Microencapsulation of Ascorbic Acid for Cosmetic by Utilizing Self-assembly of Phase Separated Polymer

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Micrometer-sized polymer particles encapsulated ascorbic acid (vitamin C; VC) were successfully prepared by the three types of the self-assembling method, those are, phase separation and self-assembly of added polymer at the oil–water interface in emulsion, microsuspension polymerization utilizing the self-assembly of phase separated polymer (SaPSeP) method, and their hybrid method. In the stability study at 50°C for 2 months, the three kinds of capsule particles exhibited effective protection of VC from the interaction with other components in cosmetic consisting of water-in-oil (W/O) emulsion. The encapsulated VC was easily released from the capsule particles by an excess of water. These encapsulation methods will be useful for the stabilization of water-soluble substances in cosmetic consisting of W/O emulsion.

Key words microencapsulation; self-assembly; ascorbic acid; microsuspension polymerization

Vitamin C (VC) has useful effects such as anti-inflammatory effect,1) improvement of acne,2) whitening effect, anti-aging effect,3) scavenger of activated oxygen,4) and the promotion of collagen synthesis.5) The compounding of VC in cosmetic is very effective for skin care and beneficial for users. However, it is very difficult in cosmetic containing components having amino group and foundation containing iron oxide pigment, because the color of cosmetic is changed with aging. As a method for stabilization of VC, the encapsulation has been investigated by various approaches, for examples, encapsulation into liposomes,6) water-in-oil-in-water (W/O/W) emulsification using microchannel,7,8) preparation of solid-in-oil suspension,9–11) and coating by spray chilling12) and spray dry.13) Since the above shell walls are generally weak and starting materials aren’t simple, it seemed of interest to encapsulate VC by common plastic shell wall using convenient methods in terms of adequate protection of VC in cosmetic.

The encapsulation by self-assembly is useful for versatile substances. Because radical polymerization14) and polycondensation15) aren’t available in many cases. Various encapsulation by self-assembly has been carried out by the formation of micelles, vesicles, nanoparticles, nanospheres or nanogels by polyion complex,15,16) a pair of copolymers,17) amphiphilic block copolymers,18–21) graft copolymers,22) amphiphilic protein,23) or cholesterol-bearing pullulan24) in an aqueous solution. But there has been no previous report on the encapsulation by commonly available homopolymer like poly(methyl methacrylate) (PMMA), which is too hydrophobic and precipitates immediately in an aqueous solution. Uyama and colleagues reported it in the simple preparation of monolith that PMMA is soluble in 80% aqueous ethanol (EtOH) at 80°C.25) We have applied it to the encapsulation by self-assembly of PMMA at oil–water interface. The method using PMMA may be a novel example among various encapsulation by self-assembly hitherto reported.15–24)

Since VC is unstable in oxidation, little attention has been paid to the encapsulation of it by radical polymerization which is simple and convenient. The encapsulation by microsuspension polymerization utilizing the self-assembly of phase separated polymer (SaPSeP) method in oil-in-water (O/W) emulsion was developed by Okubo et al.26,27) and applied to the preparation of capsule particles for cosmetic.28,29) We have further extended this technique in water-in-oil (W/O) emulsion and performed the encapsulation of VC. Furthermore, we have carried out the encapsulation of VC by the self-assembly of PMMA accompanied with microsuspension polymerization to reinforce the shell wall.

This paper is mainly concerned with the microencapsulation of VC by utilizing three types of self-assembly of phase separated polymer and the estimation of encapsulation by the stability studies of foundations consisting of W/O emulsion containing encapsulated VC.

Experimental

Materials Soybean oil (saponification value, 188–195; iodine value, 123–142; Wako Pure Chemical Industries, Ltd., Japan), polyethylene glycol (PEG) (PEG 600; average molecular weight, 560–640; Wako Pure Chemical Industries, Ltd.), poly(vinyl alcohol) (PVA) (Gohsenol EG-05; degree of polymerization, 600; degree of saponification, 86.5–89%; The Nippon Synthetic Chemical Industry Co., Ltd., Japan), and PMMA (average molecular weight, ca. 800000; Tokyo Chemical Industry Co., Ltd., Japan) were obtained commercially. Tetraclosein ricinoleate (Poem PR-100; Takeda Chemical Industries Co., Ltd., Japan) and polyethylene glycol #400 dimethacrylate (PEGDM) (NK Ester 9G; Shin-Nakamura Chemical Co., Ltd., Japan) were generously provided. All other reagents were of reagent grade and were used without further purification. Chifure Make-up Foundation N (Chifure Corporation, Japan) was used as a base in the formulation of foundation.
Particles were observed with a Microscope built-in Micromanipulator Axis Pro SS and the particle size distribution was estimated with a Horiba laser scattering particle size distribution analyzer Partica LA-950VA.

**Preparation of Capsule Particles (A) by Self-assembly of PMMA**

The procedure is illustrated in Chart 1. Water (7.0 g), VC (2.0 g), PEG (400 mg), PVA (200 mg), soybean oil (20 g), and Poem PR-100 (1.0 g) were placed in a 100 mL three-necked round flask equipped with a mechanical stirrer, a thermometer, and a distillation apparatus. The mixture was stirred at 500 rpm for 5 min at room temperature and heated up to 80°C with stirring at 200 rpm. To the emulsion warmed at 80°C, the homogeneous solution composed of EtOH (8.0 g), water (2.0 g) and PMMA (400 mg) heated at 80°C was added at 80°C with stirring at 200 rpm. The mixture was stirred at 95°C under stirring at 200 rpm for 30 min to remove an excess (5 mL) of EtOH, further stirred at 200 rpm for 1 h at room temperature, and then allowed to stand overnight. Thirty-three point five gram of the dispersion containing (A) was obtained after decantation. The dispersion was used as a raw material without isolation of (A).

**Preparation of Capsule Particles (B) by Microsuspension Polymerization Utilizing the SaPSeP Method**

The procedure is illustrated in Chart 2. Water (7.0 g), VC (1.5 g), PEG (400 mg), PVA (200 mg), PEGDM (1.0 g), V-501 (20 mg), MeOH (400 mg), soybean oil (20 g), and Poem PR-100 (1.0 g) were placed in a 100 mL three-necked round flask equipped with a mechanical stirrer and a thermometer. The mixture was stirred at 500 rpm for 5 min at room temperature, polymerized at 85°C for 2 h 30 min under stirring at 200 rpm, further stirred at 200 rpm for 1 h at room temperature, and then allowed to stand overnight. Twenty-nine point eight gram of the dispersion containing (B) was obtained after decantation. The dispersion was used as a raw material without isolation of (B).

**Preparation of Capsule Particles (C) by the Hybrid Method**

The procedure is illustrated in Chart 3. Water (7.0 g), VC (2.0 g), PEG (400 mg), PVA (200 mg), soybean oil (20 g), and Poem PR-100 (1.0 g) were placed in a 100 mL three-necked round flask equipped with a mechanical stirrer, a thermometer and a distillation apparatus. The mixture was
stirred at 500 rpm for 5 min at room temperature and heated up to 80°C with stirring at 200 rpm. PEGDM (1.0 g) and V-501 (20 mg) were added to the homogeneous solution composed of EtOH (8.0 g), water (2.0 g), and PMMA (400 mg) heated at 80°C, and further added to the emulsion warmed to 80°C in the three-necked round flask. The mixture was heated at 95°C under stirring at 200 rpm for 30 min to remove 5 mL of EtOH, further polymerized at 85°C for 2 h 30 min under stirring at 200 rpm, then stirred at 200 rpm for 1 h at room temperature, and allowed to stand overnight. Thirty-four point two gram of the dispersion containing (C) was obtained after decantation. The dispersion was used as a raw material without isolation of (C).

**Determination of VC in Dispersions Containing Capsule Particles** Water (20 mL) was added to dispersion measured precisely (ca. 1.0 g) containing capsule particles, and the mixture was extracted with diethyl ether from dispersion containing capsule particles, the water extract was subjected to iodometry14) (see Experimental). 

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\text{VC content of dispersion (\%)} = \frac{100}{\text{theoretical}}
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The mixture was heated at 95°C under stirring at 200 rpm for 30 min to remove 5 mL of EtOH, further polymerized at 85°C for 2 h 30 min under stirring at 200 rpm, then stirred at 200 rpm for 1 h at room temperature, and allowed to stand overnight. Thirty-four point two gram of the dispersion containing (C) was obtained after decantation. The dispersion was used as a raw material without isolation of (C).

**Results and Discussion** Figure 1 shows optical micrographs of the three kinds of capsule particles, indicating core-shell structure and support size distribution and \(d_n\) (number-average diameter) measured by a particle size distribution analyzer (Table 1). Each dispersion containing capsule particles was separated into capsule particles and dispersion medium by a glass microfiber filter. With the addition of a few drops of EtOH containing iodine to each of capsule particles and the dispersion medium respectively, the former showed a loss of color derived from iodine, but the latter didn’t show it. The evidence and optical micrographs supported that VC was encapsulated in the core part of capsule particles. The VC contents of dispersions containing capsule particles were almost coincident with the values calculated from the amount of VC used in the encapsulations, indicating that VC didn’t decompose in the process of encapsulation (Table 1). For the estimation of encapsulation of VC, four kinds of foundations consisting of W/O emulsion containing VC, dispersions containing (A), (B), and (C) respectively, were prepared (Table 2) and their stabilities were
estimated at 50°C for 2 months. After 2 months, liquid phase was formed upon the foundations. Though the top part of the foundation blended VC was discolored to dark brown from beige, that of the foundation containing (A) was discolored to slightly brown and those of the foundations containing (B) and (C) were hardly discolored (Fig. 2). The stabilities of VC in the foundations were also estimated by the comparison of VC content of foundation stored at 50°C and that stored at 0°C as a control. Remaining percent of VC in the foundations containing VC, (A), (B), and (C) were, respectively, 53.6, 84.5, 91.5, and 95.7%, suggesting effective stabilization of VC by the encapsulations. (B) and (C) were superior in the stabilization of VC to (A), indicating that the shell walls of the former formed by the encapsulation accompanied with cross-linking polymerization in the inside of the shell are stronger than that of the latter formed only by diffusion and adsorption of phase-separating polymer to water-oil interface. Encapsulated VC could be quantitatively extracted with an excess of water from the capsule particles (Experimental section, Table 1), indicating the possibility of release of VC from the capsule particles by the action of moisture or sweat after the administration on the skin. But the release of VC from the capsule particles and the absorption of it in vivo are not clear.

Preparation of Capsule Particles (A) by Self-assembly of PMMA In the preliminary experiments, the following findings have been obtained. By the addition of hot homogeneous aqueous EtOH containing PMMA into the mixture of water and soybean oil, PMMA was precipitated in the aqueous phase containing EtOH. PVA and PEG are water-soluble and aren’t miscible with PMMA. The addition of them to an aqueous solution not only stabilizes water droplets in the emulsion, but makes PMMA to oil–water interface diffused as promoting agents for phase-separation. Further improvements for the appropriate release from capsule particles and the absorption of VC into the corium (skin) are necessary. However, the use of simple starting materials and the convenient methods make the procedures synthetically viable for the encapsulation of VC.

Conflict of Interest The authors declare no conflict of interest.

References
6) Wechtersbach L., Ulrich P. N., Cigic B., Food Sci. Technol., 45,
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