Encapsulation of an ultraviolet filter into cationic polystyrene particles via emulsion polymerization with poly(vinyl alcohol)

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Abstract

Highly effective organic ultraviolet (UV) filters, such as diethylamino hydroxybenzoyl hexyl benzoate (DHHB), are widely used in sunscreens; however, they have remarkably low solubility in water, which limits their use in water-based cosmetics. In this study, DHHB-encapsulated cationic polystyrene particles (PS-DHHB) dispersed in water were prepared via emulsion polymerization. Before polymerization, an emulsifying styrene monomer phase containing dissolved DHHB and a water phase containing low-molecular-weight poly(vinyl alcohol) were prepared, and subsequent polymerization yielded PS-DHHB particles. Crucially, the addition of polystyrene (PS) to the monomer phase contributed to a high solid content, and PS served as a stabilizer, enhancing the encapsulation efficiency of DHHB. Notably, the synthesized PS-DHHB exhibited UV absorption in a range similar to that of DHHB. Furthermore, even when aqueous alcohol (used as an antibacterial agent) was added, no DHHB was leached from the PS-DHHB particles. Finally, we found that the cationic PS-DHHB particles suppressed hair protein loss induced by UV light (320–400 nm) more effectively than cationic PS particles without DHHB. In summary, water-dispersed PS-DHHB particles exhibiting effective UV absorption and adsorption onto hair were developed, and these particles have applications in polymer science and cosmetics.

1. Introduction

For humans and many animals, exposure to sunlight is physiologically important because it promotes the production of vitamin D \[1\], sets circadian rhythms \[2\], and improves mood \[3\]. However, prolonged exposure to sunlight could result in overexposure to ultraviolet (UV) rays, increasing the risk of sunburn and skin cancers \[4\]. To minimize the risks from UV rays, organic UV filters that absorb UVB (280–320 nm) and UVA (320–400 nm) light have been developed and are used in numerous products in daily use, including cosmetics \[5\]. As aromatic moieties often absorb UV rays, organic UV filters are often polar oils or insoluble solids. Consequently, they are not easily dissolved or uniformly dispersed in water, rendering their use in water-rich cosmetic formulations challenging. However, miniemulsion polymerization allows for the efficient preparation of aqueous dispersions of polymer particles with encapsulated materials \[6, 7\]. These particles are produced by the emulsification of an oil phase comprising a monomer (e.g., styrene) dispersed or dissolved in an aqueous phase with a surfactant and polymerization using a water-soluble radical initiator \[8, 9\]. To date, several studies have employed the miniemulsion polymerization technique to prepare UV-filter-encapsulating particles \[10–12\].

However, the current method for encapsulating UV filters by miniemulsion polymerization has few limitations. In particular, before polymerization, the monomer and water phases must be emulsified under ultrasonic or high-pressure conditions, which requires large amounts of energy and special equipment. Emulsification also requires the use of large amounts of emulsifiers, such as sodium dodecyl sulfate (SDS), which causes skin irritation as a result of its carryover into the final cosmetic product \[13–15\]. Further, even if the emulsifier is removed by washing, the effluent would be harmful to the environment. Therefore, the development of an emulsion polymerization process that employs sustainable materials...
and enhances the energy efficiency, reduces the need for special equipment, and achieves emulsification without environmentally harmful or toxic compounds is crucial.

Polymer-based emulsifiers such as poly(vinyl alcohol) (PVA) have been used for decades to stabilize emulsions [16–20]. PVA is a synthetic polymer with a range of industrial, commercial, and medical applications; further, it is safe for humans [21] and biodegradable [22]. However, most emulsion polymerization techniques that employ PVA as a stabilizer involve the synthesis of polyvinyl acetate emulsions, and only a few studies have been conducted with other polymers, such as polystyrene. Moreover, most reports to date have been limited to anionic particles, mainly because anionic radical initiators were used. For cosmetic applications, including sunscreens, UV-filter-encapsulating particles need to be adsorbed on biological surfaces, such as hair, to form a film that blocks UV rays; retaining UV absorbers on hair fiber surfaces via electrostatic interactions is an important strategy used in sunscreens. Positively charged sunscreen ingredients are desirable because of their high affinity toward anionic hair fiber surfaces [23]. However, only a few studies on cationic UV absorbers have been reported [24]. In this study, we prepared cationic polystyrene particles encapsulating a UVA filter (diethylamino hydroxybenzoyl hexyl benzoate [DHHB]) using low-molecular-weight PVA and a cationic radical initiator. We characterized the polystyrene particle size, size distribution, and surface charge of the polystyrene particles encapsulating DHHB (PS-DHHB). Accordingly, we evaluated the DHHB content within PS-DHHB and the UV absorbance of PS-DHHB to assess its potential as a UV filter. Further, the leaching of DHHB from the PS-DHHB particles in aqueous alcohol was tested because such solutions are frequently used as antibacterial agents in cosmetics. Subsequently, the differences in the adsorption of cationic and anionic PS-DHHB particles to hair fibers were determined. Finally, we evaluated hair protein loss by UVA irradiation under PS-DHHB or PS-treated conditions.

2. Material and methods

2.1. Materials

Water treated by reverse osmosis and electrodeionization (RO plus EDI) was used in all experiments. Acetonitrile, chloroform, ethanol (99.5%), potassium peroxodisulfate (KPS), tetrahydrofuran (THF), and 2,2’-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) were procured from Fujifilm Wako Pure Chemical Corporation, Japan. DHHB, styrene, 1,2-pentanediol, 1,2-hexanediol, and 1,3-butanediol were procured from Tokyo Chemical Industry Corporation, Japan. Dulbecco’s phosphate-buffered saline (DPBS, catalog number: 14190-144), Pierce™ BCA Protein Assay Kit (catalog numbers: 23225 and 23227), and Pierce™ Bovine Serum Albumin (BSA) Standard Ampules (2 mg/mL, catalog number: 23209) were purchased from Thermo Fisher Scientific, USA. Inhibitor removers (catalog number: 311340) were obtained from Sigma–Aldrich Corporation, United States. PS with a weight-average molecular weight ($M_w$) of 300000 was procured from Toyo Styrene Co., Ltd., Japan, and that with an $M_w$ of 50000 was procured from Polysciences, Inc., USA. Poly(vinyl alcohol) (PVA; JP-05: degree of polymerization, 500; degree of saponification, 87–89 mol%; JMR-3H: degree of polymerization, 110; degree of saponification,
78 mol%; these values are the manufacturer’s specifications) were supplied by Japan Vam & Poval Co., Ltd., Japan. The dynamic viscosities of 4 wt% aqueous solutions of JP-05 and JMR-3H were 4.99 ± 0.02 and 1.79 ± 0.01 mPa/s, respectively. The interfacial tensions of 0.1 wt% aqueous solutions of JP-05 and JMR-3H suspended in styrene were 6.7 ± 0.0 and 1.7 ± 0.1 mN/m, respectively. The dynamic viscosity of the PVA aqueous solution was measured using a viscometer (ViscoQC 300-L, Anton Paar, Austria). Temperature devices and sensors (PTD 80) and a spindle (SC4-18) were used for temperature control (25°C) and measurement, respectively. The interfacial tension was measured by pendant drop tensiometry using a contact angle meter (DMo-502, Kyowa Interface Science Co., Ltd., Japan). A blunt-tipped needle with an outer diameter of 0.7 mm was used to add a droplet of aqueous PVA solution to styrene. The measurements were conducted at 25 ± 1°C and 45 ± 5% humidity. Experimental droplet images were processed using the Young–Laplace equation in FAMAS (version 7.2.0, Kyowa Interface Science Co., Ltd., Japan). The densities of the styrene and PVA solution were 0.91 and 1.00, respectively, for calculation. The errors in the experimental values represent the standard deviations of three independent measurements. A representative image used for calculating the interfacial tension is shown in Figure S1.

2.2. Emulsification

Emulsification was conducted using the LABOLUTION system with Homogenizing Mixer Mark II Model 2.5 as a mixing head (PRIMIX Corporation, Japan). Styrene was processed before use by inhibitor removers to eliminate 4-tert-butylcatechol. The typical procedure was as follows. Aqueous PVA (2 wt%, 189 g) was added to a 500 mL polypropylene disposable cup, following which styrene (45 g) with dissolved DHHB (15 g) was slowly added to the aqueous PVA solution with stirring at 2000 rpm and homogenized at 6000 rpm. When polystyrene was used as a stabilizer for polymerization, it was dissolved in the styrene monomer overnight with stirring at 4°C. The average hydrodynamic diameter (Z-average) of the emulsion droplet was determined using a dynamic light scattering (DLS) instrument (Zetasizer Nano ZSP, Malvern Instruments Limited, UK) equipped with a 10 mW He–Ne laser having a wavelength of 633 nm. Dispersion Technology (version 7.13; Malvern Instruments Limited) was used to collect and analyze the data. The instrument was operated in backscatter mode at an angle of 173° (noninvasive backscatter, NIBS®), and 12 runs were performed on each sample to determine the Z-average value. After homogenization, Ar gas was bubbled through the emulsion for 10 min for deoxygenation.

2.3. Synthesis of DHHB-encapsulated cationic polystyrene particles (PS-DHHB)

The amounts of each component used for synthesizing the polymer particles are summarized in Table 1. Briefly, all emulsions were prepared in accordance with the procedure described in Section 2.2 and poured into a 300 mL reactor (inner diameter: 75 mm) equipped with a baffle board and three-necked lid. Subsequently, the temperature of the emulsion was increased, and the radical initiator VA-044 (0.47 g) dissolved in 1 g of water was added to the warmed emulsion. Polymerization was conducted with
mechanical stirring for 4 h at 60°C using a sealing mixer (UZ-SM1, Nakamura Scientific Instruments Industry Co., Ltd., Japan) and propeller-shaped stirring rod (Ø 8 × 300 mm). After polymerization, all particle dispersions were cooled to 25°C and characterized.

2.4. Characterization of PS-DHHB

The Z-average and polydispersity index (PDI) of PS-DHHB were determined using DLS and the method described in Section 2.2. The zeta potential (ζ) was evaluated using the Zetasizer Nano ZSP instrument with mixed-mode measurement-phase analysis light scattering (M3-PALS®). At least 10 runs were performed for each sample to determine the zeta potential. All measurements were performed at 25°C, and the viscosity and relative permittivity of water were 0.89 mPa s$^{-1}$ and 78.5, respectively. Henry’s function (1.5) was used to calculate the zeta potential.

The contents of PS-DHHB ($C_{PS-DHHB}$) in the dispersion were calculated from the oven dry weight of 0.3 g of the PS-DHHB dispersion.

The ultraviolet–visible (UV–vis) absorbance spectra were obtained using a spectrophotometer (BioMate™ 160, Thermo Fisher Scientific, Inc. USA). Water and THF were used as the blank in the particle dispersion and particles dissolved in THF, respectively.

The DHHB contents in the polystyrene particles ($C_{DHHB}$) were determined using a Shimadzu Prominence high-performance liquid chromatography (HPLC) system (Shimadzu Corporation, Japan) equipped with an octadecyl-silica (ODS) column (250 mm × 4.6 mm, 5 μL; OSAKA SODA CO., LTD., Japan). Acetonitrile was used as the mobile phase at a flow rate of 0.5 mL/min for 11 min. The column temperature was set to 40°C, injection volume used was 1 μL, and responses were measured at 350 nm using a UV-vis detector (SPD-20MA). LabSolutions Version 5.101 (Shimadzu Corporation, Japan) was used to collect and analyze the data. Sample preparation was performed as follows. The PS-DHHB dispersion (150 μL) was separated from the precipitate and supernatant by centrifugation at 20400 g for 15 min, after which the supernatant was removed. The precipitate was dissolved in THF (150 μL), chloroform (850 μL) was added, and the mixture was sonicated for 15 min. The dispersion was diluted by adding acetonitrile and filtered using a polytetrafluoroethylene (PTFE) membrane filter with a pore size of 0.2 μm. A standard solution was prepared by dissolving DHHB in acetonitrile.

The polymer content ($C_{Polymer}$) was calculated using Eq. (1).

$$C_{Polymer} = C_{PS-DHHB} - C_{DHHB}$$

2.5. Evaluation of DHHB leaching from particles into an aqueous alcohol solution
PS-DHHB dispersion (20 mg; Table 1, Entry 2) was added to a 1.5 mL tube, and different alcohols (ethanol, 1,2-hexanediol, 1,2-pentanediol, and 1,3-butanediol) were added to reach the minimum inhibitory concentration (MIC), as previously reported [25]. Water was added to the mixture to adjust the final PS-DHHB (0.05 wt%) and alcohol contents. Thereafter, the samples were incubated 50°C for 24 h and centrifuged at 20400 \( g \) for 45 min. After centrifugation, the supernatant (150 µL) was diluted in acetonitrile (850 µL).

The positive control (PC) was prepared as follows. The PS-DHHB dispersion (20 mg, Entry 2) and THF (100 µL) were mixed well in a 1.5 mL tube. Chloroform (600 µL) was then added to the mixture, and sonication was conducted for 15 min. The sonicated samples (10 µL) were diluted with acetonitrile (990 µL). All prepared samples were filtered through a PTFE membrane filter with a pore size of 0.2 µm.

The contents of DHHB in the supernatant containing each alcohol and positive control were determined by HPLC, as described in Section 2.4. Based on the HPLC analysis, the relative amounts of DHHB in the aqueous alcohol solutions were calculated using Eq. (2).

\[
\text{Relative amount of DHHB in aqueous alcohol solution} = \frac{C_{\text{Sample}}}{C_{\text{PC}}}
\]

Here, \( C_{\text{PC}} \) is the DHHB content in the positive control, and \( C_{\text{Sample}} \) is the DHHB content in the aqueous alcohol solution.

### 2.6. Evaluation of the hair adsorption of PS-DHHB

Bleached white human hair was used. The hair was soaked in 0.05 wt% PS (Entry S1), cationic PS-DHHB (Entry 2), or anionic PS-DHHB (Entry S2) dispersions for 10 min. The treated hair was then washed three times by soaking in water for 5 min to remove extra particles and dried at room temperature. The dried hair was cut to 1 cm for observation using scanning electron microscopy (SEM, Nova NanoSEM 450, FEI Co., USA), and SEM measurements were conducted at an accelerating voltage of 500 V and a magnification of 2000\( \times \) (Figs. 4A–C) or 8000\( \times \) (Fig. 4D). Each dried hair sample was directly attached to carbon tape and observed using SEM. The obtained images were analyzed using NaviCam (Sony Computer Science Laboratories, Inc., Japan).

### 2.7. Evaluation of hair protein loss by UVA irradiation

Bleached white human hair was used. The hair was soaked in 1 wt% PS (Entry S1) or cationic PS-DHHB (Entry 2) dispersions for 10 min. The treated hair was then dried under 25°C for two days. Subsequently, the dried hair was irradiated by UVA light using a UVA lamp (FPL27BLB, Sankyo Denki Co., Ltd., Japan).

The cumulative irradiation amount of UVA rays was calculated using Eq. (3)

\[
\text{Accumulated UVA Intensity} \left[ \text{J/cm}^2 \right] = I_{\text{UVA}} \left[ \text{mW/cm}^2 \right] \times T \left[ \text{second} \right]
\]
Here, \( I_{\text{UV}_A} \) is the UVA intensity measured by the UV ray intensity meter (catalog number: UV-340C, CUSTOM Corporation, Japan), and \( T \) is irradiation time.

The UVA-irradiated hair was partially cut from the terminal part with scissors, weighed, and the incubated in water at 40°C for 3 h to extract the protein from the hairs. The weight ratio of water to the hair was 70. The concentration of the extracted hair protein in the water was measured by the bicinchoninic acid (BCA) assay [26]. The procedure of the BCA assay was as follows. The working reagent (WR) was prepared by mixing Reagent A and Reagent B at a ratio of 50:1 (by volume). Subsequently, 25 µL of the samples and standard solutions were each prepared in a 96-well plate. Then, 200 µL of WR was added to the plate, and the plate was incubated at 37°C for 30 min. After the reaction, absorbance of the samples was measured at a wavelength of 562 nm using a microplate reader. The concentration of the BSA standard was adjusted to 0–50 µg/mL using DPBS. Statistical analysis was performed by Dunnett's test. Differences were considered significant when the \( p \)-value was less than 0.05. The hair protein loss among control (without particles), PS-treated (Entry S1), and PS-DHHB-treated samples (Entry 2) without UVA light irradiation were not significant (Fig. S2).

3. Results

3.1. Selection of the optimal type of PVA for the emulsion polymerization of styrene

To optimize the type of PVA, we focused on obtaining small emulsion droplets and maintaining a stable emulsion during the deoxygenation process before polymerization. Figure 1 shows the change with time in the Z-average of the emulsion droplets emulsified using two types of PVA: JP-05 and JMR-3H. The PVA content in the emulsion was set to 2 wt% because the emulsification tests indicated that the droplet size decreased as the JMR-3H content increased (Fig. S3). The Z-average of the emulsion droplets made with JP-05 was over 2000 nm during homogenization, and the particle size decreased as the homogenization time increased. The minimum size of the emulsion prepared using JP-05 was 2574 ± 188 nm after 30 min. During deoxygenation before polymerization, the emulsion prepared with JP-05 formed many bubbles that interrupted polymerization (data not shown). The Z-average of the emulsion droplets made with JMR-3H was less than 1000 nm during homogenization, and the minimum Z-average size was 334 ± 13 nm at 10 min. In contrast, the emulsion prepared with JMR-3H did not show foam formation during deoxygenation. Thus, we concluded that JMR-3H was more suitable for emulsion polymerization than JP-05 because it formed smaller droplets of less than 400 nm, and the emulsion was stable during deoxygenation.

3.2. Polymerization conditions for the formation of stable and DHHB-rich PS-DHHB
Table 1 summarizes the results of the characterization of the synthesized PS-DHHB prepared at 60°C. Three indicators were used. First, PDI values should be less than 0.2, indicating the absence of particle aggregation. Second, $C_{\text{polymer}}$ should be approximately 20 wt%, which implies that the styrene monomer conversion is almost 100%. Third, high $C_{\text{DHHB}}$ values are better; that is, a lower $C_{\text{DHHB}}$ means that a lower amount of DHHB is encapsulated into particles, resulting in the incomplete preparation of PS-DHHB. As shown, for all entries, the PDI values were less than 0.2, indicating a narrow size distribution and no aggregation of PS-DHHB. Entries 2–4 show that $C_{\text{polymer}}$ was 20 wt%, and Entry 1 was 13 wt%, suggesting that Entry 1 underwent incomplete polymerization. Thus, slower stirring during polymerization results in higher styrene monomer conversion. In addition, by comparing Entries 2, 3, and 4, we can see that dissolving polystyrene in the styrene monomer before polymerization increased $C_{\text{DHHB}}$. Additionally, the use of polystyrene with a smaller molecular weight tended to yield better results (Entry 4). As shown by the results for Entry 4, $C_{\text{polymer}}$ and $C_{\text{DHHB}}$ were 20 and 6 wt%, respectively, suggesting that the styrene monomer was almost completely converted into a polymer, and DHHB was almost completely incorporated into the particles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Styrene (g)</th>
<th>PS (g)</th>
<th>Rotation (rpm)</th>
<th>Z-average (nm)</th>
<th>PDI</th>
<th>$\zeta$ (mV)</th>
<th>$C_{\text{Polymer}}$ (wt%)</th>
<th>$C_{\text{DHHB}}$ (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>0</td>
<td>450</td>
<td>246</td>
<td>0.18</td>
<td>48</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>0</td>
<td>120</td>
<td>247</td>
<td>0.12</td>
<td>43</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>40.5</td>
<td>4.5 $^a$</td>
<td>120</td>
<td>227</td>
<td>0.078</td>
<td>45</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>40.5</td>
<td>4.5 $^b$</td>
<td>120</td>
<td>215</td>
<td>0.11</td>
<td>45</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

$^a M_w = 300000; \quad ^b M_w = 50000$

### 3.3. UV absorption of the synthesized PS-DHHB

The relative absorbance spectrum of the PS-DHHB (Entry 2) dissolved in THF is shown in Fig. 2a (circles). The maximum absorption wavelength ($\lambda_{\text{max}}$) was observed at 352 nm, which is consistent with the $\lambda_{\text{max}}$ of DHHB dissolved in THF (Fig. 2a, black line). Thus, these spectra are almost identical, suggesting that there was no change in the chemical structure before and after polymerization. The absorbance spectra of the PS-DHHB dispersion (Entry 2, black line), PS dispersion (Entry S1, dotted line), and absorbance spectra of the filtered clear liquid of Entry 2 (broken line) filtered through a polysulfone membrane filter (pore size: 0.2 µm) are shown in Fig. 2b. The protocol for the synthesis of Entry S1 is described in the Supporting Information, and the characterization results are shown in Table S1. The $\lambda_{\text{max}}$ value of Entry 2 was observed at 362 nm. By contrast, no absorption in the UVA range was
observed in the case of empty PS particles (Entry S1) and filtered Entry 2. This, we confirmed that UVA absorption occurs only for PS-DHHB.

### 3.4. Evaluation of DHHB leaching from PS-DHHB in aqueous alcohol

Figure 3 shows the relative content of DHHB in aqueous alcohol leached from PS-DHHB based on three independent measurements. The relative amount of DHHB in the aqueous alcohol solution was calculated using the equation described in Section 2.6. Alcohols are often used in cosmetics to prevent product degradation caused by microbial contamination [25]. The contents of the alcohols employed were as follows: 10 wt% ethanol (E_10), 20 wt% 1,3-butanediol (B_20), 10 wt% 1,2-pentanediol (P_10), and 2.5 wt% 1,2-hexanediol (H_2.5). DHHB was not detected in any solution. Thus, we confirmed that no DHHB leaked from PS-DHHB.

### 3.5. Evaluation of the hair adsorption properties of PS-DHHB

Figure 4 shows the SEM images of hair treated with water (Fig. 4a), the PS-DHHB dispersion (Fig. 4b, Entry 2), and the anionic PS-DHHB dispersion (Fig. 4c, Entry S2), and Fig. 4d shows an enlarged image of the square shown in Fig. 4b. The protocol for the preparation of Entry S2 is described in the Supporting Information, and the characterization results are listed in Table S1. The characteristic scale-like surface structure of hair can be seen in Fig. 4, and, after treatment, particles can be seen over a wide area of the hair surface (Figs. 4b and 4d). The particle diameter measured from 10 particles observed in Fig. 4d was 263 ± 19 nm, which is almost the same as that obtained by DLS measurements (Entry 2). In contrast, no particle structures were observed in Figs. 4a and 4c; hence, we confirmed that cationic PS-DHHB was strongly adsorbed onto hair, whereas anionic PS-DHHB was not adsorbed onto hair.

### 3.6. Evaluation of inhibition of protein loss from hair by UVA irradiation

Figure 5 shows the amount of protein loss from hair fibers exposed to UVA irradiation at 192 J/cm², which is almost equivalent to sunlight exposure in summer at noon for 10 h. Hair fibers without particles (non-treated samples) lost approximately 1.4 times protein compared to those treated with the particles without UVA irradiation (Fig. S2). No significant difference was observed between the non-treated hair samples and Entry S1. There was a statistically significant difference between non-treated samples and Entry 2 ($p < 0.05$). Herein, we confirmed that cationic PS-DHHB effectively suppressed hair protein loss caused by UVA irradiation, whereas cationic PS without DHHB did not sufficiently suppress hair protein loss.

### 4. Discussion
4.1. Difference in emulsification arising from the molecular weight of PVA

We found that low-molecular-weight PVA (JMR-3H) was effective for emulsifying styrene before polymerization. The sizes of the styrene emulsion droplets prepared using JMR-3H stabilized within 5 min, and the droplets were small (Fig. 1). By contrast, for JP-05, the droplet size gradually decreased up to an emulsification time of 30 min, and the droplet size did not plateau subsequently (Fig. 1). Budhlall et al. reported that PVA with almost the same degree of polymerization (480) and degree of saponification (87–89 mol%) as JP-05 behaves like a cluster consisting of multiple entangled molecules in aqueous solution. They also suggested that the movement of clustered PVA would be slower because of its internal interactions or consecutive association–dissociation processes [27]. Therefore, the slower emulsification in the presence of JP-05 than that with JMR-3H suggests that JP-05 had a clustered structure.

Furthermore, when using JP-05, there was a lower decrease in interfacial tension than when using JMR-3H (Fig. S1). We assumed that the adsorption of JP-05 at the interface of the styrene monomers would be weaker than that of JMR-3H because of the clustering of JP-05 in aqueous solution. In other words, we speculate that the clustering of JP-05 had a negative effect on the emulsification of styrene before polymerization in two ways: reducing the rate of adsorption at the interface of the styrene droplets and stabilizing the interface after adsorption. In contrast, JMR-3H was efficiently and quickly adsorbed at the interface and stabilized the droplets as a polymeric emulsifier.

4.2. Efficient preparation of PS-DHHB by emulsion polymerization

In the miniemulsion polymerization technique, two factors are important for successful capsule particle preparation. First, smaller, stable droplets are best (approximately 30–500 nm) [28]. Second, suppressing the formation of new monomer droplets caused by monomer diffusion from already formed monomer droplets is crucial because, otherwise, the encapsulated substances (i.e., DHHB) in the monomer droplets tend to precipitate because of their decreased solubility on the diffusion of monomers, which act as their solvent. Therefore, we compared the number and size of the emulsion droplets before polymerization and after polymerization [29] (see Section 4.1 for details of the polymerization procedure). There was little difference between the droplet size of the emulsion and the particle size after polymerization: the minimum droplet size was 334 ± 13 nm, and the PS-DHHB had diameters ranging from 200 to 250 nm. Therefore, JMR-3H prevents the styrene monomers from migrating to new nuclei through the DHHB-dissolved monomer droplets.

The addition of a hydrophobic agent (hydrophobe) to the monomer phase is also an effective technique for capsule particle preparation. Crucially, hydrophobes stabilize the monomer phase during miniemulsion polymerization [30–32], and Kamogawa et al. reported that the dissolution of PS improves oil droplet stability [34] and has been used for the encapsulation of compounds into particles [35, 36]. In
this study, we confirmed that use of pre-dissolved polystyrene in the monomer phase (Entries 3 and 4) resulted in enhanced DHHB encapsulation in the final particles compared to those without polystyrene (Entry 2). These results further support the idea that stabilization of the styrene monomer droplet and suppression of styrene monomer diffusion between monomer droplets results in improved encapsulation efficiency. However, a detailed investigation is needed in future work because we observed that encapsulation efficiency varied with the molecular weight of the PS.

4.3. UV absorption and hair adsorption of PS-DHHB

Comparing the $\lambda_{max}$ between the PS-DHHB dispersion and DHHB dissolved in THF, the $\lambda_{max}$ of the PS-DHHB dispersion shifted to longer wavelengths. In the case of polystyrene particles encapsulating anthraquinones, the $\lambda_{max}$ shifted to longer wavelength because of the $\pi-\pi$ stacking of the anthraquinones [36], and similar intermolecular interactions between polystyrene and DHHB may have occurred in our particles.

The outermost layer of the hair, called the cuticle, has a scale-like structure, as shown in Fig. 4. The cuticle has a strongly negative surface charge because it contains carboxyl groups derived from glutamate, aspartate, and sulfonate groups [23]. The surface of PS-DHHB has a positive charge derived from the imidazolium group of the radical initiator; therefore, PS-DHHB was more strongly adsorbed onto the surface of hair through electrostatic interactions compared to the particles with a negative charge.

4.4. Applications of PS-DHHB

PS-DHHB significantly inhibited hair protein loss due to UVA irradiation, while cationic PS did not significantly inhibit protein loss. The suppression of hair protein loss by PS-DHHB may occur in the following two steps: cationic surface-charged PS particles are adsorbed on the hair fiber via electrostatic interaction, and next the encapsulated DHHB into particles exhibited UVA protection. It is well known that hair generates free radicals under UV irradiation that cause the oxidative degradation of proteins, resulting in protein loss, fading of hair color, and loss of the aesthetic appearance of hair [24, 36–38]. Since only a few studies have reported the use of cationic UVA filters to protect hair [24], we believe that PS-DHHB can be potentially used as a new UVA filter in hair-care products.

Notably, DHHB was not leached from the particles when combined with alcohol, which is often used as an antibacterial agent in cosmetics. Based on our results, PS-DHHB is stable in water-rich cosmetic formulations. PS, DHHB, and PVA, which are the components of PS-DHHB, are each registered in the International Nomenclature of Cosmetic Ingredients (INCI) and have a proven track record in cosmetics; therefore, we believe that PS-DHHB could be treated as a cosmetic. However, the safety of PS-DHHB should be reevaluated in future research while considering its use as a cosmetic.

5. Conclusions

In this study, DHHB-encapsulated cationic polystyrene (PS-DHHB) was prepared by emulsion polymerization. In particular, the emulsification process before polymerization using low-molecular-
weight PVA was effective for the preparation of particles. Furthermore, the dissolution of polystyrene in the monomer phase as a stabilizer was effective for achieving high DHHB encapsulation. We identified three characteristics of PS-DHHB. First, the PS-DHHB dispersion exhibited absorption in the UVA region. Second, no DHHB was leached in aqueous alcohol (in the range of concentrations used for antimicrobial purposes in cosmetics). Third, cationic PS-DHHB was more strongly adsorbed onto human hair than anionic PS-DHHB. Finally, cationic PS-DHHB inhibited hair protein loss caused by UVA irradiation more effectively than cationic PS particles. Therefore, the PS-DHHB particles show promise as UV protectors in cosmetics.

**Declarations**

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**Ethical Approval**

Not applicable.

**Competing interests**

All authors are employed by Kirin Holdings Co., Ltd.

**Authors' contributions**

T.Y. and M.S. conceived the idea of the study. T.Y wrote the main manuscript text and prepared figures 4 and 5. M.S. prepared figure 1. S.Y. and R.K. prepared figures 2-3, table 1 and supporting information. T.T. supervised the conduct of this study. All authors reviewed the manuscript draft and revised it critically on intellectual content. All authors reviewed the final version of this manuscript to be published.

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**Availability of data and materials**

Not applicable.

**References**


Figures
Figure 1

Change in the emulsion droplet size with time during homogenization. Both emulsions contained 2 wt% PVA. The errors in experimental values represent the standard deviation of three independent measurements.
Figure 2

(a) Relative absorbance spectra of the PS-DHHB dispersion (Entry 2) and DHHB dissolved in THF (circles and the continuous line, respectively). Absorbance was normalized at the maximum absorption wavelength (352 nm). Entry 2 was diluted 10000 times with THF. (b) The absorbance spectra of 0.5 ppm of the PS-DHHB dispersion (Entry 2), 0.5 ppm of the PS dispersion (Entry S1), and Entry 2 filtered through a polysulfone membrane filter (pore size: 0.2 μm) are shown as black, dotted, and broken lines, respectively.
Relative amount of DHHB in aqueous alcohol based on three independent measurements: positive control (PC), 10 wt% ethanol (E10), 20 wt% 1,3-butanediol (B20), 10 wt% 1,2-pentanediol (P10), and 2.5 wt% 1,2-hexanediol (H2.5).

Figure 3
Figure 4

SEM images of hair fibers with adsorbed PS-DHHB: (a) control, (b) PS-DHHB (Entry 2), (c) anionic PS-DHHB (Entry S1), and (d) enlarged image of the area in the square frame in (b). The white scale bar in (a)–(c) is 10 μm, and that in (d) is 3 μm.
Figure 5

Amount of hair protein loss due to UVA light exposure (192 J/cm²) for hair samples treated with PS: (Entry S1) and PS-DHHB (Entry 2). *p < 0.05 by Dunnett's test, n = 3, mean ± standard deviation.

Supplementary Files

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