Efficient Transdermal Penetration and Improved Stability of L-Ascorbic Acid Encapsulated in an Inorganic Nanocapsule

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Encapsulation of L-ascorbic acid (vitamin C) within a bio-compatible layered inorganic material was achieved by coprecipitation reaction, in which the layered inorganic lattice and its intercalate of vitamin C are simultaneously formed. The nano-meter sized powders of vitamin C intercalate thus prepared was again encapsulated with silica nano-sol to form a nanoporous shell structure. This ternary nanohybrid of vitamin C-layered inorganic core-SiO_2 shell exhibited an enhanced storage stability and a sustained releasing of vitamin C. Furthermore, the nano-encapsulation of vitamin C with inorganic mineral was very helpful in delivering vitamin C molecules into skin through stratum corneum, facilitating transdermal penetration of vitamin C in topical application.

Key Words : Layered inorganic material, L-Ascorbic acid, Encapsulation, Intercalation, Transdermal penetration

Introduction

L-Ascorbic acid (vitamin C), a representative water soluble vitamin, has a variety of biological, pharmaceutical and dermatological functions; it promotes collagen biosynthesis, provides photoprotection, causes melanin reduction, scavenges free radical, and enhances the immunity (anti-virus effect), etc.[1-6] These functions are closely related to the well-known antioxidant properties of this compound. Vitamin C, however, is very unstable to air, moisture, light, heat, metal ions, oxygen, and base, and it easily decomposes into biologically inactive compounds such as 2,3-diketo-L-gulonic acid, oxalic acid, L-threonic acid, L-xylonic acid, and L-Lyxonic acid.[1] Therefore, the applications of vitamin C in the fields of cosmetics, dermatologicals, and pharmaceuticals are limited despite of its useful functions. Thus to overcome chemical instability of vitamin C, extensive studies have been tried on encapsulation and immobilization of vitamin C[4,7-10] by using liposome, microemulsions (water-in-oil or oil in water), and liquid crystals. Another way of suppressing its decomposition is to derivatize the vitamin C as a salt such as ascorbyl palmitate or magnesium ascorbyl phosphate. However, the instability problem of vitamin C still remains unsolved in cosmetic, dermatological and pharmaceutical applications. More recently we found that unstable biological and drug molecules such as DNA (deoxyribonucleic acid), As-myc (c-myc antisense oligonucleotide), ATP (adenosin-5'-triphosphate), MTX (methotrexate) can be remarkably stabilized and be effectively penetrated through various cell membranes when they were encapsulated in the layered inorganic matrix.[11-14]

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In the present study, we are also very successful in demonstrating a new method of encapsulating and stabilizing the vitamin C in an inorganic layered materials like hydrated layered metal oxide with high biocompatibility and skin affinity, so that it can be applicable as the cosmetic ingredient. Here, we describe, for the first time, a novel ternary encapsulation system in detail and discuss its physico-chemical properties along with its chemical stability, controlled release behavior, biological activity, and transdermal delivery efficiency.

Experimental Section

Synthesis. The encapsulation of vitamin C with inorganic layer was achieved by a chemical coprecipitation in an aqueous solution.[15] 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 L-ascorbate species. In a typical reaction, $Zn(NO_3)_2 \cdot 6H_2O$ (15.5 g, 0.05 mol) was dissolved in a decarbonated water (300 mL), and the aqueous metal solution was added into a solution containing L-ascorbic acid (0.1 M, 200 mL). Then the pH of the reaction solution was adjusted to 6.7 ($\pm$ 0.2) by the addition of NaOH aqueous solution (0.1 M) under vigorous stirring. During the encapsulation process, nitrogen gas was flowed through the reaction solution continuously to minimize the decomposition of L-ascorbic acid and to prevent the contamination from air. The white precipitate formed by coprecipitation reaction was aged at room temperature for 12 hrs, filtered, and washed with decarbonated water thoroughly. Thus prepared core-shell particle of vitamin C-hydrated zinc oxide hybrid was encapsulated again with nano-sized silica (SiO_2) particles through the controlled hydrolysis of tetraethylorthosilicate (TEOS, $Si(OC_2H_5)_4$). In this second

encapsulation process, 10 g of the vitamin C-hydrated zinc oxide hybrid gel was firstly re-dispersed in 35 mL ethanol and 10 g of TEOS was added slowly into the suspension. After 1 hr stirring, 15 mL decarbonated water was added to the suspension to induce the hydrolysis of TEOS, which was lasted for 24 hrs under continuous stirring. The resulting product was washed with ethanol thoroughly and dried under vacuum to form vitamin C-inorganic (ZnO/SiO_2) hybrid particles (Vitabrid-C).

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 L-ascorbic acid. Prior to the measurement, all the powdery samples were dissolved in 0.1 M HCl aqueous solution to recover pure L-ascorbic acid molecules encapsulated. Then the supernatant was analyzed after the filtration through a 0.2 μm nylon filter using an absorbance maximum of λ_{max} at 245 nm, corresponding to the typical absorption peak of L-ascorbic acid in acidic solution. High performance liquid chromatography (HPLC) spectra were recorded on an Agilent 1100 Series Instrument equipped with UV detector (λ_{max} = 245 nm in acidic solution). An octadecyl-silica reversed-phase column (4.6 mm × 250 mm, Zorbax), a mobile phase containing 0.15 mM EDTA (ethylenediamintetraacetic acid) and 25 mM potassium dihydrogen phosphate (KH_2PO_4) with the pH of 2.5, a flow-rate of 1 mL/min and 20 μL of injection volume were used. Field emission-scanning electron microscopic (FE-SEM) observation for Vitabrid-C 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 vitamin C in the core-shell particle was profiled in a 0.08% NaCl aqueous solution. At first, Vitabrid-C powder (100 mg) was dispersed in 50 mL 0.08% NaCl solution and stirred at 25 °C with a rate of 50 rpm. The released amount of vitamin C was determined periodically with UV-vis spectrum using the absorption peak at 265 nm (λ_{max} of L-ascorbic acid in neutral solution).

Stability test. The stability of pure vitamin C in Vitabrid-C powder in an aqueous solution was evaluated by monitoring the retention of vitamin C at the different storage periods. For the evaluation, 400 mg of Vitabrid-C powder (containing 100 mg of vitamin C) and 100 mg of pure vitamin C (sodium L-ascorbate, 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 vitamin C was analyzed periodically with HPLC.

***In vitro* L-DOPA oxidase inhibition test**. L-DOPA oxidase inhibition activity was determined by a conventional method after minor modifications.[16-19] The 0.1 M phosphate buffer solution (PBS) of pH 6.8, 4 mM L-DOPA (β-(3,4-dihydroxylphenyl)-L-alanine) and aqueous suspension of Vitabrid-C powder with the solid content of 0.1, 1, 10, and 100 μg/mL were prepared in advance. The aqueous solutions of L-ascorbic acid (pure vitamin C) with the content of 0.1, 1, 10, and 100 μg/mL were also prepared as the reference in the L-DOPA oxidase inhibition tests. All the solutions and suspensions were stored in ice bath before the test. Each suspension (Vitabrid-C) and solution (pure vitamin C) for the test was added into 135 μL PBS and gently mixed for 5 minutes. Then 20 μL of 50 μg/mL tyrosinase was added into the solutions, followed by the addition of 40 μL of 4 mM L-DOPA to start the enzymatic reaction of L-DOPA to dopachrome at 37 °C for 10 minutes. Then the absorbance at 475 nm of dopachrome was measured. L-DOPA oxidase inhibition value was evaluated by the following equation;

$$\text{L-DOPA oxidase inhibition (\%)} = [1-\{(B-A)/(D-C)\}] \times 100$$

A & B : absorbances of the sample before and after the reaction
C & D : absorbances of the blanks before and after the reaction

DPPH assay. The free radical scavenging activity of Vitabrid-C and pure vitamin C was evaluated by the previously reported method after minor modifications.[4,20-22] The free radical scavenging activity of Vitabrid-C and L-ascorbic acid was assayed using a relatively stable free radical, DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl). 70% ethanol, 0.15 mM DPPH solution, aqueous suspensions of Vitabrid-C powder with the solid content of 0.1, 1, 10, and 100 μg/mL, and pure vitamin C solutions of 0.1, 1, 10, and 100 μg/mL were stored in an ice bath, respectively. 5 μL of the test samples (Vitabrid-C suspensions or pure vitamin C solutions) were added into 115 μL of 70% ethanol, separately. After mixing the solution homogeneously, 80 μL of 0.15 mM DPPH solution was added into the solutions, respectively. Then the redox reaction was carried out at room temperature for 20 minutes, and the free radical scavenging activity of each antioxidant was quantified by comparing the change of absorbance at 517 nm. Radical scavenging activity was evaluated by the following equation;

$$\text{Radical scavenging activity (\%)} = [1-\{(B-A)/(D-C)\}] \times 100$$

A & B : absorbance of the samples before and after the reaction
C & D : absorbance of the blanks before and after the reaction

Transdermal transfer test. The passive permeability of vitamin C in Vitabrid-C powder, w/o emulsion containing Vitabrid-C and o/w emulsion containing L-ascorbic acid was investigated by Franz diffusion cell method[23-25] using hairless mouse skin with an effective diffusional area of 0.64 cm^2. The skin samples were hydrated in advance in PBS for 15 minutes before setting them to the chambers. Receiver compartment vehicle consisted of 5.2 mL of 0.0001% dithiothreitol (DTT), 0.00042% EDTA, and 25 mM potassium dihydrogen phosphate buffer solution (pH 4.5). 0.04 g of Vitabrid-C powder and 1 g of emulsion samples were added into each donor compartment, respectively, and sealed with parafilm to prevent evaporation. In case of Vitabrid-C powder, a few drops of 0.8 wt% NaCl aqueous solution was added into the donor compartment to wet the powder sample, thus to mimic an artificial sweat condition on human skin. Permeated L-ascorbic acid was withdrawn from receiver

(100 μL) chambers periodically and analyzed by HPLC. And then the same volume of pure vehicle was added to the receiver to maintain a constant volume.

Results and Discussion

The encapsulation of L-ascorbic acid with inorganic layer was achieved by chemical coprecipitation in an aqueous solution. L-ascorbic acid is a weak acid ($pK_1 = 4.17$),[1] and it becomes deprotonated in aqueous solution at the pH > 4.17 to form an anionic L-ascorbate species. Since the hydrated zinc oxide has a positive surface charge, the anionic ascorbate molecules would be adsorbed onto the inorganic surface during coprecipitation, leading to the encapsulation of vitamin C molecule in the inorganic layers.

Figure 1 shows the X-ray diffraction patterns of the vitamin C- hydrated zinc oxide hybrid obtained during the first encapsulation process (a) and the silica modified one (Vitabrid-C) (b). As can be seen from Figure 1(a), primary L-ascorbic acid-inorganic hybrid shows a layer character with the basal spacing of 14.5 Å. This suggests that L-ascorbate anions are inserted between the zinc hydroxide sheets to form an intercalate with 1 : 1 layer sequence along the c-axis where L-ascorbate molecules are encapsulated by inorganic layers as depicted in the inset. Upon encapsulation of the L-ascorbic acid-inorganic hybrid within the shell of nano-sized silica particles, the crystalline phase disappears as shown in XRD (b), suggesting that the silica deposition on the primary L-ascorbic acid-inorganic hybrid gives rise to a drastic suppression of long range ordering. According to the elemental analysis and vitamin C content, the primary hybrid was found to be composed of 49.0 wt% ZnO_2, 42.1 wt% vitamin C, and 8.9 wt% H_2O. On the other hand, the silica coating on the hybrid led to a change in composition with 40.7 wt% SiO_2, 29.0 wt% ZnO, 25.0 wt% vitamin C, and 5.3 wt% H_2O.

Figure 2 represents the scanning electron micrograph (SEM) image of Vitabrid-C powders, which consist of spherical aggregates with a homogeneous particle size of ~0.5 μm and size distribution. In addition, one can see clearly nano-sized silica particles to form nanoporous shell structure.

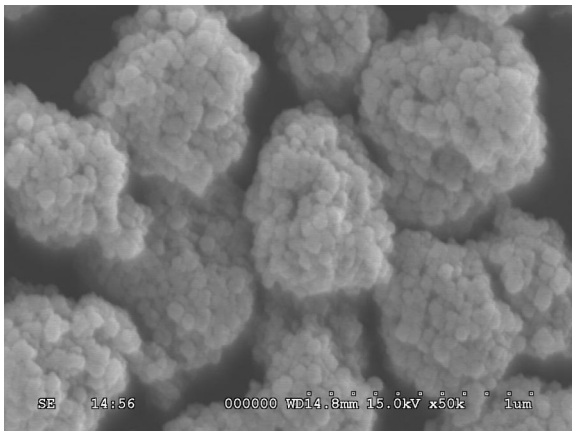

Figure 2. Scanning electron micorgraph of Vitabrid-C powder.

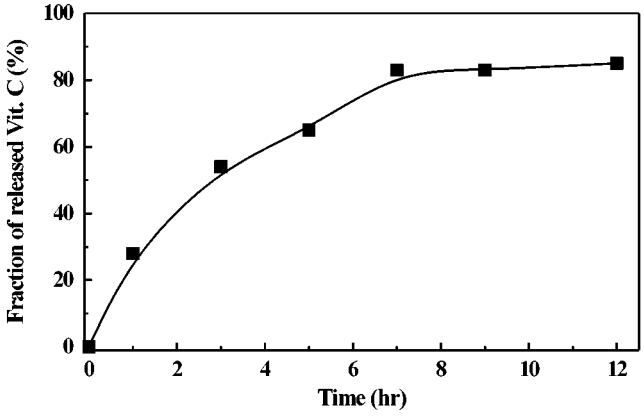

Figure 3. Time controlled releasing curve of vitamin C in Vitabrid-C powder.

The controlled release of vitamin C from Vitabrid-C powder could be demonstrated as shown in Figure 3. The vitamin C 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

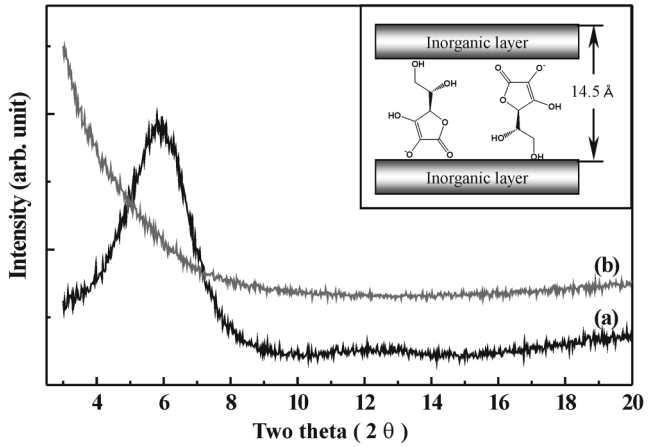

Figure 1. Powder X-ray diffraction patterns of (a) vitamin C-hydrated zinc oxide nanohybrid and (b) Vitabrid-C, respectively.

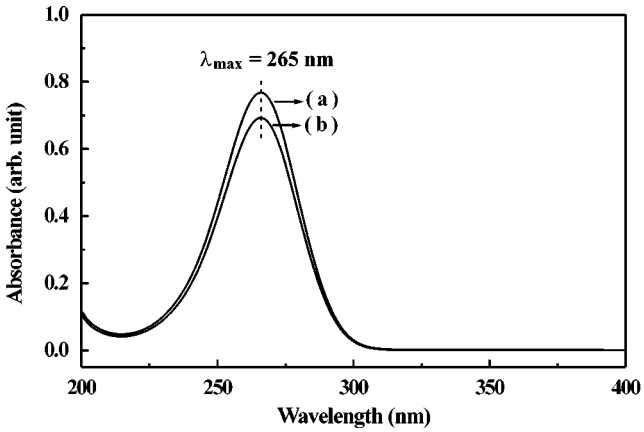

Figure 4. UV-vis spectra of (a) released vitamin C from Vitabrid-C and (b) pure vitamin C (sodium L-ascorbate).

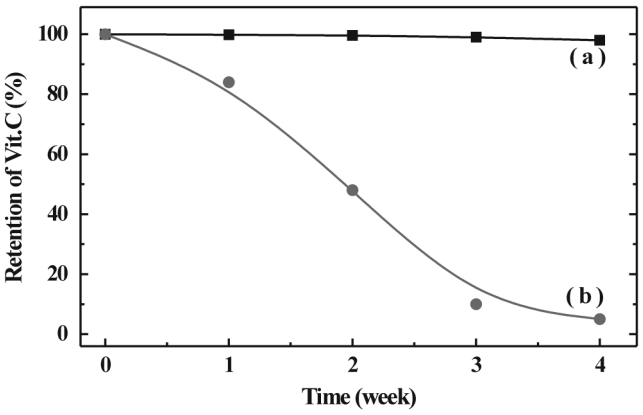

Figure 5. Retention of vitamin C in (a) Vitabrid-C and (b) pure vitamin C (sodium L-ascorbate) at 42 °C in aqueous medium.

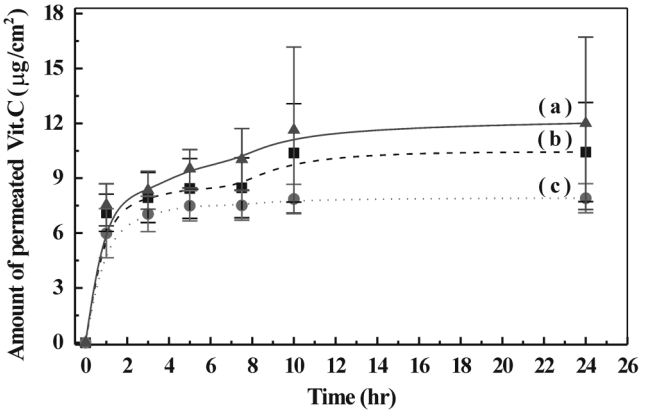

Figure 6. Skin permeation profiles of vitamin C in (a) Vitabrid-C powder, (b) Vitabrid-C-containing w/o emusion, and (c) pure vitamin C-containing o/w emulsion, respectively.

0.08% NaCl and released in a time-controlled manner. The released vitamin C is comfirmed to be the pure one by comparing the UV-vis specta for both, since they show the same absorption maximum (Fig. 4).

Figure 5 compares the retention stability of pure vitamin C and Vitabrid-C powder in an aqueous solution at 42 °C. The active vitamin C content in an aqueous solution of pure vitamin C decreases rapidly and down to < 10% within 4 weeks. While the vitamin C molecules in Vitabrid-C remain almost constant up to 4 weeks. More than 95% of vitamin C molecules are retained in this hybrid system after 4 weeks. Such an excellent stabilization of vitamin C is mainly due to the encapsulation of vitamin C molecules with inorganic nano-layer on a molecular level through the interfacial surface charge interaction between L-ascorbate and inorganic zinc hydroxide layers.

The results of L-DOPA oxidase inhibition activity test and radical scavenging activity one (DPPH assay) for Vitabrid-C and pure vitamin C are summarized in Table 1. According to the L-DOPA oxidase inhibition activity test, Vitabrid-C shows the inhibition activity equivalent to pure vitamin C. Vitabrid-C has the activities of 46.8% and 99.7% when the Vitabrid-C contents are 10 μg/mL and 100 μg/mL, respectively. The value of ID_{50}, a critical concentration of sample required to reduce the L-DOPA oxidase activity to 50%, of Vitabrid-C is determined to be 15.4 μg/mL which is very close to the ID_{50} value for pure vitamin C of 16.9 μg/mL. Therefore, it is evident that the vitamin C encapsulated by inorganic matrix has a biological activity equivalent to pure vitamin C.

The antioxidant potentials of Vitabrid-C and pure vitamin C were also evaluated by measuring the DPPH radical concentration, and found to be the same irrespective of the concentration, indicating that Vitabrid-C could play an effective role as the radical scavenger. From the L-DOPA oxidase inhibition and DPPH tests, it is concluded that vitamin C molecules are encapsulated safely by inorganic materials without loss of their biological activity.

The profiles of transdermal transport of vitamin C in Vitabrid-C powder (a), Vitabrid-C-containing w/o emulsion (b), and pure vitamin C-containing o/w emulsion (c) are shown in Figure 6. The cumulative amount of vitamin C permeated though the skin is expressed per cm^2 in the graph. The overall features of permeation patterns are quite similar one another, suggesting the similar penetration mechanism irrespective of the sample forms. However, the absolute amounts of permeated vitamin C after 24 hrs are more or less different with the following order; Vitabrid-C powder (12.0 μg/cm^2) > Vitabrid-C-containing w/o emulsion (10.4 μg/cm^2) > pure vitamin C-containing o/w emulsion (7.9 μg/cm^2). This indicates clearly that the inorganically encapsulated vitamin C shows higher penetration rate than the pure vitamin C. Though the delivery mechanism should be further studied, it becomes evident that the encapsulation of vitamin C in an inorganic nano-capsule and its controlled release behavior are important factors for the effective penetration of vitamin C molecules through the skin barrier.

Table 1. DOPA oxidase inhibition activity and radical scavenging activity of Vitabrid-C and pure vitamin C

Concentration	L-DOPA Oxidase Activity (%)		DPPH Test (%)	
	Pure vitamin C (Reference)	Vitabrid -C	Pure vitamin C (Reference)	Vitabrid -C
0.1 μg/mL	9.9	8.7	2.6	0.8
1 μg/mL	20.5	22.9	11.2	11.5
10 μg/mL	45.8	46.8	69.5	68.2
100 μg/mL	100	99.7	88.3	88.3

The proposed releasing and delivering mechanism of vitamin C molecules in Vitabrid-C is schematically represented in Figure 7. In Vitabrid-C, the vitamin C molecules are adsorbed and immobilized between inorganic layers with positive surface charge, and further coated with nano-sized silica particles, forming a nanoporous shell structure. Due to its well developed nanoporous structure, the Vitabrid-C absorbs effectively the skin wastes, sebums, and sweats discharged from the human skin. Actually, the Vitabrid-C shows a large oil adsorption capacity more than 150%.[26] The absorption of chemical species such as NaCl and fatty acids in sweat and skin wastes into the nanopores of Vitabrid-C

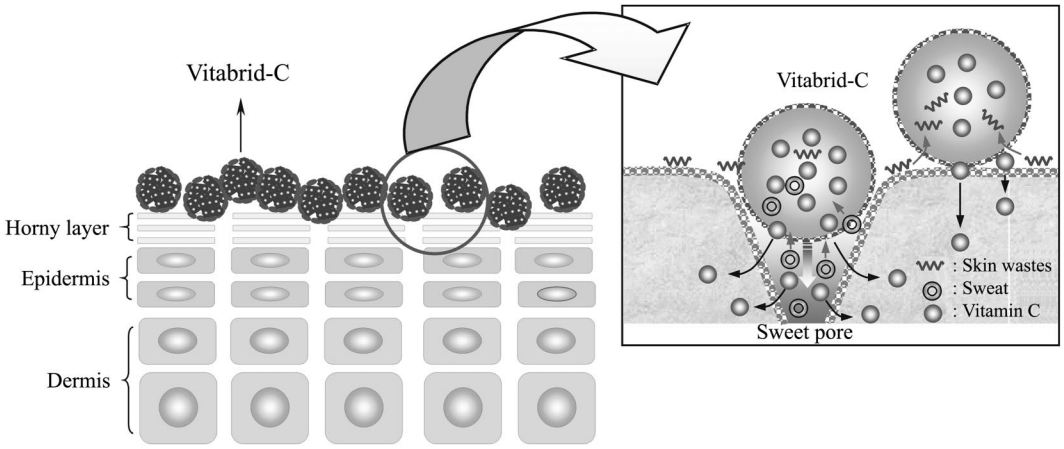

Figure 7. The proposed releasing and delivering mechanism of vitamin C in Vitabrid-C.

gives rise to a release of vitamin C in the pore by the exchange reaction between them, in such a way the vitamin C molecules could be slowly diffused out from the inorganic shell and delivered into the epidermis in skin.

Conclusion

L-Ascorbic acid 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 vitamin C (Vitabrid-C) shows a superior storage stability in aqueous medium compared to the pure sodium L-ascorbate, and an excellent time-controlled releasing behavior. Furthermore the Vitabrid-C has a biological activity (L-DOPA oxidase inhibition and free radical scavenging activity) equivalent to the pure L-ascorbic acid. It is, therefore, quite evident that the reversible inclusion and the time controlled release properties of vitamin C stabilized in Vitabrid-C are important factors for allowing the vitamin C molecules to penetrate the skin barrier effectively thanks to the nanosized inorganic delivery carrier.

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References

1. Machlin, L. J. *Handbook of Vitamins*, 2nd Ed.; Marcel Dekker, Inc.: 1991.
2. Doba, T.; Burton, G. W.; Ingold, K. U. *Biochim. Biophys. Acta* **1985**, *835*, 298.
3. Bossi, A.; Piletsky, S. A.; Piletska, E. V.; Righetti, P. G.; Turner, A. P. F. *Anal Chem.* **2000**, *72*, 4296.
4. Yamamoto, I.; Tai, A.; Fujinami, Y.; Sasaki, K.; Okazaki, S. *J. Med. Chem.* **2002**, *45*, 462.
5. Tsuchiya, H.; Bates, C. J. *J. Nurt. Biochem.* **1998**, *9*, 402.
6. Horino, Y.; Takahashi, S.; Miura, T.; Takahashi, Y. *Life Science* **2002**, *71*, 3031.
7. Gallarate, M.; Carlotti, M. E.; Trotta, M.; Bovo, S. *Int. J. Pharm.* **1999**, *188*, 233.
8. Austria, R.; Semenzato, A.; Bettero, A. *J. Pharm. Biomed. Anal.* **1997**, *15*, 795.
9. Spiclin, P.; Gasperlin, M.; Kmetec, V. *Int. J. Pharm.* **2001**, *222*, 271.
10. Semenzato, A.; Austria, R.; Dall'Aglio, C.; Bettero, A. *J. Chromatogr. A* **1995**, *705*, 385.
11. Choy, J. H.; Kwak, S. Y.; Park, J. S.; Jeong, Y. J. *Angew. Chem. Int. Ed.* **2000**, *39*(22), 4042.
12. Choy, J. H.; Kwak, S. Y.; Park, J. S.; Jeong, Y. J.; Portier, J. *J. Am. Chem. Soc.* **1999**, *121*, 1399.
13. Choy, J. H.; Kwak, S. Y.; Park, J. S.; Jeong, Y. J. *J. Mater. Chem.* **2001**, *11*(6), 1671.
14. Choy, J. H.; Park, J. S.; Kwak, S. Y.; Jeong, Y. J.; Han, Y. S. *Mol Cryst. & Liq. Cryst.* **2000**, *341*, 425.
15. Hwang, S. H.; Han, Y. S.; Choy, J. H. *Bull. Korean Chem. Soc.* **2001**, *22*, 1019.
16. Riley, P. A. *Cell. Mol. Biol.* **1999**, *45*, 951.
17. Han, W. S.; Yoo, J. Y.; Youn, S. W.; Kim, D. S.; Park, K. C.; Kim, S. Y.; Kim, K. H. *J. Dermatol. Sci.* **2002**, *30*, 10.
18. Kubo, I.; Kinst-Hori, I. *J. Agric. Food Chem.* **1999**, *47*, 4121.
19. Jimenez, M.; Chazarra, S.; Escribano, J.; Cabanes, J.; Garcia-Carmona, F. *J. Agric. Food Chem.* **2001**, *49*, 4060.
20. Tachibana, Y.; Kikuzaki, H.; Lajis, N. H.; Nakatani, N. *J. Agric. Food Chem.* **2001**, *49*, 5589.
21. Kim, D. O.; Lee, K. W.; Lee, H. J.; Lee, C. Y. *J. Agric. Food Chem.* **2002**, *50*, 3713.
22. Sawai, Y.; Moon, J. H. *J. Agric. Food Chem.* **2000**, *48*, 6247.
23. Simonsen, L.; Petersen, M. B.; Groth, L. *Eur. J. Pharm. Sci.* **2002**, *17*, 95.
24. Wissing, S. A.; Muller, R. H. *J. Control. Release* **2002**, *81*, 225.
25. Youenang Piemi, M. P.; De Luca, M.; Grossiord, J. J.; Seiller, M.; Marty, J. P. *Int. J. Pharm.* **1998**, *171*, 207.
26. Oil adsorption capacity was measured by KS (Korean Industrial Standard) method.