EFFECT OF ANTIOXIDANT ON THE STABILITY AND EFFICACY ON ANTI-WRINKLE OF INDOLE-3-ACETIC ACID

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Abstract

A recent development in cosmetics has been the pursuit of wrinkling in the skin. The cosmetics composed of anti-wrinkle agent stand out from the point of view of environmental contamination and pollution. Among them, Indole-3-acetic acid (IAA), studied with wrinkling pigmentation and swelling conditions in the area of the eye, showed clinically significant reduction in depth of lines during one month trial using skin treatment. But, IAA has shown some problems when used in cosmetic formulations, such as stability, permeability and toxicity. The results of the clinical examination were shown that its permeability and toxicity didn't matter. To increase the stability of IAA, antioxidants such as Licorice, ubiquinone, tocopherol, Baicalin, ferulic acid, BHT, ascorbic acid, sodium metabisulfite, and so on were employed in cosmetic formulations.

Our main purpose is the study for the stability efficiency and effect of each other of cream formulations containing optimal dosage antioxidants (o/w type emulsion). This study evaluated wrinkle reduction effect of IAA, which is used in cosmetics.

Introduction

According to the results of a recent questionnaire on female skin problems, wrinkles were one of the major concerns for women along with spots or freckles.[1]

They are involved in general processes of the intrinsic and extrinsic aging.[2-6] The intrinsic processes resemble similar processes described for other tissues. The extrinsic processes include the dose of the UV rays skin was exposed to and the oxidation associated with UV-expose.

Due to these factors, a great deal of research has been done on the cosmetic preparation of human wrinkles and their improvements. Some effective substances such as retinoid compounds have been developed.[17-24] In this research, we will introduce the indole-3-acetic acid (IAA) for the wrinkle's improvements.

The IAA has long been recognized as their demonstrated plant-growth regulating properties and anti-inflammatory activities.[25-27] IAA as cell migration agent is useful in treating wrinkled skin and can minimize the effects of photo aging on the skin by itself. In addition, through the test in vitro, we could determine the possible cell growth promotion activity.

But, IAA is unstable under the some condition like light, oxygen, certain temperature, and lipid peroxide and water presence, and the instability of IAA makes it difficult to incorporate these ingredients in cosmetic products without using stabilization techniques.

To increase the stability of IAA, some antioxidants are employed in cosmetic formulations. Although the use of antioxidants in cosmetic products is therefore highly recommended, we found that an effect is different through the antioxidant species used in this study.

In addition, wrinkle reduction effect of IAA has been evaluated and compared with a placebo, which are widely used in cosmetics.

Materials and Measurements

Materials :

For this experiment, we formulated an anti-aging cream as an oil-in-water emulsion containing 300 ppm of IAA. We used materials such as Licorice, ubiquinone, tocopherol, Baicalin, ferulic acid, BHT, ascorbic acid and sodium metabisulfite as antioxidants, and the formulation pH was adjusted to 3.0 – 3.5.

We used purified water that had passed through the anion-cation exchange resin column. All materials used were cosmetic grade. This formula has been commercialized and cannot be presented here.

Measurements :

Emulsion droplets were examined with the optical microscope (model BX 40 F-3).

To measure the temperature stabilities, IAA was prepared in homogeneous state in o/w emulsion. Samples were stored at 25 ℃ and 40 ℃ as long as 6 month. Periodically, 6.6 g of each sample were weighted and diluted with methanol. The amount of residual IAA was measured by HPLC. Detector by ultraviolet spectrophotometer set at 280 ㎚. The mobile phase, consisting of acetonitrile/K_2HPO_4(50 : 50 v/v, with 0.01 M concentration of K_2HPO_4 to adjust at pH 4.5), was pumped at 1.0 ㎖/min.

The stationary phase applied was a Luna OAS 5 ㎛(250 * 4.6 ㎜) with a 10 ㎜ precolumn of the same material. Column temperature controlled at 25 ℃ and electrochemical detection was performed at + 0.4 V of potential. Each time 20 ㎕ samples were injected for analysis.

Wrinkle reduction tests :

Clinical studies were performed to 26 female volunteers, the ages of 25 over. All volunteers gave informed consent before participation and signed a written Informed Consent Form. Each volunteer used either an emulsion containing 300 ppm IAA or a placebo.

The emulsions were applied twice a day for 12 weeks. Wrinkle measurements were taken before and after the 12 weeks period. The following methods were used to determine wrinkle reduction effect : (a) examination with the unaided eye, and (b) R-value analysis with Visiometer SV 600 taking before and after photographs of wrinkle shadows in the main deep wrinkled area in the captured image of the replica.

Measuring principle of Visiometer is the same as following. The CCD-chip measures the light having penetrated the replica. The intensity of light is measured according to the Lambert and Beer's law of absorption.

Results and Discussion

Characteristics :

The mixtures used for experiment were prepared in our laboratory and photomicrograph of an o/w emulsion is shown in Figure 1. The figure shows distributed particles of uniform sizes in a continuous bulk water phase. This result indicates the o/w emulsion formulation.

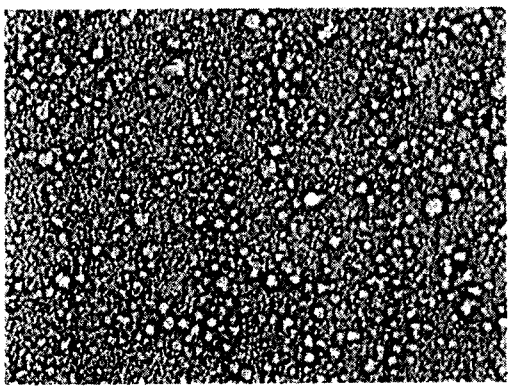


Figure 1. Photomicrographs of o/w emulsion containing 300 ppm IAA. Magnification of 400×.

There appeared a characteristic peak in the about 4.2 min for retention time (Figure 2). The HPLC peaks of IAA for the standard sample and the o/w emulsion kept at 25 ℃ for 6 month showed that IAA amount was decrease.

In the creams formulated with IAA, light and heat reduce the content of IAA. This stability of IAA may be maintained by adding antioxidants, but this adjustment is not always successful.

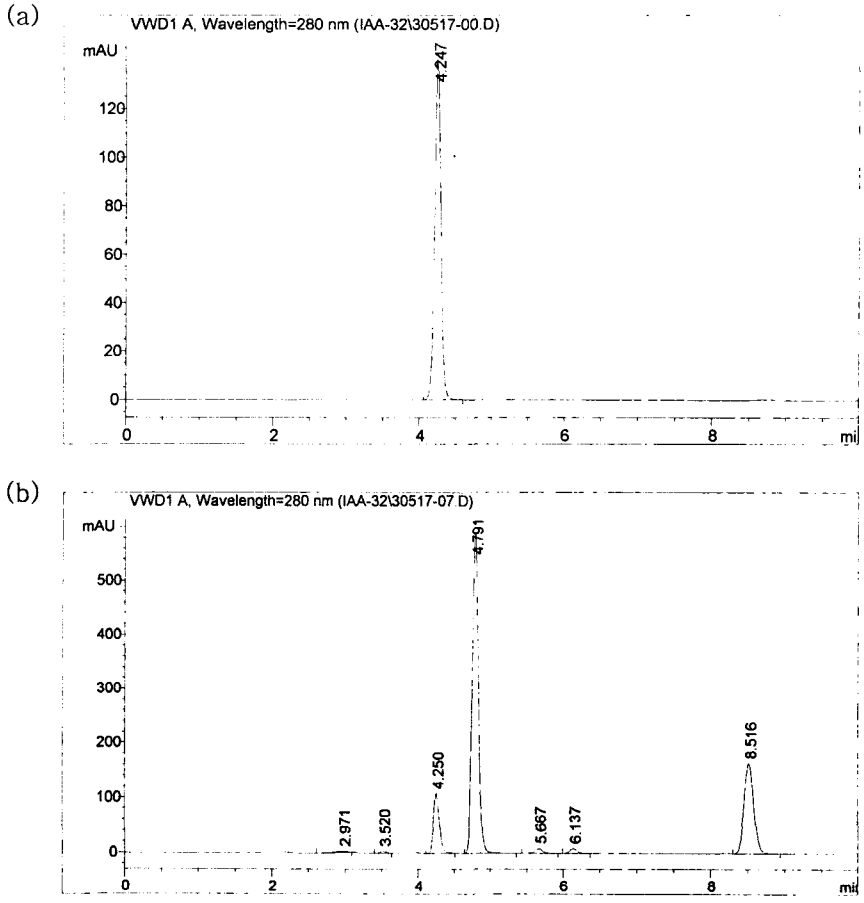
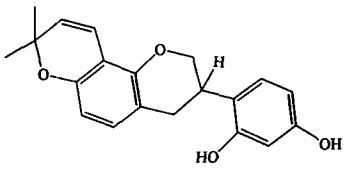
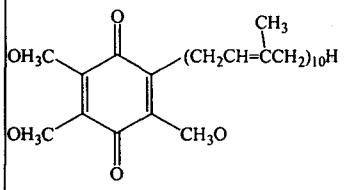
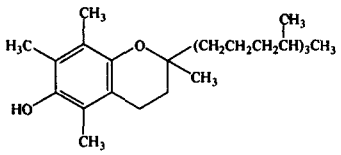
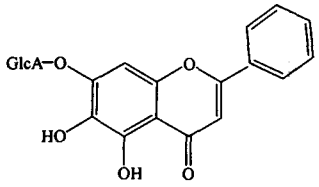
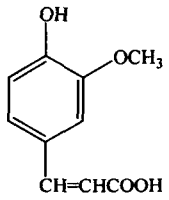
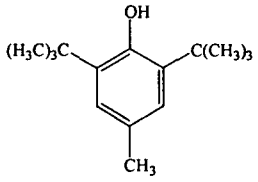
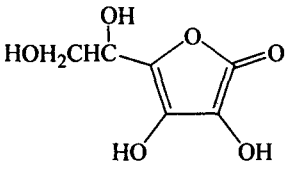
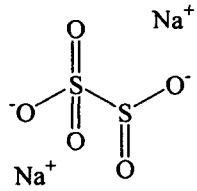


Figure 2. HPLC-UV (280 nm) chromatogram obtained from (a) standard IAA, (b) o/w emulsion containing of 300 ppm IAA after 6 month storage at 25 ℃.

The effects of antioxidants ;

The main objective of the present study is to evaluate the antioxidant effect on the stability of IAA. The antioxidants shown in Table 1 were added to the o/w emulsion cream used in our experiments.

Table 1. Antioxidant chemical formaula

Antioxidants	Chemical formula	Antioxidants	Chemical formula
Licorice		Ubiquinone	
Tocopherol		Baicalin	
Ferulic acid		BHT	
Ascorbic Acid		Sodium Metabisulfite	

Using HPLC to determine how the content of 300 ppm IAA change during storage at 25 ℃ and 40 ℃ during 6 month produced the data shown in Figure 3 - Figure 5.

Figure 3 showed the results of the stabilities under the condition mentioned in Table 2. The condition B and E showed positive effect at 25 ℃ than condition A (cream with no antioxidant). At 40 ℃, the effect of Baicalin is considerable, but Licorice did not affect to the stability.

On the other hand, tocopherol has not significant effect of it at both temperature.

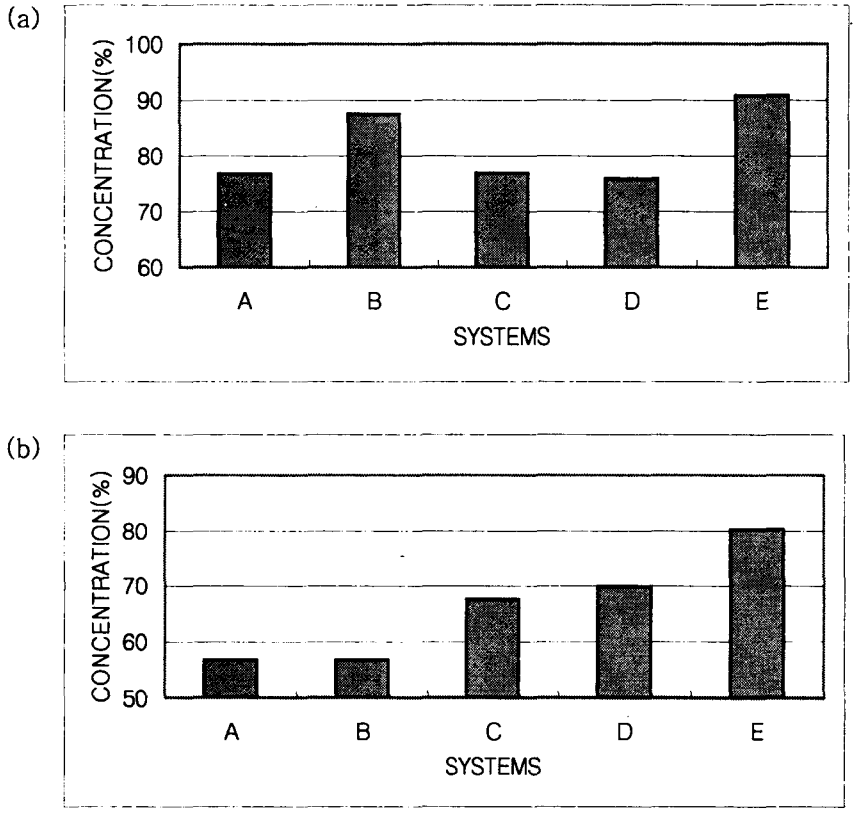


Figure 3. The percentage of IAA remaining in tested-creams during 6 month of storage at (a) 25 ℃ and (b) 40 ℃.

Table 2. Antioxidant's species in o/w emulsion containing 300 ppm IAA.

Condition	A	B	C	D	E
Antioxidents	Blank	Licorice	B/ ubiquinone	C/ tocopherol	D/ Baicalin

Then other antioxidant was tested.

Ascorbic acid is somewhat improved stability at 25 ℃ than blank experiment, but at 40 ℃, stabilization effect of this material was not satisfied for the practical application.

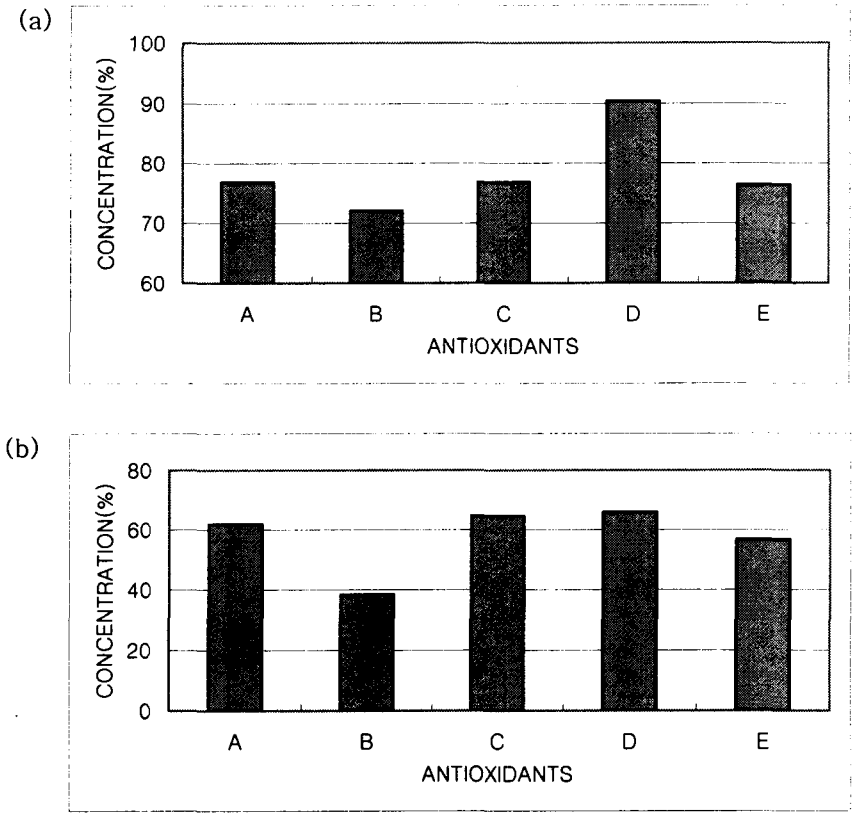


Figure 4. The Percentage of IAA remaining in tested-creams containing (A)none, (B)ferulic acid, (C)BHT, (D)ascorbic acid, (E)sodium metabisulfite as antioxidants using during 6 month of storage at (a) 25 ℃ and (b) 40 ℃.

The suitable antioxidant was selected based on the above experiment and the Figure 5 showed the results of effort to decide the optimal dosage of the antioxidant. Although tocopherol as antioxidant did not affect to the stability showing Figure 3, we found that tocopherol is an important antioxidant, and some believe that it donates reducing equivalents to lipid peroxides formed through oxidation in the cell membrane. Due to the fact, we examined the function of tocopherol as the antioxidant in our experimental cream.

The increasing the amount of tocopherol caused the decreasing of the stability. We should experiment with tocopherol below 0.07 % to find its optimal concentration for the stability of IAA. On the other hand, optimal concentration of Baicalin was 0.02 %.

The increasing dosage of sodium metabisulfite used for the general antioxidant was significantly affected on stability increasing.

According to the result of above, we could determine that the superior stability against reduction in the content of IAA was analogous as follows :

tocopherol 0.07 % = Baicalin 0.02 % = sodium metabisulfite 0.012 %

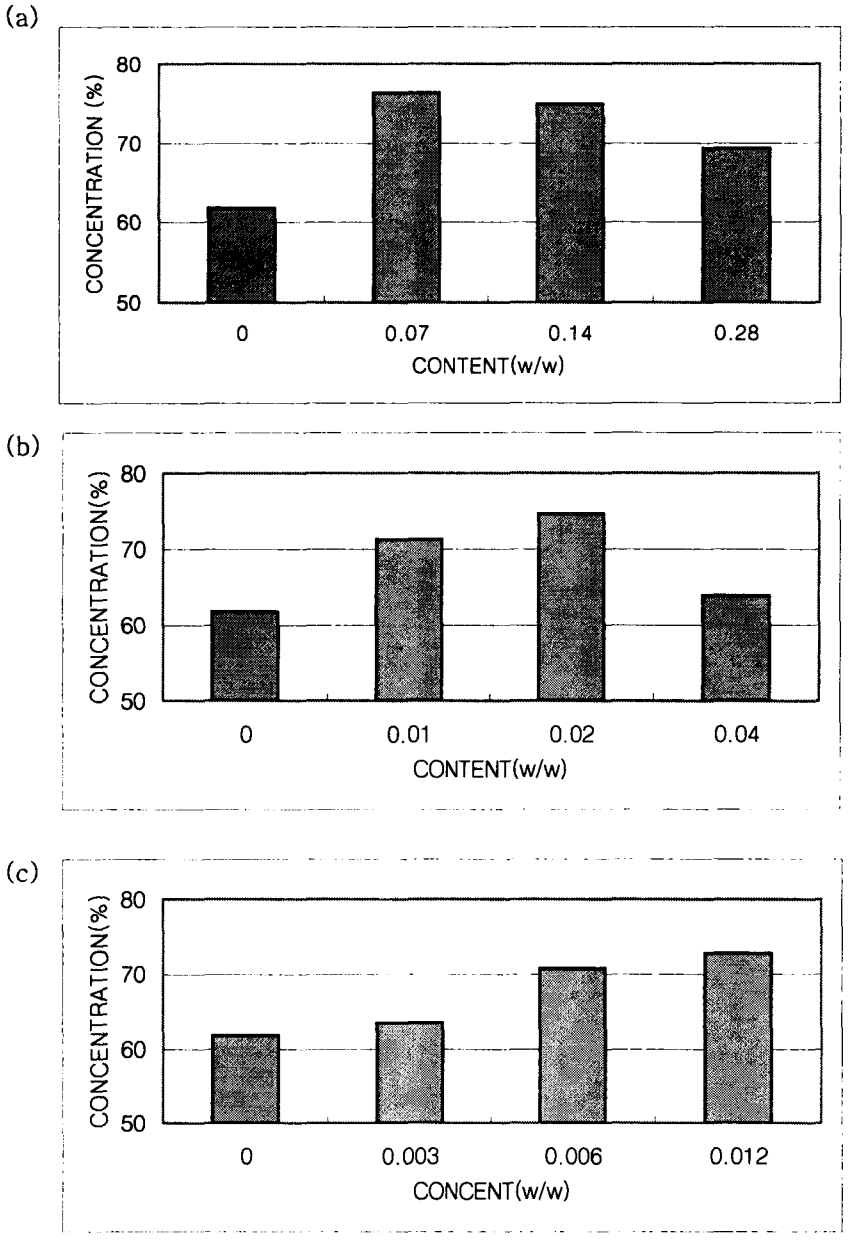


Figure 5. The percentage of IAA remaining in tested-creams containing (a)tocopherol, (b)Baicalin, (c)sodium metabisulfite as antioxidant during 6 month of storage at 40 ℃.

Wrinkle reduction

Wrinkle area value is a measure of the roughness of a surface profile. We used this value to evaluate the anti-wrinkle effectiveness of either an emulsion containing 300 ppm IAA or a placebo.

The change in wrinkle area values before and after the 12-week treatment is significant for both treatment groups. These results are shown in Table 3 and Table 4.

Table 3. The p-value of selected time interval between emulsion containing 300 ppm IAA and placebo without IAA (examination with the unaided eye)

Time after treatment	p-value
4 week	0.238
8 week	0.052
12 week	0.006*

Table 4. The p-value of selected time interval between emulsion containing 300 ppm IAA and placebo without IAA (analysis with Visiometer)

Time after treatment	R-value	p-value
4 week	R1	0.111
	R2	0.034*
	R3	0.023
	R4	0.370
	R5	0.098
8 week	R1	0.012*
	R2	0.001*
	R3	0.001*
	R4	0.276
	R5	0.345
12 week	R1	0.001*
	R2	0.002*
	R3	0.005*
	R4	0.758
	R5	0.609

* significantly different at $p<0.05$ compared with placebo

- R1 = Skin Roughness, R2 = Maximum Roughness, R3 = Average Roughness,
- R4 = Smoothness Depth, R5 = Arithmetic Average Roughness

The quantitative contest between the parameters is as follows.

$$R1 \geq R2 \geq R3 \geq R4 \geq R5$$

In further support of the clinical grading, there was a statistically significant improvement ($p<0.05$) in skin smoothness via silicone replica analysis at week 12th. Skin roughness and the depth were improved, and results from the examination with the unaided eye were favorable. Most participants noticed the anti-aging effect on their skin after the treatments.

In analysis using a Visiometer, Maximum Roughness is significantly different from after four weeks of use.

Additionally, Skin Roughness and Average Roughness are significant improvement in after eight weeks of use.

Although the detail mechanism of IAA action has not been known, IAA was recognized and marketed as cosmetic actives material with skin-hydration powers. The published data about quantify of these powers are surprisingly scare.

Even with the currently available stabilized forms of IAA, one would probably have to follow various precautions to achieve this stability and consequently the desired physiological effect in the skin.

Conclusion

In this study, we prepared o/w type creams containing 300 ppm of IAA and different antioxidants. We investigated the effect of antioxidant on the stability of IAA and whether IAA could improve the moisturizing potential of a cosmetic emulsion when present in the concentration of 300 ppm. During the study, the following facts were revealed.

- The cream containing tocopherol(0.07 %), Baicalin(0.02 %) and sodium metabisulfite(0.012%) showed superior stability against reduction in the content of IAA.
- The IAA content changes over the course of time when any of the creams is stored at 25 ℃, or 40 ℃ for 6 month.
- The other creams containing ferulic acid or ubiquinone as antioxidants showed a rapidly reduction in the content of IAA.
- The short-term trial revealed no significant difference between the wrinkle reduction effect of IAA o/w emulsion and the placebo.
- The long-term study detected a significant increase in the performance of IAA

treatment, compared with the placebo.

These results suggest that IAA would be a good anti-wrinkle agent for enhancing bioavailability and stability of IAA with antioxidant such as tocopherol, Baicalin, sodium metabisulfite, and so on.

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