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Poly(vinyl alcohol) hollow microcapsules prepared by emulsification, salting out, and photo cross-linking method

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Abstract−Coumarin residues were conjugated to poly(vinyl alcohol) (PVA) by reacting epoxypropoxy coumarin (EPC) with the polymer. According to the peak areas on the ^{1}H NMR spectrum, EPC was calculated to be conjugated to every 283 repeating units (vinyl alcohols). A cyclic photo-dimerization and dedimerization of EPC of PVA-EPC conjugate were observed under a cyclic irradiation of 365 nm and 254 nm. The salting-out of the conjugate significantly took a place in the range of 0-2.0 M NaCl, and the phenomenon was observed at a lower concentration than that of unmodified PVA was. Oil-in-water emulsion was prepared as a template for the preparation of hollow microcapsules using chloroform as an oil phase and PVA-EPC as an emulsifier. The emulsion was stable for 24 hr in terms of droplet size. The wall surrounding droplets was built-up by the salting-out of PVA-EPC, and it was cross-linked by the irradiation of 365 nm. After chloroform was evaporated and salt was removed by a dialysis, hollow microcapsules were successfully obtained.

Key words: Poly(vinyl alcohol), Coumarin, Salting-out, Emulsion, Photo-crosslinking Hollow Microcapsules

INTRODUCTION

Stimuli-sensitive polymeric hollow microcapsules have attracted considerable attention recently due to their potential applications in cosmetics, controlled drug delivery systems, and bioanalytics [1,2]. Hollow microcapsules have been fabricated by spray drying, emulsion/interfacial polymerization, phase separation, and self assembling [3,4] Hollow microcapsules for ultrasound echogenic contrast agents could be prepared by spray drying [5]. Hollow microcapsules built by layer by layer assembly were prepared by dissolving the melamine formaldehyde (MF) templates coated with six double layers of poly (sodium 4-styrene sulfonic acid) (PSS) and poly(ethylene imine) (PEI) using hydrochloric acid [6]. Thermo-sensitive poly (N-isopropylacrylamide) (PNIPAM) hollow microcapsules were prepared by interfacial polymerization at the interface of W/O single emulsions at a temperature below the lower critical solution temperature (LCST) of PNIPAM [7]. The NIPAM monomer-contained W/O emulsion droplets could be obtained by shirasuporous-glass (SPG) membrane emulsification technique. And then the monomers in W/O emulsions were polymerized at 20 °C by UV irradiation to prepare hollow PNIPAM microcapsules. Recently, monodisperse chitosan hollow microcapsules were developed using the SPG membrane emulsification technique [8]. Calcium alginate microspheres as core particles were prepared by mixing W/O emulsions containing alginate and calcium inos and were coated with chitosan. The microspheres were then treated with tripolyphophate to remove the core and to crosslink the shell. Also, photo-crosslinkable chitosan emulsions were deposited onto the calcium alginate microspheres. Next they were subjected to UV irradiation to crosslink the chitosan shell, and tripolyphosphate was added to solubilize the core.

In this study, photo-responsive hollow capsules were prepared by a process consisting of emulsification, salting out, photo-crosslinking, evaporation and dialysis. First, PVA having coumarin as a pending group (PVA-coumarin) was prepared by reacting the polyol

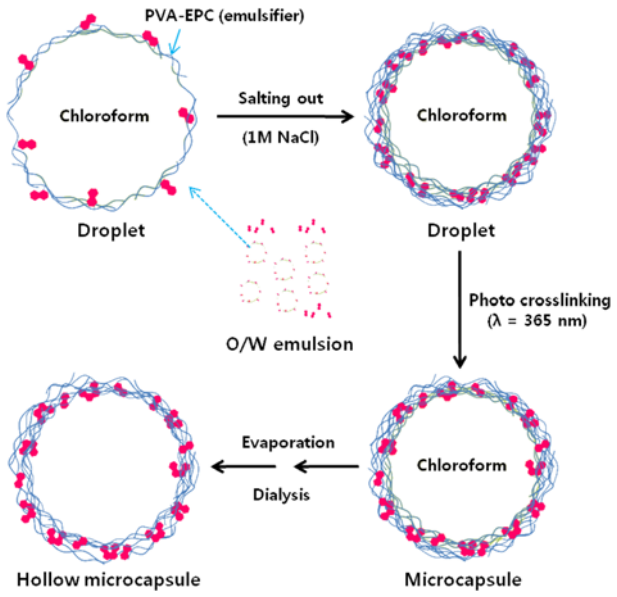

Fig. 1. Schematic representation for preparation of PVA-EPC hollow microcapsules by emulsification, salting out, photo-crosslinking, evaporation and dialysis.

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with epoxypropoxy coumarin (EPC) in a strong alkali condition. Then an oil-in-water (O/W) emulsion was prepared using chloroform as an oil phase and PVA-EPC as an emulsifier. Subsequently, the wall surrounding oil droplets was built-up by the addition of NaCl to the O/W emulsion (causing the salting-out of PVA-EPC), and the PVA-EPC wall was photo cross-linked under the irradiation of λ=365 nm. To obtain hollow microcapsules, the oil phase was evaporated and the salt was removed by a dialysis. The preparation of hollow microcapsules is depicted in Fig. 1.

EXPERIMENTAL

1. Materials

7-Hydroxycoumarin, poly(vinyl alcohol) (PVA, M.W. 30,000-70,000), epichlorohydrin (EPI), Potassium hydroxide (KOH), Sodium chloride (NaCl) and chloroform were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade.

2. Preparation of Epoxypropoxy Coumarin

Epoxyproxy coumarin (EPC) was prepared using 7-hydroxycoumarin as a starting material [9]. Epichlorohydrin (20 ml) was added to the alkalized alcoholic solution of 7-hydroxycoumarin (3.24 g/(100 ml ethanol+5 ml KOH solution)). The derivertization was done at 95-100 °C for 2.5 hr with a reflux. After solvent was evaporated, the dry residue of reaction mixture was dissolved in the mixture solvent of distilled water/chloroform (80 ml/100 ml) in a 250 ml-separation funnel, and then it was left to stand for 1 hr at room temperature. Water phase containing impurities was removed, and the remaining oil phase was contacted with fresh distilled water (80 ml) for 1 hr to further remove impurities from the oil phase. After the solvent of the oil phase was being evaporated, the dry residue was re-crystallized in ethanol. The white solid was filtered and dried in a vacuum oven thermostated at 40 °C.

3. Preparation of PVA-EPC Conjugates

EPC was conjugated to PVA following a method described in a previous report [10]. In brief, 10 ml of EPC solution in dimethylsulfoxide (10% (w/v)) was added to 40 ml of an alkalized PVA solution (25% (w/v)) in a dropwise manner, and the conjugation was done for 48 hr at 30 °C. The reaction mixture was dialyzed against distilled water using a dialysis membrane (MWCO 3500-5000, Spectra/Por ®) and the product was freeze-dried for further use.

4. ^{1}H NMR Spectroscopy of EPC and PVA-EPC

EPC was dissolved in $CDCl_3$ and PVA-EPC conjugate was dissolved in D_2O. ^{1}H NMR spectra were obtained on a Bruker Avance 600 spectrometer (Karlsruhe, Germany, in the Central Laboratory Center of Kangwon National University).

5. Dimerization Degree of PVA-EPC under Cyclic Irradiations

Cyclic dimerization and de-dimerization of EPC residues of PVA-EPC conjugate were investigated by a method described previously [11]. PVA-EPC was dissolved in distilled water so that the concentration was 0.05%. The PVA-EPC solution was subjected to the irradiation of λ=365 nm (400 W, HPA 400/30SD, Philips) for 15 min and the irradiation of λ=254 nm (6 W, Vilber Lourmat, Marne la Vallee, France) for 5 min in a cyclic manner. The degree of dimerization was determined as follows [12].

$$\text{Dimerization (\%)} = (1 - A_t/A_o) \times 100$$

Where, A_o is the absorbance of EPC residue at 327 nm before irradiating a UV light, and A_t is the absorbance after irradiating a UV light for a certain period.

6. Observation of Salting-out of PVA and PVA-EPC

Each of PVA and PVA-EPC was dissolved in distilled water so that the concentration was 2% (w/v). NaCl was dissolved in each polymer solution so that the concentration was 0 M, 0.05 M, 0.1 M, 0.2 M, 0.4 M, 1.0 M and 2.0 M. After the solutions were stirred using a magnetic bar for 30 min at room temperature, the viscosities of solutions were measured on a viscometer (Brookfield, Model DV-2, USA), and the turbidities of solutions were measured with a UV spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, U.K.).

7. Preparation of PVA-EPC Hollow Microcapsules

PVA-EPC was dissolved in distilled water so that the concentration was 2% (w/v). 2 ml of chloroform was put to 20 ml of PVA-EPC solution and it was emulsified for 10 min with a homogenizer (DIAX 900, Germany) operating at level 2. To enrich the emulsifying layer (PVA-EPC layer surrounding oil droplet) through the salting-out of PVA-EPC, NaCl was dissolved in the emulsion so that the concentration in the water phase was 1.0 M. To cross-link PVA-EPC layers surrounding oil droplets, the emulsion was subjected to the 2 hr-irradiation of 365 nm. The oil phase was then evaporated at room temperature by blowing air in the emulsion for 48 hr.

RESULTS AND DISCUSSION

1. ^{1}H NMR Spectroscopy of EPC and PVA-EPC

In ^{1}H NMR spectrum of EPC, the total area of the aromatic proton signals (6.3, 6.8, 6.9, 7.4, 7.65 ppm) was 5, and the total area of epoxypropoxy proton signals (3.4, 4.0, 4.3 ppm) was 3. EPC is believed to be highly purified because the area ratio is close to 1 : 1. The result is in a good agreement with a previous work (10). In ^{1}H NMR spectrum of PVA-EPC, the area of aromatic proton signals (6.3, 7.0, 7.5, 7.9 ppm) was 5 and the area of the -CH_2- groups of PVA (1.6 ppm) was 565. By taking advantage of the peak areas, the molar ratio of EPC residue to PVA was calculated to be 4.02/1, assuming the average molecular weight of PVA was 50,000 (corresponding to 1136 repeating units). It indicates that EPC was attached to PVA every 283 repeating units of vinyl alcohol. In a previous work [10], EPC was conjugated to PVA of higher molecular weight (MW 85,000) by the same method as employed in the present work. EPC was reported to be conjugated to every 444 repeating units, quite different from the value (283 repeating units) obtained in the present work. The molar ratio of PVA to EPC in the previous work was 1 : 200, corresponding to the vinyl alcohol to EPC molar ratio of 1 : 0.1. The molar ratio of PVA to EPC in the present work was 1 : 100, corresponding to the vinyl alcohol to EPC molar ratio of 1 : 0.08. Even though the reactive sites molar ratio (the vinyl alcohol to EPC molar ratio) in the present work was lower than the ratio used in the previous work, EPC density on PVA chain (1/283) was higher than that reported in the previous report (1/444). How is that possible? The concentrations of PVA and EPC in the reaction mixture in the present work were 4.58% (w/v) and 2.0% (w/v), respectively, and the concentrations were around double those in the previous work (2.17% (w/v) and 1.11% (w/v), respectively). The higher concentration may account for the higher density of EPC on PVA chain.

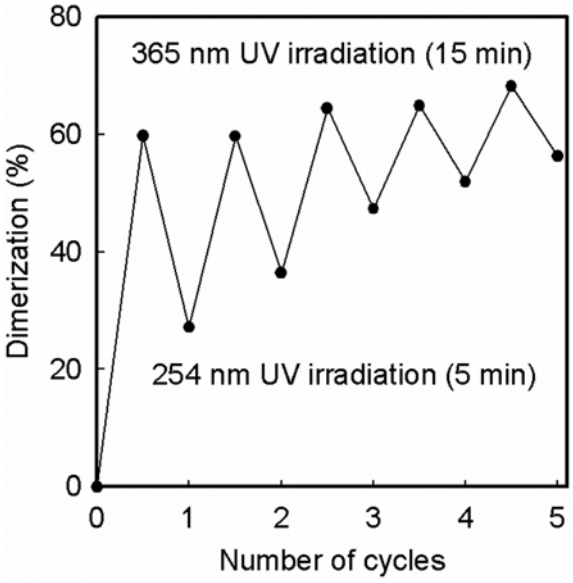

Fig. 2. Dimerization degrees of EPC residues of PVA-EPC under cyclic irradiation of 365 nm and 254 nm.

2. Dimerization Degrees of PVA-EPC under Cyclic Irradiations

Fig. 2 shows the dimerization degrees of EPC residues of PVA-EPC under cyclic irradiation of 365 nm and 254 nm. EPC could be photo-dimerized by the 15 min-irradiation of 365 nm (400 W), and the dimerization degree was about 60%. Even when attached to PVA as pendant groups, coumarin residues were photo-dimerized, and PVA-bearing coumarin became a hydrogel under the irradiation of 365 nm [10]. On the other hand, the dimerization degree decreased to 27% upon a subsequent 5 min-irradiation of 254 nm. The cyclobutane bridges, formed by the irradiation of λ>310 nm, between two coumarin molecules were reported to be broken down by the irradiation of λ<260 nm [13]. A series of cyclic dimerizations and de-dimerizations were observed by further cyclic irradiations, indicating that the photo-dimerization is irreversible. The result described above is in a good agreement with the result reported in previous works [10].

3. Observation of Salting-out of PVA and PVA-EPC

Fig. 3 shows the viscosities of PVA solution and PVA-EPC solution with NaCl concentration. The viscosity of PVA solution was kept almost constant, while the salt concentration increased up to 2.0 M. However, the viscosity of PVA-EPC solution substantially increased when the salt concentration increased from 0.2 M to 0.5 M, and the value markedly increased when the concentration increased from 1.0 M to 2.0 M. Salt can break the hydrogen bonding between the hydroxyl groups of PVA and water molecules, and the dehydrated PVA molecules can interact inter/intra-molecularly through hydrogen bonding among the hydroxyl groups [14,15]. The salt concentration of 0.05-2.0 M, adopted in the present work, may induce a salting-out of PVA. But, based on the result that no significant change in the viscosity of PVA solution occurred in the range of 0.05-2.0 M, the concentrations might be insufficient to induce the salting-out of PVA. In case of PVA-EPC, beside the inter/intra molecular interaction caused by the hydrogen bonding among hydroxyl groups, the hydrophobic interactions among EPC residues could take a place, and it could promote the inter/intra molecular interaction of PVA-EPC. This would account for why the viscosity of PVA-EPC solution markedly increased in the salt concentration range tested (0.05-2.0 M).

Fig. 4 shows the turbidity of the PVA solution and PVA-EPC solution with NaCl concentration. PVA solutions were kept to be clear while the salt concentration increased up to 1.0 M. The turbidity of the solution significantly increased when the salt concentration increased from 1.0 M to 2.0 M. The turbidity increase is possibly due to the salting out effect of PVA molecules and they interact inter/intra molecularly through the hydrogen bonding among hydroxyl groups. On the other hand, the turbidity of PVA-EPC solution began to increase when the salt concentration was 0.2 M. As described

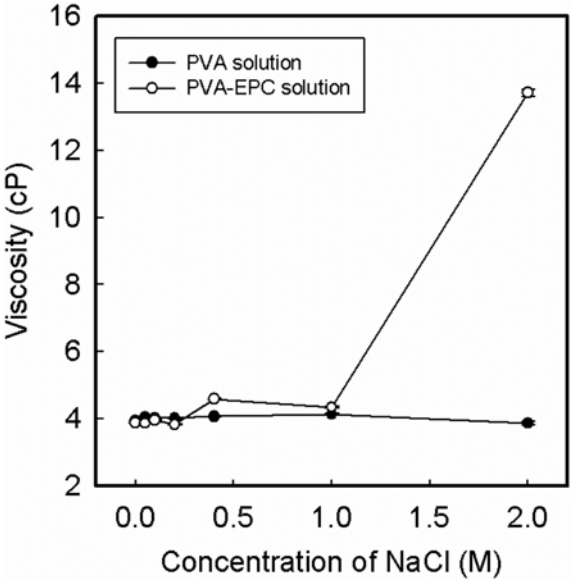

Fig. 3. Viscosities of PVA solution and PVA-EPC solution with NaCl concentration.

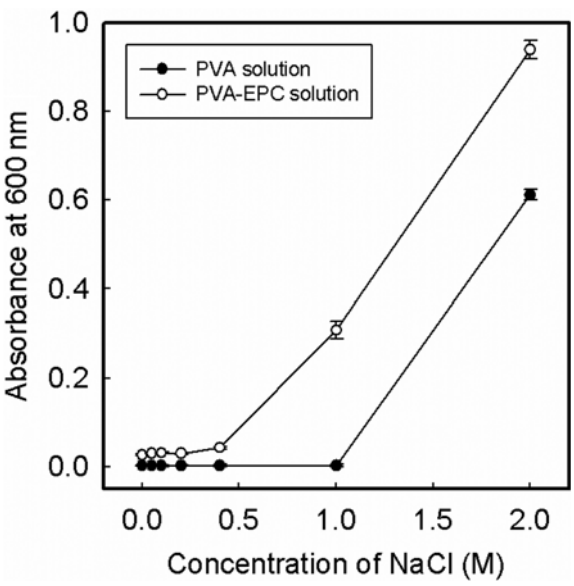

Fig. 4. Turbidity of the PVA solution and PVA-EPC solution with NaCl concentration.

previously, along with the salting-out effect, the hydrophobic interactions among coumarin residues could accelerate the inter/intra molecular interaction of PVA-EPC. This could account for why PVA-EPC solution became turbid at lower concentration of salt than PVA solution did.

4. Preparation of PVA-EPC Hollow Microcapsules

Fig. 5 shows the photomicrographs of emulsion, salt-added emulsion, and salt-added/photo-treated emulsion. The mean size of droplet in each emulsion, determined using an image analyzer, was 25.9 μm, 26.0 μm and 27.5 μm, respectively. By adding salt to the O/W emulsion, PVA-EPC will be salt-outed (Fig. 3 and Fig. 4) and it is likely to be deposited on the O/W interface, possibly leading to an increase in the size of oil droplets. By irradiating the light of 365 nm, PVA-EPC surrounding oil droplets will be cross-linked (Fig. 2)

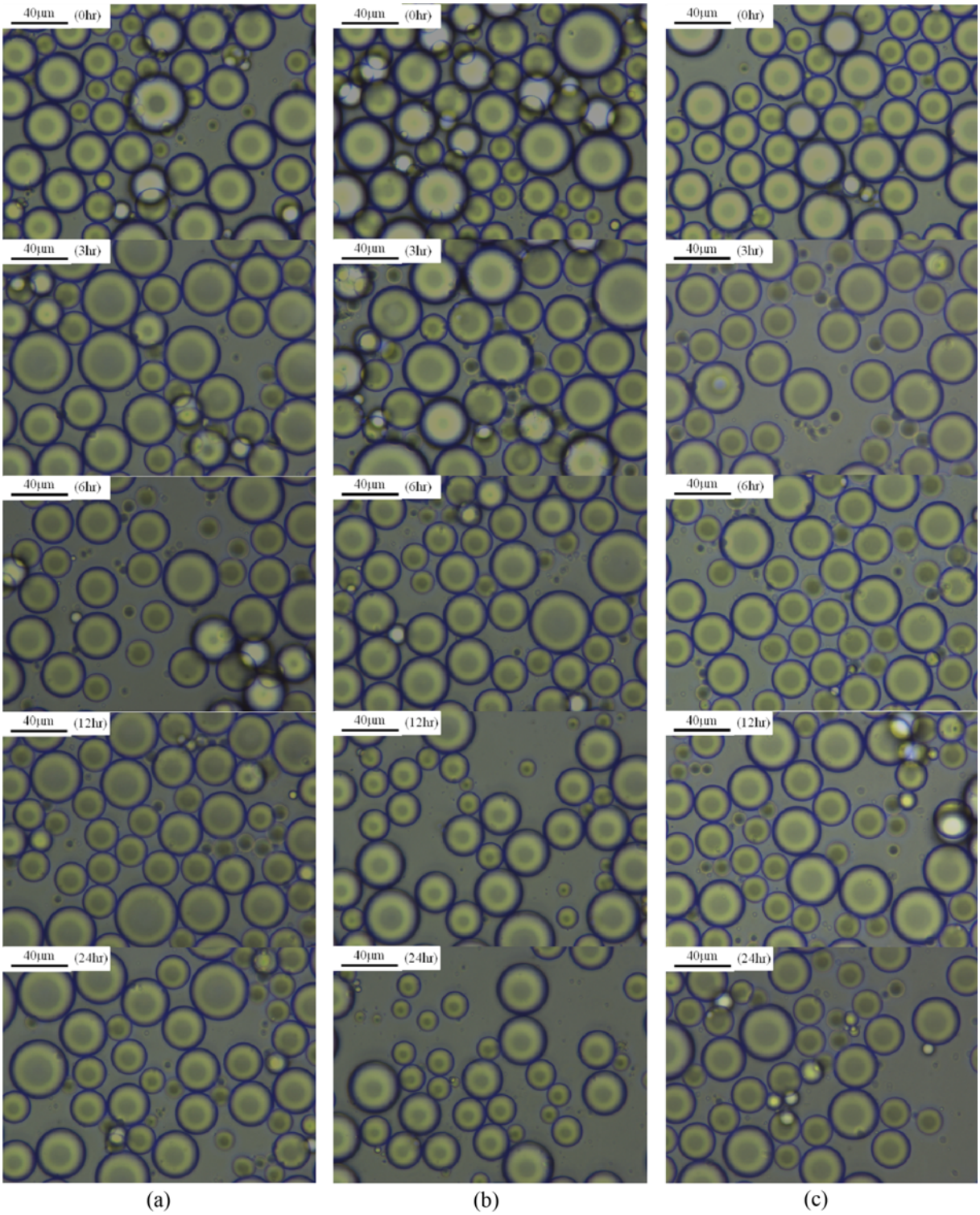

Fig. 5. Photomicrographs of emulsion (a), salt-added emulsion (b), and salt-added/photo-treated emulsion (c) (Magnification ×400).

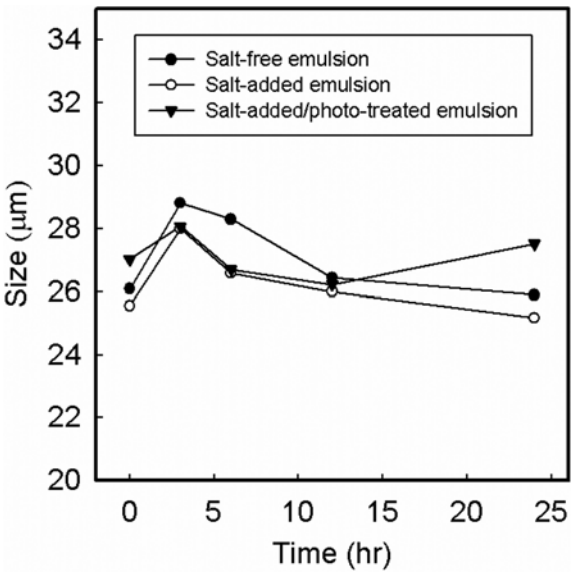

Fig. 6. Size change of droplet in salt-free emulsion, salt-added emulsion, and salt-added/photo-treated emulsion with time lapse.

and PVA-EPC layer is hardened, possibly resulting in a decrease in the size of oil droplets. However, no significant change in size and shape was observed after being salt-added (Fig. 5(b)) and subsequently being photo-treated (Fig. 5(c)). The volume of oil droplet will be almost constant with respect to the salt addition and the light irradiation. In addition, although PVA-EPC may have an effect on the droplet size through its salting-out and its cross-linking, the thickness of PVA-EPC layer would be relatively small compared with the diameter of oil droplets. This may explain why not only the salting-out of PVA-EPC (Fig. 5(b)), but also the cross-linking of the conjugate (Fig. 5(c)), had little effect on the size of oil droplets.

Fig. 6 shows the size change of droplet in salt-free emulsion, salt-added emulsion, and salt-added/photo-treated emulsion with time lapse. No significant change in droplet size was observed for 24 hr, whatever the emulsion was. PVA is known to be an emulsifier for the preparation of O/W emulsion. It is adsorbed at O/W interface, forming a hydrophilic protecting layer and preventing the fusion of oil droplets. PVA-EPC is believed to act as an emulsifier for O/W emulsion in the same way, because only one of 281 hydroxyl groups is substituted with EPC, so the emulsifying property of PVA-EPC will be similar to that of PVA. In fact, PVA-EPC exhibited a significant air/water interfacial activity. Furthermore, the PVA-EPC layer surrounding oil droplets will be hardened after being salting-outed and subsequently being cross-linked, so the oil droplets will hardly have a chance to fuse one another. Accordingly, the stability of the emulsion droplets could be ascribed to the hydrophilic protecting layer of PVA-EPC, and the layer hardening caused by the salting-out and the photo- cross-linking.

Fig. 7(a) shows the photomicrographs of hollow microcapsules obtained after oil phase (chloroform) and salt were removed from the salt-added/photo-treated emulsion. The shape was somewhat irregular, but the size was comparable that of the oil droplets of emulsion. Obviously, a hollow core and shell structure was observed on the photo. Fig. 7(b) shows the photo of hollow microcapsules obtained after oil and salt being removed from the salt-added/photo-untreated

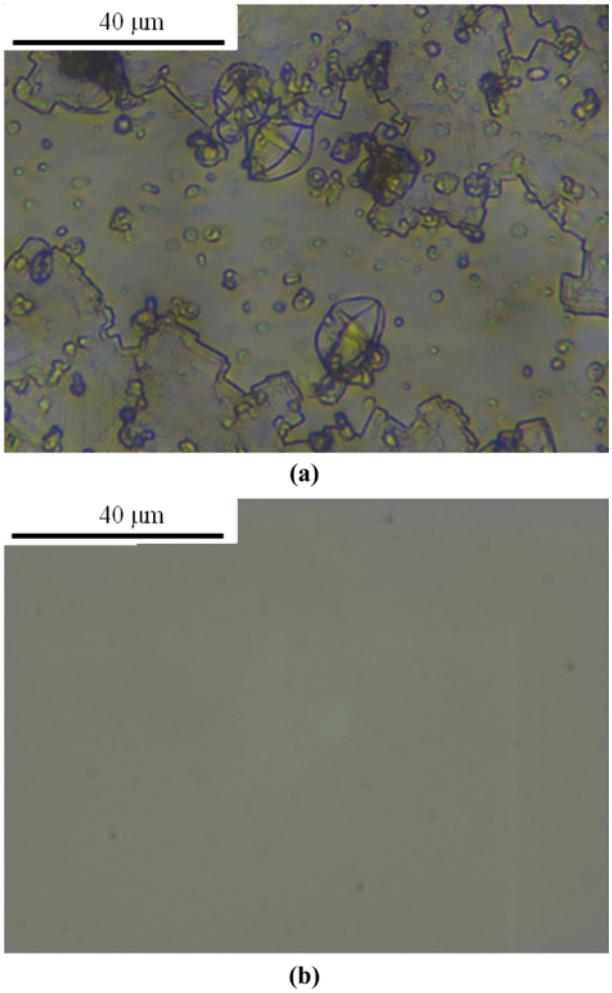

Fig. 7. Photomicrographs of hollow microcapsules obtained after oil phase (chloroform) and salt being removed from salt-added/photo-treated emulsion (a) and salt-added/photo-untreated emulsion (b) (Magnification ×400).

emulsion. After oil was evaporated and salt was removed by dialysis, the turbid emulsion became somewhat transparent solution. As a result, there was no trace of particles on the photograph. The PVA-EPC wall hardened and solidified by the salting-out will be dissolved upon dialysis because the hydroxyl groups of PVA-EPC become fully hydrated with water molecules when salt is absent. Since the microparticles having core/shell structure were obtained from photo-treated emulsion even after the salt was removed by dialysis, it is concluded that the walls (PVA-EPC layers) of microcapsules in Fig. 7(a) were photo cross-linked.

CONCLUSIONS

PVA-coumarin conjugate was successfully prepared by reacting the polyol with epoxyproxy coumarin (EPC). Oil-in-water (O/W) emulsion could be prepared using chloroform as an oil phase and PVA-coumarin as an emulsifier. The wall surrounding oil droplets was built-up by the addition of NaCl to the O/W emulsion, and the PVA-coumarin wall was photo cross-linked under the irradiation of λ=365 nm. Hollow microcapsules were obtained after the oil

phase was evaporated and the salt was removed by dialysis. Hollow microcapsules prepared in the present work would be used as a photo-responsive drug carrier, since the cross-linking wall of microcapsule can be controlled by photo irradiation.

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