# The Stability of Double-Capsulated Retinol in O/W Emulsion

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## Abstract

Using the *all-trans-retinol* which is double-capsulated with matrix (MDC®), we investigated its stability and the change of the epidermal thickness. The proprietary MDC® comprise two steps of capsulation of retinol, i.e., primary microcapsulation with collagen and then secondary capsulation with gellan gum.

We compared the activity of *all-trans-retinol* in various forms such as (1) simply in O/W, (2) in W/O emulsion, (3) in primary capsulated form in O/W emulsion, or (4)in MDC® in O/W emulsion. After storage at 45℃ for 4 weeks, retinol in MDC® in O/W emulsion retained 92% of the activity compared to the standard material upon HPLC analysis, whereas the primary capsule gave 70%, the O/W emulsion form 47% and the W/O emulsion 78%. The retinol in MDC® in O/W induced the siginificant increase in epidermal thickness compared to the vehicle.

# Introduction

Retinoid, such as retinol, retinoic acid, retin aldehyde, and their erivatives, are used as an active ingredient in cosmetics. Among the etinoid, which are suppoded to induce thickening of the epidermis and ıin the stratum coreum, retinol is reguarded as desirable. Also, retinol ; an endogeneous compound that is generated naturally in the body, etinol takes part in the epidermal growth, differentiation and eproduction. Retinol is superior to the retinoic acid in safety, because .n excessive of quantity of retinol is stored in the inactive ester form, nostly retinyl palmitate, some retinyl acetate, in the body.

The O/W emulsion has many advantages, for exampls, non-occlusive, ıon-greasy, good compatability with other products, excellent washing ›roperty from the skin, good feeling used, economical in manufacture.

We take note of these aspects, we study the method that improve he stability and safety of retinol in O/W emulsion by the MDC® double capsulated matrix) technique using the collagen and gellan ;um. *all-trans-retinol* is used in this study, which has a higher ›hysiological activity than other isomers. We compared the activity of *ıll-trans retinol* in various forms such as (1) simply in O/W, (2) in *N*/O emulsion, (3) in primary-capsulated form in O/W emulsion , or 4) in MDC® in O/W emulsion by means of reverse-phase ıigh-performance liquid chromatography (HPLC).

# Materials and methods

## Test samples

(1) simply in O/W, (2) in W/O emulsion, (3) in primary-capsulated form in O/W emulsion , or (4) in MDC® in O/W emulsion, each samples contains 2500IU *all-trans-retinol*.

## Materials

*all-trans-retinol* (315,000IU/g) was purchased from BASF in German. Retinol was stored in a small, opaque vessel under liquid nitrogen.

Gellan gum from Kelco Co. in U.S.A.

## Instruments

Reverse-phase high-performance liquid chromatography (HPLC) grade solvents were used for extractions and chromatography. HPLC was carried out under the conditions;

Column: CAPSEU PAK(C18) Shiseido, type-UG 120Å, 5μ, size-4.6mmΦ *250mm

Detectior: Spectoflow 757 (ABI Analytical Kratos Division)

Flow rate:0.8ml/min

Oven Temp: 25℃

Mobible phase: Methanol(99.9%, Prolabo)

Operating pressure(*1000psi): 1.8

## First microcapsulation

Materials 1~9 were mixed in beaker. At this time, pH of the mixture was controlled about 3.5 by material 2. Later, the mixture were neutralized with the material 10. After neutralizing completely, when this mixture were stood, the capsules were floated. The obtained

capsules were washed 2~3 times with distilled water, and then added to the base which were prepared by adding materials 12~14 to material 11.

## Preparation the MDC®

Material 2 was added to material 1 and then heated up to 70 ℃. After dissolving, the mixture was cooled to room temperature. Then, after adding material 3 to the mixture, obtained mixture was added to material 4 and mixed properly. This mixture was added to the solution which were prepared by adding material 5 to material 6, and stirred at about 100 rpm for 10~30 minutes. After stirring, the matrix-double capsule was obtained by washing the mixture with distilled water.

First microcapsulation

| Materials | Content(g) |
|---|---|
| 1. Distilled water | 61.5 |
| 2. Acid(Citric acid) | q.s. |
| 3. Collagen | 0.5 |
| 4. Glycosaminoglycan | 0.5 |
| 5. BHT | 0.1 |
| 6. Tocopherol | 0.1 |
| 7. Mineral oil | 12.0 |
| 8. Soya oil | 25.0 |
| 9. Vitamin A (retinol) | 0.28 |
| 10. Potassium hydroxide | q.s. |
| 11. Distilled water | 50.0 |
| 12. Xanthin gum | 0.5 |
| 13. Butylene glycol | 15.0 |
| 14. Preservative | q.s. |

Preparation the MDC®

| Materials | Content(g) |
|---|---|
| 1. Distilled water | 19.8 |
| 2. Gelan gum | 0.2 |
| 3. Capsule of prepa ration 1 | 25.0 |
| 4. Liquid paraffin | 20.0 |
| 5. $CaCl_2$ | 0.2 |
| 6. Distilled water | to 350 |

## Framing the standard curve

Weigh exactly the 100mg of pure *all-trans-retinol* (standard material),

and fill the 100ml volumetric flask with absolute alcohol. mingle absolutly for 30 min with the magnetic stirrer. In this time, use the brown-colored bottles for preventing the retinol from decomposition from UV, and carry out the experiment in hood filled with innert gas, argon. Each sample in the volumetric flask is taken by 1ml, 2ml, 3ml, 4ml, and add the absolute ethanol to 50ml. put the prepared samples into the cell for the HPLC, measure the absorbance of the retinol in 325nm. repeat the same experiment under the same condition, method. After checking the same result, fram the standard curve using the arithmetic average.

## Extraction of Retinol

Each sample, emulsion1; simply in O/W, emulsion2; in W/O emulsion, emulsion3; in primary-capsulated form in O/W emulsion, emulsion4 in MDC® in O/W emulsion, is prepared by table 1. All of them are stored at 45℃ for 4 weeks. 3g of the samples are weighed accurately. Especially, emulsion 3, emulsion4 need the freeze-drying treatment for absolute extraction from the capsule. So, all the samples are positioned on a liquid nitrogen precooled stage (<-180℃) in a modified evaporator (Polaron model 6000) equipped for high vacuum freeze-drying at -40℃. After a high vacuum was established ($<10^{-4}$Pa), a liquid nitrogen cooled cold trap was placed over the sample holder, after freeze-drying (more than 16 hours) overnight, the sample holder was heated slowly (10℃/15min) to 0℃, and allowed to reach room temperature until the next morning. After breaking the vacuum of the freeze-dryer, absolute alcohol is added into the samples until becoming the volume of 25ml. These samples are stirred with magnetic stirrer

for 15 hours overnight. Then, in order to prevent the decomposition of retinol from UV, we should use the pure yelloow-colored flourescent light, brown-colored bottles, and glove box. The possibility of bringing the retinol into contact with the oxygen is negated by using argon gas blanket for preparation, conservation, measurement. Filtered out throuh 0.45μm membrane filter, put into the tube for HPLC measurement of the absorbance.

## The Experiment of Changing the Epidermal Thickness

**Test subjects:** 7-8 week old female albino hairless mouse were obtained from Charles River Laboratories.

**Test method:** Test samples, stored at 45℃ for 4 weeks, were applied once a day for 3 weeks, store them in the dark room. Skin samples processed for hematoxylin and eosin were read at light microscopy level.

# Result

- We compared the activity of *all-trans-retinol* in various forms after storage at 45℃ for 4 weeks, Emulsion4 showed siginificantly high percentage of *all-trans-retinol* after storing them at 45℃ for 4 weeks. Retinol in MDC® in O/W emulsion retained 92% of the activity upon HPLC analysis, whereas the primary capsule gave 70%, the O/W emulsion form 47% and the W/O emulsion 78%.

- Emulsion4, in MDC® in O/W emulsion, induced the siginificant increase in epidermal thickness conpared to the vehicle.

# Discussion

It is very difficult to use the retinol in the cosmetics because it is easily oxidized in the air or in the water solution, and deteriorated in stability. For example, the products containg the retinol are mostly W/O emulsion because in O/W emulsion it is very difficult to stabilize the retinol . But, in W/O emulsion the continuous phase is composed of oils, so isolation of the retinol and oxygen reduce the oxidation of retinol to the minimum.

There are factors exerted and evil influence, for examples, type of the product (O/W, W/O), the ratio of water and oil in the same type, the kinds and quantity of surfactant, pH of the product, UV, manufacturing and storing temperature, trace elements in the product. Especially, the reasonable adjusted ratio of the oil phase and water phase is necessary to prohibit the decomposion of the retinol into anhydroretinol due to increasing the quantity of water and the thermal isomerization to 13-cis-retinol due to increasing the quantity of the oil.

Emulsion4, Using the *all-trans-retinol* which is double-capsulated with matrix (MDC®), gives results superier to the other samples in satability and the enhancement of epidermal thickness. On the other hand, emusion2 and emulsion3. more or less, are better results than emulsion1, but, they are not enough to show the satisfing results. Since the MDC® has matrix structure in which microcapsules are embedded *all-trans-retinol* seemed to be released into the matrix through capillary of the primary capsule wall without direct exposure to the continuous phase. In this manner, direct release of the capsule content, retinol, into continuous phase or influx of water into the capsule can be prevented and siginificantly reduced. Therefore, the

MDC® has the controlled released effect of active ingredient on the skin because microcapsules in proximity to the outer wall of matrix are released first through the capillary of the matrix. The MDC® has additional merit of processing at room temperature.

# Referance

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*Figure 1. Conversion of all-trans-Retinol*

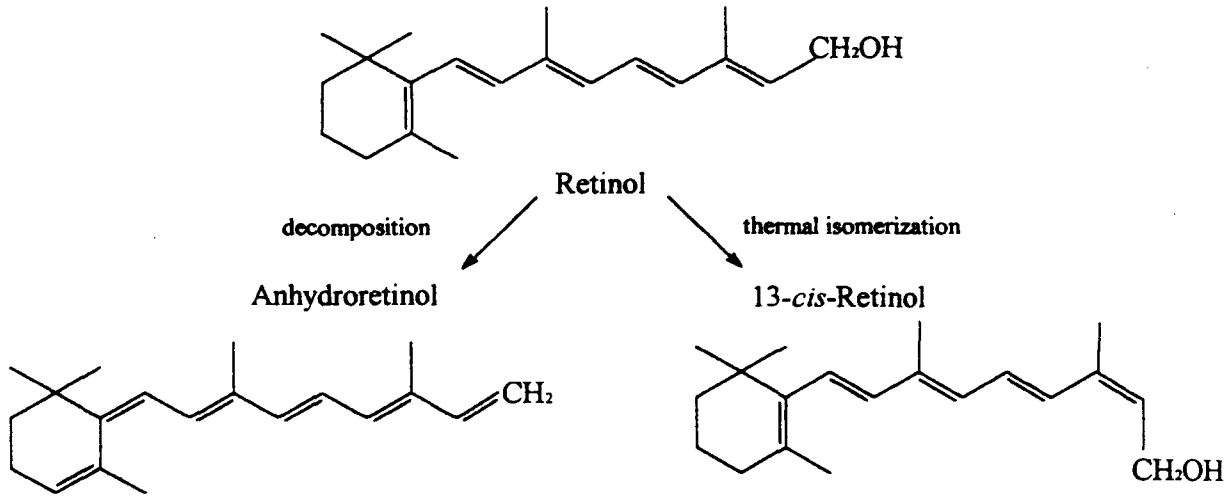
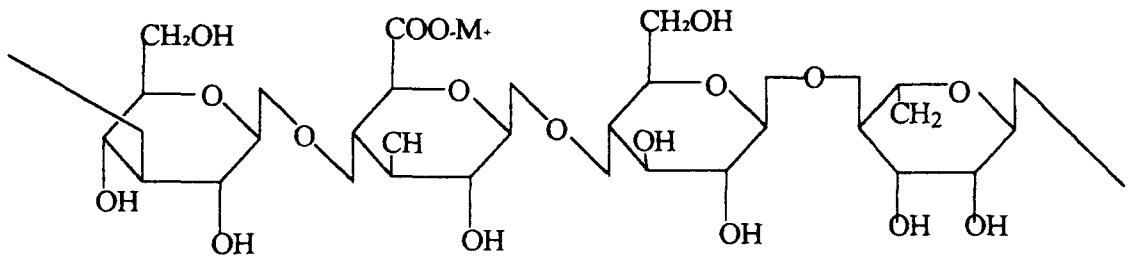



*Figure 2. Structure of Gellan gum*




–3)-*B*-D-Glcp-(1–4)-*B*-D-GlcpA-1–4-*B*-D-Glcp-(1–4)-*a*-L-Rhap-(1–)

*Table 1. Formulations of the Test Samples*

Emulsion1 : simply in O/W emulsion

| Ingredients | Concentration |
|---|---|
| Water | to 100 |
| EDTA-2Na | 0.1 |
| Glycerin | 10.0 |
| Allantoin | 0.1 |
| Liquid Paraffin | 8.0 |
| Squalane | 6.0 |
| Sorbitan stearate | 1.5 |
| Cetostearyl alcohol | 1.0 |
| BHT | 0.05 |
| Tocopheryl acetate | 1.5 |
| Methyl pafaben | 0.1 |
| Perfume | 0.08 |
| All-trans-Retinol | 2500IU |

Emulsion2 : simply in W/O emulsion

| Ingredients | Concentration |
|---|---|
| Liquid Paraffin | 16.0 |
| Dimethicone copolyol | 2.0 |
| Polymethyl methacrylate | 0.5 |
| Squalane | 9.0 |
| Methyl paraben | 0.1 |
| Tocopheryl acetate | 0.5 |
| BHT | 0.1 |
| Water | to 100 |
| EDTA-2Na | 0.02 |
| Glycerin | 7.0 |
| Sodium chloride | 1.5 |
| Perfume | 0.08 |
| All-trans-Retinol | 2500IU |

Emulsion3 : in primary-capsulated form *in O/W emulsion*

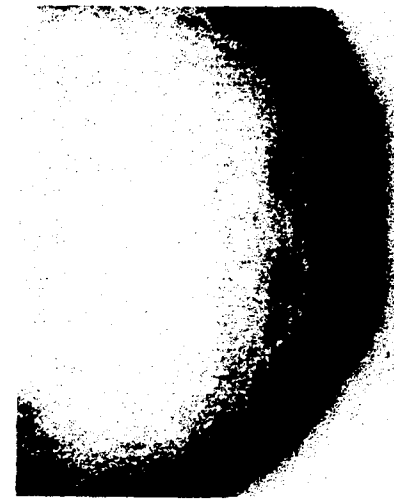
| Ingredients | Concentration |
|---|---|
| Water | to 100 |
| EDTA-2Na | 0.1 |
| Glycerin | 10.0 |
| Allantoin | 0.1 |
| Liquid Paraffin | 8.0 |
| Squalane | 6.0 |
| Sorbitan stearate | 1.5 |
| Cetostearyl alcohol | 1.0 |
| BHT | 0.05 |
| Tocopheryl acetate | 1.5 |
| Methyl pafaben | 0.1 |
| Perfume | 0.08 |
| All-trans-Retinol | 2500IU |

Emulsion4 : in MDC® in O/W emulsion

| Ingredients | Concentration |
|---|---|
| Water | to 100 |
| EDTA-2Na | 0.1 |
| Glycerin | 10.0 |
| Allantoin | 0.1 |
| Liquid Paraffin | 8.0 |
| Squalane | 6.0 |
| Sorbitan stearate | 1.5 |
| Cetostearyl alcohol | 1.0 |
| BHT | 0.05 |
| Tocopheryl acetate | 1.5 |
| Methyl pafaben | 0.1 |
| Perfume | 0.08 |
| All-trans-Retinol | 2500IU |

*Figure 3. The Shape of Capsules*

Primary Capsule

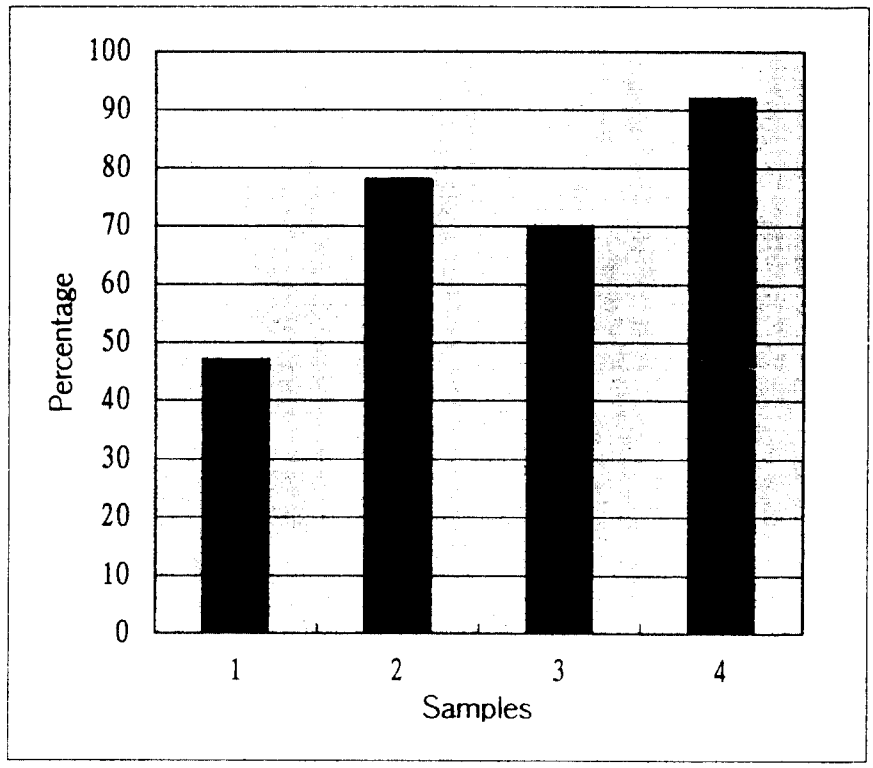


**MDC®**

*Table2. The Concentration and Percentage of the all-trans-Retinol*

| *Samples* | *Absorbance Area* | *Concentration (mg)* | *%* |
|---|---|---|---|
| Emulsion 1 | 1.6251E+06 | 0.4477 | 47 |
| Emulsion 2 | 2.6772E+06 | 0.7429 | 78 |
| Emulsion 3 | 2.4061E+06 | 0.6667 | 70 |
| Emulsion 4 | 3.1498E+06 | 0.876 | 92 |

*Figure 4. Results of the Retinol Concentration according to the Formulation Types*

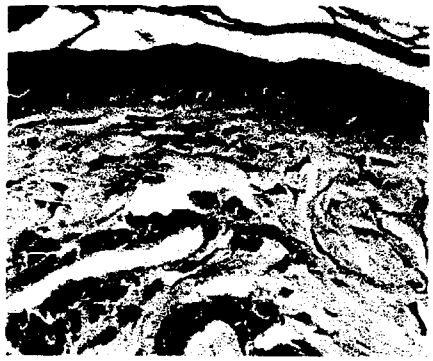



Sample 1 : simply in O/W
Sample 2 : in W/O emulsion
Sample 3 : in primary capsulated form in O/W emulsion
Sample 4 : in MDC in O/W emulsion

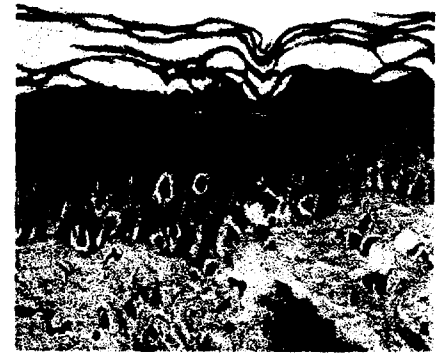
*Table 3. The Changes of the Epidermal Thickness*

| Group | Epidermal thickness | Granular layer thickness |
|---|---|---|
| Vehicle | 17±3.8 | 1.0±0.08 |
| Emulsion 4 | 34±2.3 | 1.4±0.24 |

*Figure 5. The Effects of the Retinol on the Epidermal Thickness*



*Vehicle*



*Emulsion 4*