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CLINICAL EFFICACY EVALUATION OF O/W EMULSION CONTAINING INDOLE-3-ACETIC ACID WITH THE FUNCTION OF ANTI-WRINKLE

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Abstract

Indole-3-acetic acid (IAA) molecule has been successfully encapsulated in biocompatible inorganic matrix in order for the cosmetic application such as superfacial fine line reduction. The encapsulation was realized through chemical reaction involving simultaneous formation of inorganic lattice and IAA giving rise to an IAA-inorganic nanohybrid (IAA-brid) which shows excellent storage stability and sustained releasing property of indole-3-acetic acid. The clinical efficacy of essence cream containing IAA-brid as anti-wrinkle formulation was also carefully evaluated by measuring the roughness of the skin replica before and after treatment. Upon administration of the cream on the eye-area, the fine-line is drastically reduced.

Keywords : Indole-3-acetic acid, Encapsulation, Inorganic material, Anti-wrinkle

Introduction

Indole-3-acetic acid (IAA), the most abundant and wide spread auxin, is a plant growth hormone found in aloe vera and all higher plants. In the cosmetics and dermatologicals, IAA initiates healthy fibroblast cell migration to wound areas, where they proliferate and produce collagen, elastin. IAA also act as antioxidant which decreases the lipid peroxidation.[1-4] In spite of these promising features of IAA as cosmetic and pharmaceutical ingredient, the use of IAA in cosmetic and pharmaceutical products has been limited due to its low storage stability.

In recent, we succeeded in encapsulation and stabilization of IAA using inorganic layered materials with high biocompatibility and skin affinity, so that could be applicable as the cosmetic ingredient. Here we discuss its physico-chemical properties along with its chemical stability and controlled release behavior. We also evaluate the clinical efficacy on anti-wrinkle effect of o/w emulsion cream containing IAA encapsulated with inorganic matrix.

Experimental Section

Synthesis

The encapsulation of indole-3-acetic acid (IAA) with inorganic layer was achieved by a chemical coprecipitation in an aqueous solution.[5-6] Hydrated zinc oxide, $ZnO \cdot xH_2O$, was used as an inorganic matrix since it has positive surface charge, and it can thus immobilize anionic indole-3-acetate species. During the encapsulation process, nitrogen gas was flowed through the reaction solution continuously to minimize the decomposition of IAA and to prevent the contamination from air. The white precipitate formed by coprecipitation reaction was washed with the mixed solution of 50 % ethanol and 50 % decarbonated water thoroughly. Thus prepared IAA-hydrated zinc oxide hybrid was encapsulated again with nano-sized silica (SiO_2) particles through the controlled hydrolysis of tetraethylorthosilicate (TEOS, $Si(OC_2H_5)_4$). The resulting product was washed with ethanol thoroughly and dried under vacuum to form IAA-inorganic (ZnO/SiO_2) hybrid particles (IAA-brid).

Chracterization

Powder X-ray diffraction patterns (XRD) were obtained with a Philips PW 3710 diffractometer with Ni-filtered Cu-Kα radiation (λ = 1.5418 Å) for the samples spread on slide glass. UV-vis spectra were obtained on a Perkin-Elmer Lambda 35 spectrophotometer to determine the content of IAA. Prior to the measurement, all the powdery samples were dissolved in the mixed solution of 0.1 M HCl aqueous solution and acetonitrile(0.1M HCl : acetonitrile = 2 : 98, volume ratio) to recover pure IAA molecules encapsulated. Then the supernatant was analyzed after the filtration through a 0.2 μm PTFE filter using an absorbance maximum of λ_{max} at 280 nm, corresponding to the typical absorption peak of IAA. To determine the content of IAA, high performance liquid chromatography (HPLC) spectra were also recorded on an Agilent 1100 Series Instrument equipped with UV detector (λ_{max} = 280 nm). An octadecyl-silica reversed-phase column (4.6 mm×250 mm, Zorbax), a mobile phase containing 0.1 M potassium hydrogen phosphate (K_2HPO_4)

aqueous solution with the pH of 4.5 and acetonitrile (0.1 M K_2HPO_4 : acetonitrile = 55 : 45, volume ratio), a flow-rate of 1 mL/min and 10 μL of injection volume were used. Field emission-scanning electron microscopic (FE-SEM) observation for IAA-brid was performed using a Hitachi S-4300. Prior to the observation, the powder sample was coated with Pt-Pd for 150s in vacuum.

Controlled realeasing test

Time controlled releasing behavior of the encapsulated IAA was profiled in a 0.08 % NaCl aqueous solution. At first, IAA-brid powder (100 mg) was dispersed in 100 mL 0.08 % NaCl solution and stirred at 25 °C with a rate of 50 rpm. The released amount of IAA was determined periodically with UV-vis spectrum using the absorption peak at 280 nm (λ_{max} of IAA).

Stability test

The stability of pure IAA in IAA-brid powder in an aqueous solution was evaluated by monitoring the retention of IAA at the different storage periods. For the evaluation, 100 mg of IAA-brid powder (containing 24 mg of IAA) and 24 mg of pure IAA (as a reference) were separately added into the vials containing 10 mL of decarbonated water and sealed carefully with the caps, and stored in an oven with the constant-temperature of 42 °C. The content of IAA was analyzed periodically with HPLC.

Preparation of essence cream containing IAA-brid

The essence cream with IAA-brid (IAA-brid cream) and without IAA (placebo cream) were prepared on the basis of the recipe summarized in Table 1. All of the o/w emulsion creams were composed of the same ingredients except for the amount of IAA-brid. The contents of IAA of IAA-brid cream was fixed to 750 ppm.

Clinical Efficacy Test

The clinical test was conducted to investigate the wrinkle diminution effect of IAA-brid cream in the eye-area. Twenty-seven (27) subjects, all female ranging in age from 35 to 54 years exhibiting obvious wrinkling and superficial line conditions of the skin especially around the eye-zone, were impaneled. The test article was applied twice daily, morning and evening on the eye area for a period of 12 weeks. Quantitative analysis of wrinkles[7] was conducted using skin-visiometer SV600 (from CK electronic GmbH, Germany), instrument for the determination of the skin roughness by light transmission by means of a silicon replica, before and after applications at 4, 8, and 12 weeks. All repilicas were taken in the room at 20 ~ 22 °C and 48 ~ 50 % relative humidity because the skin configurations change according to environmental temperature and humidity. Every replica was measured three times and each time circular measuring lines were used. The skin roughness parameters investigated were R1 (skin roughness), R2 (maximum roughness), R3 (average roughness), R4 (smoothness depth), R5 (arithmetic average roughness).

Results and Discussions

The encapsulation of IAA with inorganic matrix was achieved by chemical coprecipitation in an aqueous solution. IAA is a weak acid (pK_a = 4.75),[8] and it becomes mostly deprotonated in aqueous solution at pH > 4.75 to form an anionic indole-3-acetate species. Since the hydrated zinc oxide has a positive surface charge, the anionic indole-3-acetate molecules would be adsorbed onto the inorganic surface during coprecipitation, leading to the encapsulation of IAA molecule in the inorganic matrix.

Figure 1 shows the X-ray diffraction patterns of the IAA-hydrated zinc oxide hybrid obtained during the first encapsulation process (a) and the silica modified one (IAA-brid) (b). As can be seen from Figure 1 (a), primary IAA-inorganic hybrid shows a layer character with the basal spacing of 21.0 Å. This suggests that indole-3-acetate anions are inserted between the hydrated zinc oxide sheets as a double layer configuration along the c-axis where indole-3-acetate molecules are encapsulated by inorganic layers. Upon encapsulation of the IAA-inorganic hybrid within the shell of nano-sized silica particles, the crystalline phase attenuates as shown in XRD (b), suggesting that the silica deposition on the primary IAA-inorganic hybrid gives rise to a suppression of long range ordering. According to the elemental analysis and IAA content, the primary hybrid was found to be composed of 47.9 wt% ZnO_2, 41.2 wt% IAA, and 10.9 wt% H_2O. On the other hand, the silica coating on the hybrid led to a change in composition with 41.2 wt% SiO_2, 30.0 wt% ZnO, 24.0 wt% IAA, and 4.8 wt% H_2O.

Figure 2 represents the scanning electron microscopic (SEM) image of IAA-brid powders, which consist of plate-like aggregates with a particle size of 0.1 ~ 0.5 μm.

The controlled release of IAA from IAA-brid powder could be demonstrated as shown in Figure 3. The IAA molecules encapsulated in the interlayer space of inorganic layers are replaced gradually by foreign chloride anions *via* ion-exchange process in an aqueous solution of 0.08 % NaCl and released in a time-controlled manner. The released IAA is confirmed to be the pure one by comparing the UV-vis specta for both, since they show the same absorption maximum (Fig. 4).

The retention stability of pure IAA and IAA-brid powder in an aqueous solution at 42 °C was compared in Figure 5. The active IAA content in an aqueous solution of pure IAA decreases rapidly and down to < 10 % within 2 weeks. While the IAA molecules in IAA-brid remain almost constant up to 8 weeks. More than 85 % of IAA molecules are retained in this hybrid system after 8 weeks. Such an excellent stabilization of IAA is mainly due to the encapsulation of IAA molecules with inorganic nano-layer on a molecular level through the interfacial surface charge interaction between indole-3-acetate and hydrated inorganic zinc oxide layers.

Figure 6 illustrates the microscopic image of IAA-brid cream which shows that oil droplet with size of about 15 μm is uniformly dispersed in the continuous water phase.

For evaluation of efficacy of IAA-brid cream on anti-wrinkles, the replicas of outer corner of the eyes from the subjects were investigated using skin-visiometer. The skin roughness parameters measured by skin-visiometer were averaged and analyzed using a paired t-test to determine whether the effect produced by the application of IAA-brid cream was significantly different from the before application or from the application of placebo cream. The percent changes of R values before and after application were evaluated by the following equation;

$$\text{Percent change of R value (\%)} = [\,R_{\text{after application}} - R_{\text{before application}}\,] / R_{\text{before application}} \times 100$$

Figure 7 shows the percent changes of R values which are statistically significant. After 4, 8, and 12 weeks from application of IAA-brid cream, R1, R2 and R3 values are significantly reduced compared to those of before application. After 4 weeks of using IAA-cream, the skin roughness parameters (R1, R2, and R3) were drastically reduced, by approximately 13.8 %, 15.0 %, and 11.5 %, respectively, compared to before application. Finally after 12 weeks, the skin roughness parameters were further reduced to 14.9 %, 21.4 %, and 15.8 %, respectively. This suggests that the fine wrinkle in eye area is greatly improved by the administration of IAA-brid cream. The difference of efficacy between IAA-brid cream and placebo cream was evaluated by the following equation;

$$\text{Mean difference} = [\,R_{\text{after application}} - R_{\text{before application}}\,]_{\text{IAA-cream}}$$

$$- [R_{\text{after application}} - R_{\text{before application}}]_{\text{placebo-cream}}$$

Mean difference for R1, R2 and R3, and p-value depending on application period were summarized in Table 2. In comparison with IAA-brid cream and placebo cream, there is no significant difference until 4 weeks. After 8, and 12 weeks from application, statistically significant differences were observed for R1, R2, and R3 parameters in comparison with IAA-brid cream and placebo cream, representing that IAA molecules in IAA-brid cream play an important role in reducing the wrinkle.

Conclusions

IAA molecules with various biological, pharmaceutical and dermatological functions are encapsulated and immobilized successfully with bio-compatible and skin-friendly inorganic materials by wet chemical method. The encapsulated IAA (IAA-brid) shows a superior storage stability in aqueous medium compared to the pure IAA, and an excellent time-controlled releasing behavior. Through the clinical efficacy test on anti-wrinkle effect, IAA-brid has the potential to decrease the appearance of wrinkles and fine lines.

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Table 1. Recipe of o/w emulsion cream containing IAA-brid.

	IAA-brid cream	Placebo
Deionized water	60 ~ 70	60 ~ 70
Butylene glycol	3 ~ 5	3 ~ 5
Glycerine	3 ~ 5	3 ~ 5
β-Glucan	2 ~ 4	2 ~ 4
Sodium chondroitin sulfate	1 ~ 3	1 ~ 3
Glyceryl monostearate	0.5 ~ 1.5	0.5 ~ 1.5
PEG40 stearate	0.5 ~ 1.5	0.5 ~ 1.5
Cetostearyl alcohol	1 ~ 2	1 ~ 2
Stearic acid	0.5 ~1	0.5 ~1
Bees wax	1 ~ 2	1 ~ 2
Euthanol GM 2-octyldodecyl myristate	1 ~ 3	1 ~ 3
Cyclomethicone	1 ~ 2	1 ~ 2
Squalane	7 ~ 11	7 ~ 11
IAA-brid powder	0.31	-

Table 2. Evaluation of the difference of efficacy between IAA-brid cream and placebo cream depending on applied periods

Parameter	Applied Period (weeks)	Mean Difference ($\times 10^{-2}$)	Statistical Significance (p)
R1	4	2.07 ± 1.17	0.083
	8	5.30 ± 1.48	0.001*
	12	4.26 ± 1.41	0.004*
R2	4	2.19 ± 1.16	0.064
	8	8.52 ± 1.70	0.000*
	12	6.89 ± 1.80	0.000*
R3	4	0.19 ± 0.67	0.783
	8	3.41 ± 1.04	0.002*
	12	3.70 ± 1.01	0.001*

* : statistically significant at $p < 0.05$

Figure 1. Powder X-ray diffraction patterns of (a) IAA-hydrated zinc oxide hybrid and (b) IAA-brid, respectively.

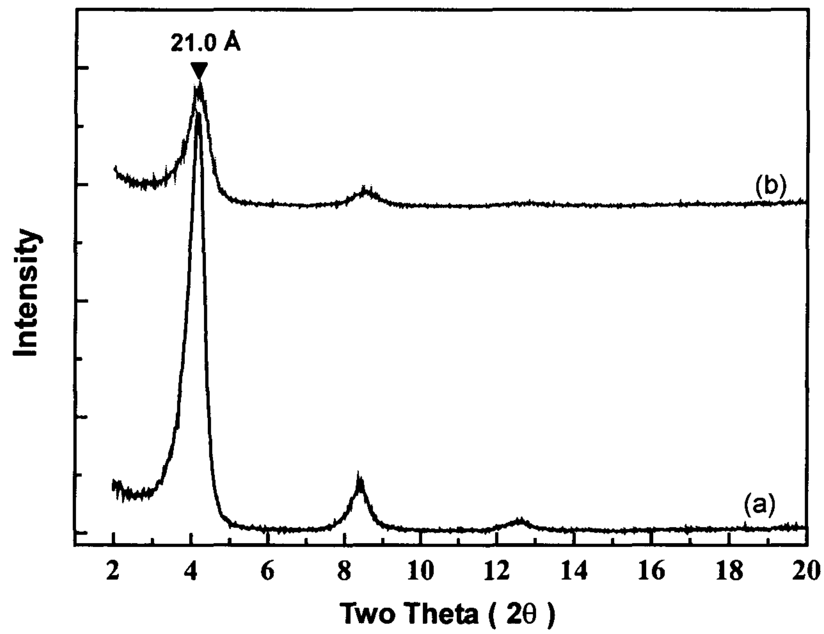

Figure 2. Scanning electron micrograph of IAA-brid powder.

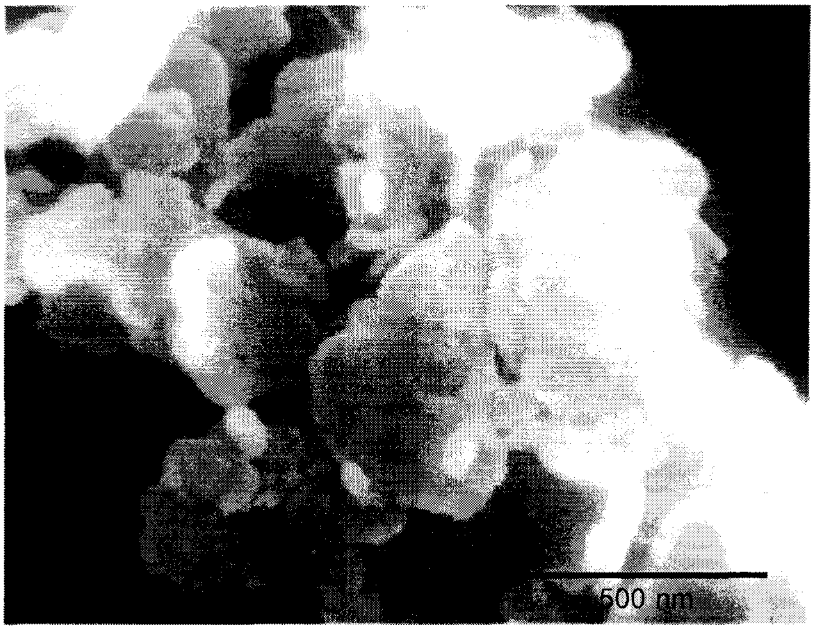

Figure 3. Time controlled releasing curve of IAA in IAA-brid powder.

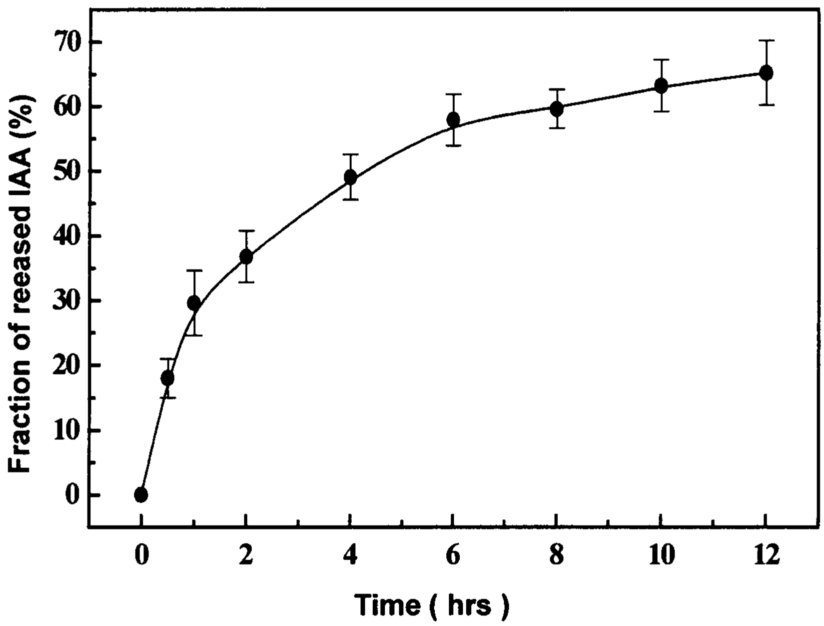

Figure 4. UV-vis spectra of (a) released IAA from IAA-brid and (b) pure IAA

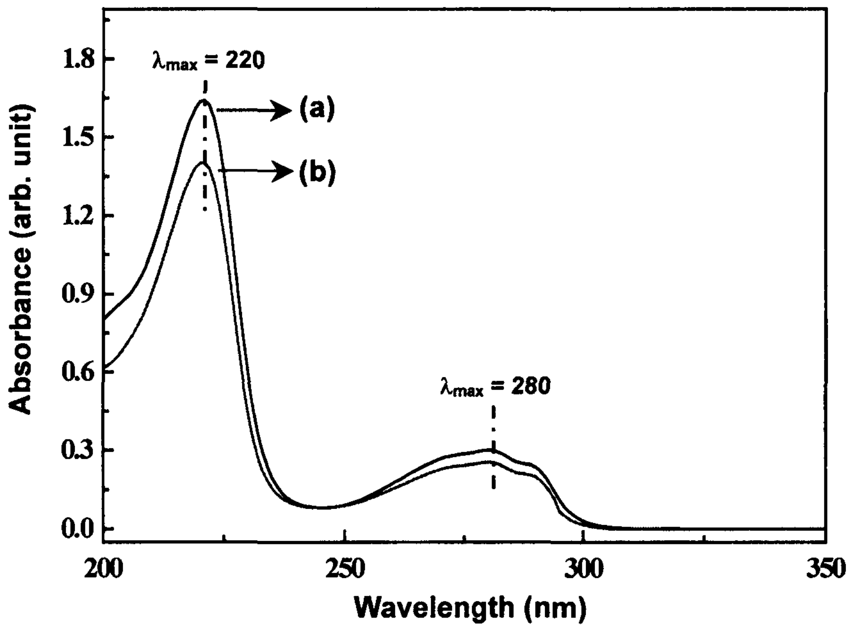

Figure 5. Retention of IAA in (a) IAA-brid and (b) pure IAA at 42 °C in aqueous medium.

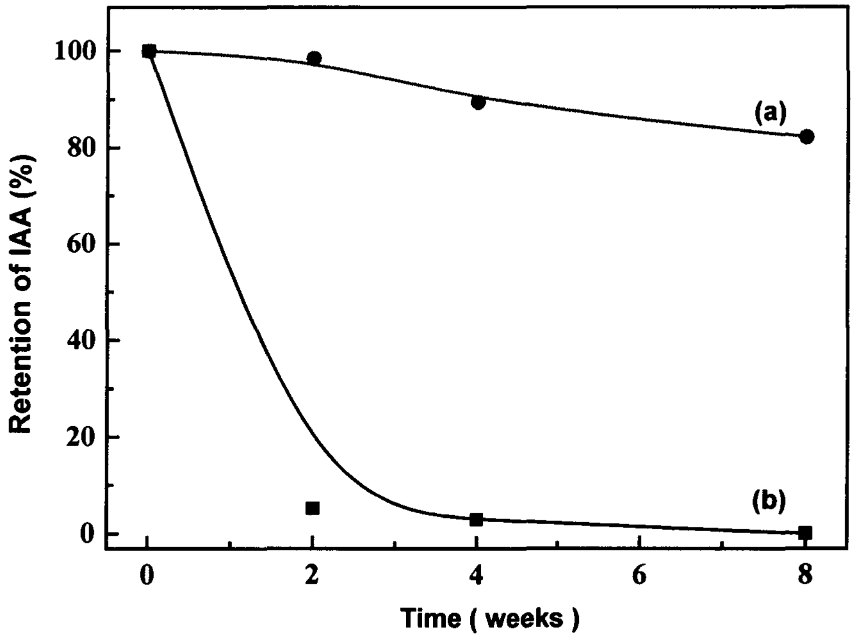

Figure 6. Microscopic image of IAA-brid cream

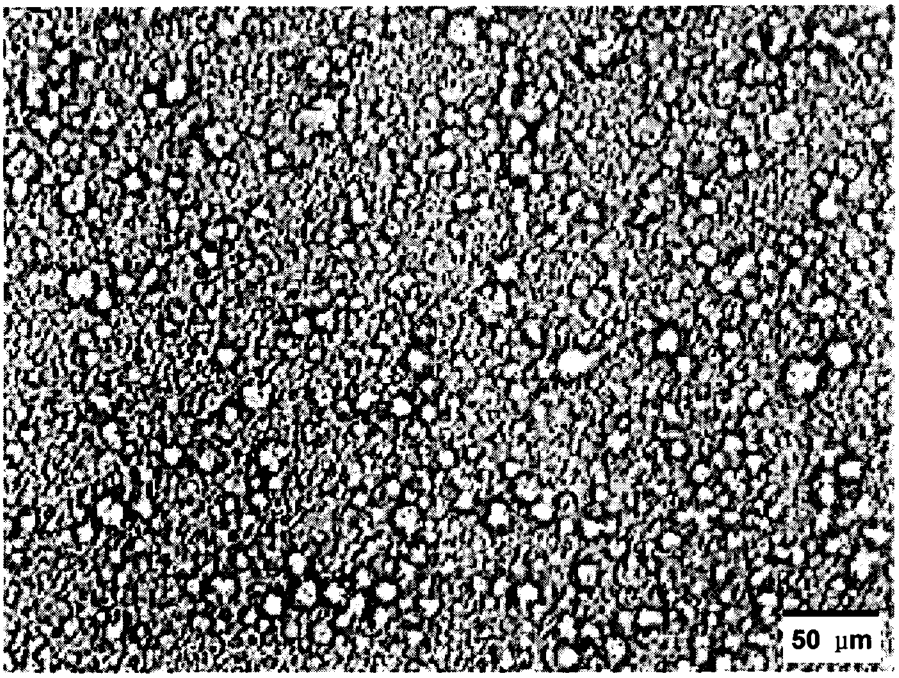

Figure 7. The percent changes of R values before and after application according to applied periods