of retinol up to 12M. Retinol was most stable at 5 °C and showed significant decrease under higher temperature(40 °C). In this design, silica-coated triple encapsulated retinol revealed that 20 %, 40 % and 80 % of retinol was decomposed up to 12M at each temperature condition (5 °C, 25 °C, 40 °C)

## Retinol Stability Profile under UV-visible Spectrophotometer

UV-Vis spectrophotometer was just applied to simply compare the degradation profile among chassis. 0.1 g of each specimen on 25 °C and 40 °C was mixed with 10 ml water and vigorously sonicated to get homogeneous phase. And 5 ml of solution was diluted with 5 ml water 2 times to obtain the clear transparency for light transmittance. In this experiment, the measuring wavelength was set on from 200 nm to 500 nm to check the detecting peak profiles throughout the ranges including at 325 nm. The result showed the similar order of tendency with liquid chromatography as expected. LC-036 and LC-037 containing lecithin, ceramide and cholesterol structured with lamellar crystals showed the highest absorption at 325nm, while non-lamellar crystal structure chassis (LC-039, LC-040) exhibited significant lower absorption. And LC-038 consisting of lecithin and cholesterol and not containing ceramide also provided almost equal absorption with LC-036 and LC-037. Comparing the absorption peak at 25 °C with 40 °C, peak at 25 °C exhibited a high and clear and solid absorption, while peaks at 40 °C were not clear and curved out, indicating that retinol was almost decomposed. (**Fig. 6**)

## Decomposition Profile of Encapsulated Retinol under Different Temperatures

The encapsulated retinol degradation in multiple chassis was assayed per 3 months till 12 months in 5 °C, 25 °C and 40 °C. Under 5 °C the retinol appeared to be significantly more stable than at 25 °C and 40 °C, exhibiting that about 80 % of encapsulated retinol in concentration retained up to 12 months versus original content. However when retinol was exposed to 25 °C and especially to 40 °C, the decomposition happened significantly faster and it just remained close to 55 % and 10 % respectively at 12 months. When analyzed per each chassis, LC-036 and LC-037 containing lecithin and ceramide and cholesterol showed much better stability with significance on each condition, implicating that lamellar crystal structures consisting of lecithin and ceramide contributed to enhancing the stabilization of retinol. And LC-038 just containing lecithin and cholesterol and not containing ceramide also provided better stabilization of retinol with slow decomposition compared with LC-039 and LC-040. LC-040 which do not contain both lecithin, ceramide, cholesterol and any fatty amphiphiles represented not stabilizing the retinol effectively due to its simple hydrogel structure, resulting in about 30 % remaining retinol in concentration under 25 °C at 12 months and 2.3 % under 40 °C at 12 months. (**Fig. 5**)

## Comparison of Retinol Stability Plot between LC and UV-visible Spectrophotometer

To compare the retinol decomposition profile between liquid chromatography with UV-Vis. spectrophotometer, both results measured by each equipment were plotted. Overall tendency of decomposition represented similar profile up to

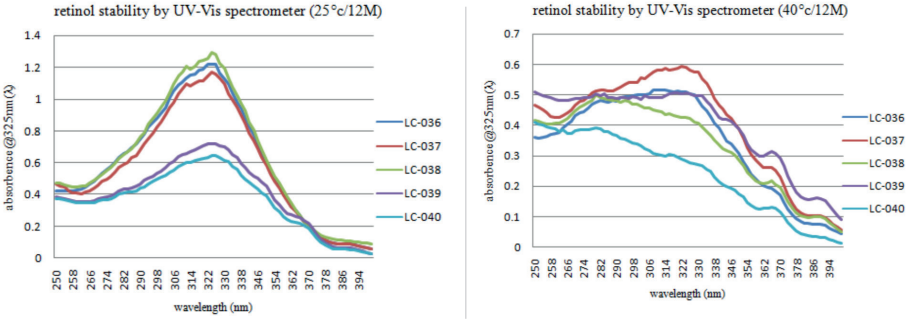



**Fig. 6** UV-Vis spectrophotometric technique to observe the degradation of retinol at 12M. It also represented the similar tendency in degrading order as with HPLC, that lamellar crystal emulsion with lecithin provided better stability.

20 months. Both measurement tools were identified to provide the meaningful accuracy against retinol decomposition. Liquid chromatography was useful to gaining quantitative figures in concentration with high accuracy, while UV-Vis. spectrophotometer was useful for qualitative measure for initial screening purposes in the wide range. (**Fig. 7**)

### Decomposition Profile of Encapsulated Retinol per Package Component

Since retinol is vulnerable to air, oxygen and light, it is apt to be decomposed when exposed. Therefore to protect the retinol decomposition, the finished bulk emulsion is recommended to be filled into specially designed high performances of barrier packages for cosmetic application. To investigate the decomposition profile by package component, three kinds of barrier-reinforced packages with 3, 5 and 7 layer PE and AL-laminated tubes were applied. When measured up to 9 months under 25 °C, 7 layer AL-laminated tube showed the most excellent high performance to barrier against retinol decomposition compared with 5 layer AL tube and 3 layer PE tube. In case of 3 layer PE tube, it showed significant decomposition versus AL-laminated tubes, implicating that package design would be one of most considerable levers in designing retinol stabilization. (**Fig. 8**)

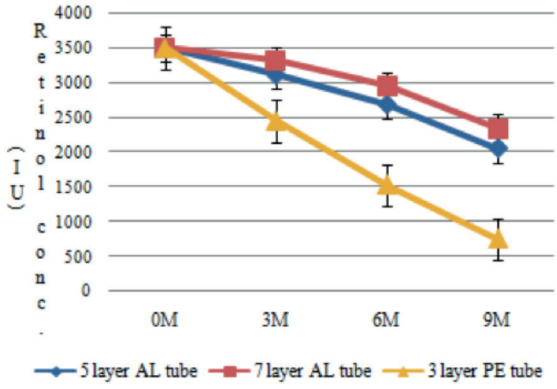



**Fig. 8** Retinol decomposition by package component. 7 layer AL-laminated PE tube demonstrated the most stable performance.

## Discussion

Decomposition profile of silica-coated triple encapsulated retinol in 5 specially designed cosmetic chassis was investigated according to thermal temperature, chassis structure and package component. As observed in microscopy, the cosmetic chassis containing the lecithin, ceramide and cholesterol (LC-036~037) provided the birefringent orthorhombic lamellar crystal features, resulting in better long-term stability of retinol in cosmetic chassis. Since silica-coated triple encapsulated retinol was directly added into lecithin-based lamellar crystal chassis, it was penetrated and stabilized more in the lamellar layers, resulting in enlarged d-spacing in SAXS measurement. These robust structures in emulsion demonstrated to enhance the stabilization of retinol 15~20 % better than other no lamellar layered structures, e.g. hydrogel and simple oil-in-water emulsion.

Some of studies [8, 9] investigated the stabilization of retinol in emulsion and NLC structures, but these all applied pure ingredient of retinol into emulsion or into NLC even for the short period. According to these results, retinol was decomposed about 20 % in 10 days at 25c, 80 % in 10days at 50c and 90 % in 10days at 80c [8]. Compared with these results, silica-coated triple encapsulated retinol in lamellar crystal chassis exhibited 40 % decomposed in 12M at 25 °C and 85 % decomposed in 12M at 40 °C, indicating that multiple encapsulation of retinol significantly contributed in enhancing the stability.

The shelf life of cosmetics is generally longer than 12M and the retinol needs to be maintained without decomposition during a use for both efficacy and against skin potential irritation. However to my knowledge there are few reports published on long-term stability of retinol in final finished basis considering the shelf life over 12M. This study revealed that the stability of retinol was significantly affected by temperature even in the AL-laminated barrier-re-inforced package and represented remarkable decrease under 40 °C versus 5 °C and 25 °C, and remained just 10~20 % under 40 °C and 50~60 % under 25 °C when passed 12M, indicating an importance of temperature control to retinol-based emulsion.

UV-Vis spectrophotometer was applied to check the degree of correlation with liquid chromatography and applicability to monitor the status of retinol decomposition. This tool showed the consistent decomposition profiles with

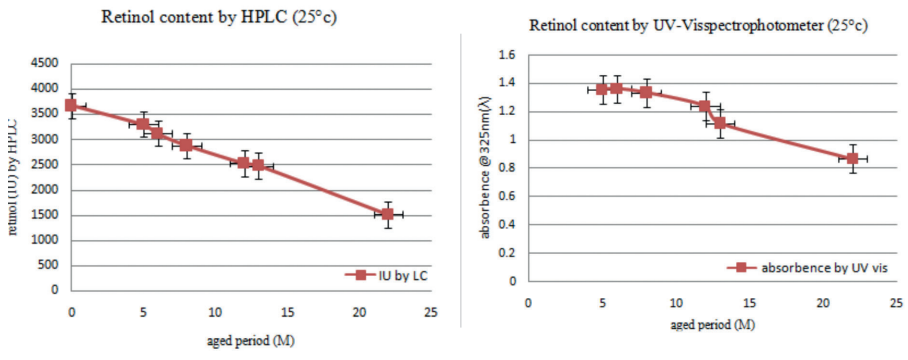



**Fig. 7** Plotting the long-term decomposition of retinol measured by HPLC and UV-Vis spectrophotometer. Silica-coated triple encapsulated retinol exhibited the gradual degradation by time.

LC and demonstrated usefulness as initial screening and fast confirmation technique.
Different component type of packaging was applied to investigate the effects of stabilizing retinol. 7 layer AL-laminated PE tube represented the most stable barrier performances compared with AL-laminated 5 layer and no AL PE tube, demonstrating that AL-lamination greatly contributed in prolonging the decomposition.

## Summary

To summarize, we could identify 12M long-term decomposition profile of silica-coated multiple encapsulated retinol to different temperature conditions for the unique design of cosmetic chassis. Decomposition of retinol was represented to be largely dependent on temperature, chassis structures and packaging component. Future works will follow to define and optimize these factors and continue to find key variables to stabilize the retinol more for cosmetic application.

### Acknowledgements
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