The Transdermal Absorption of Retinol and Retinyl Palmitate in Cosmetics Formulations

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Abstract

Background: Since The Cosmetic Ingredient Review (CIR) Expert Panel concludes that retinol and retinyl palmitate are safe as cosmetic ingredients in the present practices of use and concentration in 1987. There are numerous studies concerning about the safety and stability of retinol and retinyl palmitate thereafter. Retinol and retinyl palmitate are commonly used in topical antiaging preparations. A clear understanding about the transdermal absorption of retinol and retinyl palmitate is essential for the safety evaluation of these ingredients in cosmetics.

Methods: In this study, the effect of formulation, type and concentration of enhancers on the transdermal absorption of retinol and retinyl palmitate have been investigated. Three types of formulation, including essence, w/o and o/w emulsions, were prepared for this study. Propylene glycol, 1,3-Butylene glycol or glycerin in the concentration of 5% or 10% were used to study the transdermal absorption enhancement effect for essence. The transdermal absorption test procedure is refer to the OECD Test Guideline 428 - Skin Absorption: In Vitro Method.

Results: The results show that retinol has stronger transfer ability to diffuse into and delivered across of the dermal layer than retinyl palmitate, this should be due the molecular size difference. Higher molecular weight of retinyl palmitate is harder to move cross the stratum corneum, which is demonstrated by the determined amount on stripping tape, which is around 3-6% for retinol and around 11-20% for retinyl palmitate for all the tests.

Conclusion: For cosmetics, use retinyl palmitate as active gradient with enhancer may be able to decrease the active gradient concentration in the formulation and still achieve the effective concentration with less safety concern.

Keywords: Transdermal Absorption; Retinol; Retinyl Palmitate

Introduction.

Since The Cosmetic Ingredient Review (CIR) Expert Panel concludes that retinol and retinyl palmitate are safe as cosmetic ingredients in the present practices of use and concentration in 1987. There are numerous studies concerning about the safety and stability of retinol and retinyl palmitate thereafter. Retinol and retinyl palmitate are commonly used in topical antiaging preparations. Retinol and its derivatives have shown great benefit in treating the signs of aged skin.[1][2][3][4] However, Vitamin A is currently not regulated in the Annexes to the Cosmetics Regulation (EC) No 1223/2009. In 2016, the SCCS issued the opinion SCCS/1576/16 on Vitamin A concluding that its use as a cosmetic ingredient is safe at given concentrations for body lotions and face creams, leave-on (other than body lotions) and rinse-off products.[5] Excessive intakes of vitamin A has been linked to increased risk for hypervitaminose A and whether frequent application of skin care products containing retinol or retinyl esters could result in long term effects in skin. Therefore, a clear understanding about the transdermal absorption of retinol and retinyl palmitate is essential for the safety evaluation of these ingredients in cosmetics.

Transdermal absorption can be affected by numerous factors including the structure and concentration of active ingredients, type of formulations, temperature and etc.. The transdermal absorption efficiency can by increased by using enhancer. There are three possible models for enhancer to increase the percutaneous penetration of active ingredient.[6] The first model is to change the lipid bilayer structure and hence to increase the diffusion of active ingredient into the skin. The second model is to interact with protein in keratinocytes and is less suitable for skin care product application. The third model is to increase the partition coefficient of active ingredient in skin. The enhancing mechanism can be due to a single model or combined with multiple models.[7]

In this study, the effect of formulation, type and concentration of enhancers on the transdermal absorption of retinol and retinyl palmitate have been investigated. Three types of formulation, including essence, w/o and o/w emulsions, were prepared for this study. Propylene glycol, 1,3-Butylene glycol or glycerin in the concentration of 5% or 10% were used to study the transdermal absorption enhancement effect for essence. The transdermal absorption test procedure is refer to the OECD Test Guideline 428 - Skin Absorption: In Vitro Method.

Materials and Methods.

The chemicals used in this study includes retinol, vitamin A palmitate, mineral oil, propylene glycol, 1,3-butylene glycol, glycerin, xanthan gum, germaben II, EDTA-2Na, Tween 20, Tween 80, Olivem 1000, caprylic/capric triglyceride, isopropyl palmitate, squalane, Olivem 900, ethanol, methanol, NaCl and piglet skin. The formulation of test samples with retinol or retinyl palmitate are shown in Table 1 and Table 2.

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Table 1. The formulation of test sample with retinol

Dosage Forms	essence							o/w emulsion	w/o emulsion
Formulation	Base 1	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
Phase A									
Olivem 1000								3.00	
Caprylic/ Capric Triglyceride								6.00	6.00
Mineral oil								15.00	15.00
Isopropyl palmitate								3.00	3.00
Squalene								6.00	6.00
Olivem 900									5.00
Phase B									
Propylene Glycol	0.00	5.00	10.00					5.00	5.00
1,3-Butylene Glycol				5.00	10.00				
Glycerin						5.00	10.00		
Distilled Water	59.10	54.10	49.10	54.10	49.10	54.10	49.10	50.20	48.20
Xanthan Gum(1%)	30.00	30.00	30.00	30.00	30.00	30.00	30.00	3.00	3.00
Germaben II	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
ETDA-2Na	0.10	0.10	0.10	0.10	0.10	0.10	0.10		
Phase C									
Retinol 46% and Polysorbate 20 and BHT and BHA	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
p23	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Tween 80	2.00	2.00	2.00	2.00	2.00	2.00	2.00		

*w/w%

Table 2. The formulation of test sample with retinyl palmitate

Dosage Forms	essence							o/w emulsion	w/o emulsion
Formulation	Base 2	No.11	No.12	No.13	No.14	No.15	No.16	No.17	No.18
Phase A									
Olivem 1000								3.00	
Caprylic/ Capric Triglyceride								6.00	6.00
Mineral oil								15.00	15.00
Isopropyl palmitate								3.00	3.00
Squalene								6.00	6.00
Olivem 900									5.00
Phase B									
Propylene Glycol	0.00	5.00	10.00					5.00	5.00
1,3-Butylene Glycol				5.00	10.00				
Glycerin						5.00	10.00		
Distilled Water	59.10	54.10	49.10	54.10	49.10	54.10	49.10	50.20	48.20
Xanthan Gum(1%)	30.00	30.00	30.00	30.00	30.00	30.00	30.00	3.00	3.00
Germaben II	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
ETDA-2Na	0.10	0.10	0.10	0.10	0.10	0.10	0.10		
Phase C									
retinyl palmitate 43% and Arachis Hypogaea oil and Tocopherol	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
p23	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Tween 80	2.00	2.00	2.00	2.00	2.00	2.00	2.00		
*w/w%									

For dosage form of essence, ingredients in phase B are mixed first until become transparent before ingredients in phase C is added into it and stir for 10 minutes. For dosage form of o/w emulsion and w/o emulsion, ingredients in phase A and phase B are first heated to 85^{o}C separately. Then phase A is dropped into phase B for o/w emulsion and phase B is dropped into phase A for w/o emulsion, stir for 5 minutes and waiting for the temperature cool down to 45^{o}C before adding phase C and stir for 10 minutes.

The transdermal absorption test was performed by apply 0.01g sample (essence, w/o or o/w emulsions) on excised pig skin with exposed area of 0.64cm^2 at 37^{o}C for 10 hours with 6 duplicates. The data is accepted only when the mass balance of test chemical before and after test were smaller than 15% ◦ The mass of test chemical after test was collected from

the amount in flush collected residue sample, the amount on cotton buds for dry the skin, the amount on stripping tape, the amount diffused into the skin and the amount delivered across the skin collected in receptor determined by HPLC.

The conditions for HPLC is using Agilent HC-C18 (4.6×250mm, 5μm) as stationary phase by isocratic elution with 95% methanol aqueous solution as mobile phase at flow rate of 1.0mL/min and detected at 345nm. The quantification range of retinol and retinyl palmitate is between 1 and 50 μg/mL.

Results.

The results of transdermal absorption of essence with retinol is shown in Table 3.

Table 3. The results of transdermal absorption of essence with retinol

Formulation	Base 1	No.1	No.2	No.3	No.4	No.5	No.6
Applied mass (μg)	367.5±16.5	356.6±14.1	356.9±12.9	398.5±28.0	387.1±21.6	331.0±11.6	350.3±12.6
Residue mass in donor cell (μg)	143.0±35.0	206.3±33.0	239.9±42.9	206.3±38.5	187.4±65.4	192.5±19.6	215.4±28.6
amounts on cotton bud (μg)	106.4±23.0	51.9±33.0	55.4±4.1	68.8±16.7	105.9±19.3	59.4±38.5	89.0±42.7
amount on stripping tape (μg)	19.8±3.6	14.0±11.3	18.6±9.4	24.6±6.1	22.8±9.3	11.9±4.1	12.7±2.0
amount diffused into the skin (μg)	40.5±11.6	35.8±10.6	29.3±16.0	57.4±6.6*	49.3±19.7	41.2±12.5	41.6±17.1
amount collected in receptor (μg)	1.3±0.9	35.8±16.2*	12.4±3.9*	15.1±10.0*	6.2±3.6*	23.8±17.0*	6.3±4.0*
transdermal absorption amount (μg)	41.8±13.5	71.2±19.6*	41.7±14.9	72.5±9.8*	55.5±22.1	65.0±19.7*	48.0±18.6
Recovery (%)	84.6±7.5	96.4±10.0	99.6±14.9	93.4±11.5	96.0±17.8	99.3±8.9	104.2±9.5

*p<0.05 X±SD (n=6)

The results of transdermal absorption of essence with retinyl palmitate is shown in Table 4.

Table 4. The results of transdermal absorption of essence with retinyl palmitate

Formulation	Base 2	No.11	No.12	No.13	No.14	No.15	No.16
Applied mass (μg)	320.5±9.6	354.2±14.9	344.0±8.6	330.6±9.9	342.0±13.8	350.8±12.3	321.2±9.6
Residue mass in donor cell (μg)	66.9±14.0	132.7±20.2	102.3±14.9	129.3±17.2	105.9±33.8	93.1±42.7	98.7±36.8
amounts on cotton bud (μg)	142.6±50.3	102.1±39.6	118.1±17.1	95.1±41.8	118.3±33.0	127.9±26.2	136.8±32.8
amount on stripping tape (μg)	74.9±12.4	51.9±8.0	67.3±19.2	53.6±19.0	68.3±18.1	70.6±7.8	51.3±21.2
amount diffused into the skin (μg)	28.8±14.6	71.3±11.1	64.8±9.4	61.8±12.3	53.0±13.5	63.5±4.6	53.3±20.5
amount collected in receptor (μg)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
transdermal absorption amount (μg)	28.8±14.6	71.3±11.1*	64.8±9.4*	61.8±12.3*	53.0±13.5*	63.5±4.6*	53.3±20.5
Recovery (%)	97.7±14.4	101.1±7.6	102.5±7.0	102.8±10.0	100.4±8.4	101.2±8.7	98.9±11.5

*p<0.05 X±SD (n=6)

It was observed that for dosage form of essence with 5% propylene glycol as enhancer, the transdermal absorption amount is higher than using other concentration of propylene glycol or 1,3-butylene glycol as enhancer. Therefore, 5% propylene glycol was used as enhancer in the study of the influence of formulation type on transdermal absorption.

The results of transdermal absorption of different formulation type with retinol is shown in Table 5.

Table 5. The results of transdermal absorption of of different formulation type with retinol

Formulation	No.1	No.7	No.8
Applied mass(μg)	356.6±14.1	397.9±28.0	368.0±11.0
Residue mass in donor cell(μg)	206.3±33.0	131.0±44.1	153.9±45.0
amounts on cotton bud(μg)	51.9±33.0	175.1±64.0	150.8±33.2
amount on stripping tape(μg)	14.0±11.3	13.2±3.7	21.1±4.8
amount diffused into the skin(μg)	35.8±10.6	61.4±10.8*	48.7±10.8
amount collected in receptor(μg)	35.8±16.2	10.6±5.5	2.9±0.5*
transdermal absorption amount(μg)	71.2±19.6	72.0±7.1	51.7±11.2**
Recovery (%)	96.4±10.0	98.4±28.6	102.6±14.2

X±SD (n=6)

*p<0.05 significant difference to essence

**p<0.05 significant difference to o/w emulsion

The results of transdermal absorption of different formulation type with retinyl palmitate is shown in Table 6.

Table 6. The results of transdermal absorption of of different formulation type with retinyl palmitate

Formulation	No.11	No.17	No.18
Applied mass(μg)	354.21±14.88	344.23±13.76	354.60±15.96
Residue mass in donor cell(μg)	132.68±20.16	104.40±16.44	147.82±25.63
amounts on cotton bud(μg)	102.13±39.63	138.80±35.54	118.23±37.39
amount on stripping tape(μg)	51.91±7.98	42.72±8.88	41.76±4.33
amount diffused into the skin(μg)	71.28±11.11	55.66±17.10	37.32±17.17
amount collected in receptor(μg)	0.00±0.00	0.00±0.00	0.00±0.00
transdermal absorption amount(μg)	71.28±11.11	55.66±17.10	37.32±17.17*
Recovery (%)	101.07±7.57	99.29±12.37	97.33±7.03

X±SD (n=6)

*p<0.05 significant difference to essence

The results all show good recovery between 84% and 104%.

Discussion.

The results show that retinol has stronger transfer ability to diffuse into and delivered across of the dermal layer than retinyl palmitate, this should be due the molecular size difference. Higher molecular weight of retinyl palmitate is harder to move cross the stratum

corneum, which is demonstrated by the determined amount on stripping tape, which is around 3-6% for retinol and around 11-20% for retinyl palmitate for all the tests.

Without enhancer, the transdermal absorption amount, the amount diffused into the skin and the amount delivered across the skin, of retinyl palmitate is around two third of that of retinol. With 5% propylene glycol as enhancer in essence, the transdermal absorption amount of retinol and retinyl palmitate are both increased to around 20% and retinyl palmitate is all stayed at the dermal layer.

For comparing the effect of formulation type on the transdermal absorption, the transdermal absorption amount of retinol is almost the same for essence and o/w emulsion, however the transdermal absorption amount of retinyl palmitate is slightly lower in o/w emulsion than in essence. Nevertheless, the transdermal absorption amount of retinol and retinyl palmitate in w/o emulsion is the lowest among the three formulation types.

About the effect of enhancer type and concentration on the transdermal absorption, it was studied only for essence. Generally, essence with enhancer, propylene glycol gives better transdermal absorption result than 1,3-Butylene glycol and then glycerin. 5% propylene glycol, 1,3-Butylene glycol or glycerin also shows better transdermal absorption result than 10% propylene glycol, 1,3-Butylene glycol or glycerin. It was also noticed that retinyl palmitate is all retained on the dermal layer without transfer across it. Furthermore, 5% propylene glycol and 5% glycerin are giving higher amount delivered across the dermal layer.

Conclusion.

The retained amount in dermal layer of retinyl palmitate from essence and o/w emulsion are higher than retinol. For cosmetics, use retinyl palmitate as active gradient with enhancer may be able to decrease the active gradient concentration in the formulation and still achieve the effective concentration with less safety concern.

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Conflict of Interest Statement. NONE.

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