Photo-responsive microgels composed of polymeric β-cyclodextrin and Tween 20-coumarin conjugate

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Abstract−Photo-responsive microgels were prepared by taking advantage of the interaction between Tween 20-coumarin conjugate (TCC) and polymeric β-cyclodextrin (PβCD). TCC was prepared by covalently attaching coumarin to the hydroxyl group of the head (PEO segment) of Tween 20. The molar ratio of coumarin residue and Tween 20 was 1 : 1, determined on the ^{1}H NMR spectrum. PβCD was prepared by reacting epichlorohydrins (EPI) with the hydroxyl groups of βCDs. The primary hydroxyl groups participated in the polymerization reaction, evidenced by the ^{13}C NMR spectrum. The CD content in PβCD, determined by a colorimetric method, was 37.4%. Microgels were prepared by adding TCC to the aqueous solution PβCD (20%, w/v) so the molar ratio of TCC to βCD residue was 0.72 : 1. The shape was spherical on SEM photo and optical microscopic photo, and the diameter, measured on light scattering equipment, was a few micrometers. The size dramatically decreased by the irradiation of λ at 365 nm, possibly due to the photo-dimerization of coumarin residue of TCC. Thr size of the photo-treated microgel markedly increased by the irradiation of λ at 254 nm, possibly due to the de-dimerization of the coumarin residue dimers.

Key words: Coumarins, Tween 20, Polymeric β-Cyclodextrin, Photo-responsive Microgel

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucose residues. The cyclic oligosaccharides of six, seven, and eight glucose residues are called α-, β-, and γ-CD, respectively, and each has a hydrophobic cavity of diameter 0.57 nm for α-CD, 0.78 nm for β-CD, and 0.95 nm for γ-CD. They can form inclusion complexes with various hydrophobic compounds owing to the hydrophobic interaction of their cavities with the compounds [1,2]. The inclusion complexation property allows for the utilization of CDs as deodorants [3], separation materials [4], textiles [5,6], solubiliz-ers [7] and catalyzers [6,8].

CD polymers and CD hydrogels were prepared as drug vehicles, and they were endowed with a stimuli-sensitivity by including or associating a stimuli-responsive material into or with the cavities of CD residues. pH-responsive microgels were prepared by including a basic aromatic compound (e.g., (2-Hydroxyethyl) trimethylammonium chloride benzoate) in the cavity of a CD polymer and allowing the compound to electrostatically interact with an oppositely charged polymer (e.g., carboxymethylcellulose) [9]. Because the ionization degree of the included compound depends on the pH of medium, the microgel exhibited a pH-sensitive release. In addi-
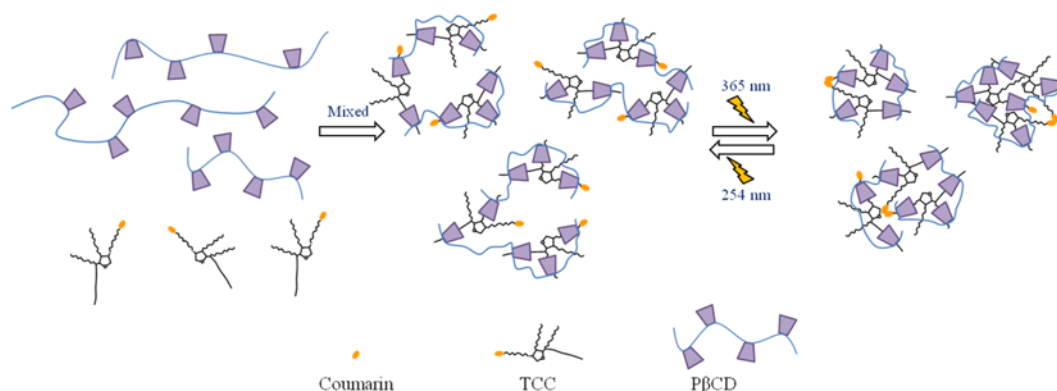

Fig. 1. Schematic illustration for microgel formation and its light-responsive size change. The coumarin moiety, the PEO segments and the hydrocarbon chain of TCC interact with the CD cavities of PβCD. As a result, Tween 20-coumarin conjugate can act as a physical cross-linker for PβCD, leading to the formation of microgel. Since the microgels contain a photo-dimerizable molecule, coumarin, they can change their properties (e.g., size) in response to a light irradiation.

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tion, a pH-responsive microgel was prepared by including an acidic aromatic compound (e.g., 1-Naphthaleneacetic acid) in the cavity of a CD polymer [10]. On the other hand, a temperature-responsive hydrogel was prepared by incorporating a hydrophobically modified thermo-sensitive polymer in a CD hydrogel [11].

In this study, a light-responsive microgel was prepared by including a light-sensitive surfactant in the cavity of a CD polymer. The light-sensitive surfactant was prepared by conjugating coumarin to the head group (PEO segments) of Tween 20. The coumarin moiety attached to the head (PEO segment) of the conjugate can interact with the CD cavities of PβCD. Coumarins were reported to interact with the CD cavity [12]. PEG segment, the head of the conjugate, is also known to interact with the CD cavity [13]. And the hydrocarbon chain of the conjugate is believed to hydrophobically interact with the CD cavities of PβCD. As a result, Tween 20-coumarin conjugate can act as a physical cross-linker for PβCD. Due to the interaction between the conjugates and the CD cavities, microgels can be formed as illustrated in Fig. 1. Since the microgels contain a photo-dimerizable molecule, coumarin, they can change their properties (e.g., size) in response to light irradiation (Fig. 1).

MATERIALS AND METHODS

1. Materials

β-Cyclodextrin (βCD, M.W. 1135) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), epichlorohydrin (EPI, M.W. 92.53), 7-hydroxycoumarin (M.W. 162.14), methylsulfonyl chloride (M.W. 114.55), triethylamine (M.W. 101.19), cesium carbonate (M.W. 325.82) and Tween 20 (M.W. 1228) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Water was doubly distilled in a Milli-Q water purification system (Millipore Corp.) until the resistivity was 18 MΩ/cm. All other reagents were in analytical grade.

2. Preparation of PβCD

βCD polymer (PβCD) was prepared by a method reported in a previous report [14]. βCD, 5 g, was dissolved in an aqueous solution of NaOH (8 ml, 30%, w/w) in a 100 ml-beaker, and then EPI (3.34 ml) was dropped to the βCD solution so that the molar ratio of βCD to EPI was 1/10. The polymerization was undertaken at 30 °C for 2 hr and 15 min with the reaction mixture being stirred. After the pH of the reaction mixture was adjusted to pH 12 using 6 N HCl, the reaction mixture stood overnight in an oven thermostated at 50 °C. After being cooled to room temperature and neutralized using 6 N HCl, the reaction mixture was dialyzed against distilled water using a dialysis bag (MWCO 1,000). The product was precipitated out in 1,000 ml of acetone, filtered through a filter paper (24.0 cm cycles, Whatman, England), and dried in an oven kept at 50 °C.

3. Characterization of PβCD

After the product, PβCD, was further dried with P_2O_5 under reduced pressure, it was dissolved in D_2O and the ^{13}C NMR spectrum was taken on a Bruker Avance 400 spectrometer (Karlsruhe, Germany, located in the Central Laboratory of Kangwon National University).

The contents of βCD were determined following a method reported in a previous report [15]. Various amounts of βCD solution (0.25 ml-4 ml) were mixed with 2 ml of sodium carbonate solution

(0.028 M), 2 ml of phenolphthalein solution (0.33 mmol/ml, pH 11.0) was mixed with the βCD solution containing sodium carbonate, and distilled water pre-adjusted to pH 11.0 was added to the mixture solution so that the final volume was 25 ml. The calibration curve was established by plotting the absorbance at 550 nm versus βCD concentration. For the determination of βCD contents, 4 ml of PβCD solution (10 mg/ml) underwent the same procedure as used for the establishment of the calibration curve.

4. Preparation of Photo-sensitive Surfactant

Photo-sensitive surfactant was prepared by covalent attaching coumarin to the head of Tween 20 [16,17]. First, 7-methylsulfonyl-coumarin was obtained by chemically modifying 7-hydroxycoumarin. 5 g of 7-hydroxycoumarin (30.86 mmol) and 6.45 ml of triethylamine (46.3 mmol) were co-dissolved in 50 ml of dichloromethane, and 2.88 ml of methylsulfonyl chloride (37.04 mmol) was dropped to the mixture solution kept around 0 °C. After being stirred for 2 hr at 0 °C and then for 24 hr at room temperature, the reaction solution was washed with distilled water and the solvent was evaporated under reduced pressure around 50 °C. After the product, 7-methylsulfonylcoumarin, was further dried with P_2O_5 under reduce pressure, it was dissolved in $CDCl_3$ and the ^1H NMR spectra were taken on a Bruker Avance 400 spectrometer (Karlsruhe, Germany) in the Central Laboratory of Kangwon National University. And then, 7-methylsulfonylcoumarin was conjugated to Tween 20 following a method described in a previous report [17]. 2 g of Tween 20 (1.6 mmol), 1.92 g of 7-methylsulfonylcoumarin (8.0 mmol) were subsequently dissolved in DMF (25 ml). After 1.6 g of Cs_2CO_3 (4.9 mmol) was added to the solution, the conjugation reaction was done by stirring the reaction mixture at 60 °C under nitrogen atmosphere for 48 hr. Cs_2CO_3 was filtered and the solvent in the filtrate was evaporated under vacuum at 60 °C. The crude viscous product was dialyzed against ethanol for 48 hr and then against distilled water for 48 hr. Finally, the purified product (Tween 20-cumarin conjugate, TCC) was freeze-dried for further use.

5. Characterization of Photo-sensitive Surfactant

After the product (TCC) was dried with P_2O_5 under reduced pressure, it was dissolved in DMSO and the ^1H NMR spectra was taken on a Bruker Avance 400 spectrometer. The photo-dimerization of TCC was investigated with and without PβCD. TCC was dissolved in distilled water so that the concentration was 9.5×10^{-3} M. In parallel, it was dissolved in PβCD solution in distilled water so that the concentration was the same. The solutions were subjected to the cyclic irradiation, 365 nm (400 W) for 10 min and then 254 nm (6 W) for 20 min. The dimerization degree was determined as follows [18].

$$Dimerization\ (\%) = (1 - A_t/A_0) \times 100\%$$

Where, A_0 was the absorbance at 324 nm before irradiating by UV light, and A_t was the absorbance after irradiating by UV light.

6. Preparation of Photo-responsive Microgels

0.4 g of PβCD was dissolved in 2 ml of distilled water contained in a 10 ml vial so that the concentration was 20% (w/v). And then, 0.131 g of photo-sensitive surfactant (TCC) was mixed in 2 ml of PβCD solution so that the concentration was 6.6% (w/v).

7. Characterization of Photo-responsive Microgels

The size of microgels was measured on a light scattering machine (ZetaPlus 90, Brookhaven Instrument Co., USA) before and after

being subjected to UV irradiation. The microgel suspension was subjected to the 3 hr-irradiation of λ=365 nm and then it underwent 0.5 hr irradiation of λ=254 nm. The microgel suspensions were diluted with distilled water so that the counter per second of the machine was 50-200. In parallel, PβCD solutions without TCC were subjected to size measurement. The shapes of microgels were observed on a photomicroscope (400X, AE 31, Motic Group CO., LTD) and on a scanning electron microscope (SEM, Jeol JSM-840A). Freeze-dried PβCD/TCC suspensions were put on metal stubs, coated with gold, and observed on the scanning electron microscope. In parallel, PβCD solution without Tween 20-coumarin conjugate was subjected to shape observation.

RESULTS AND DISCUSSION

1. Characterization of PβCD

Fig. 2 shows the ^{13}C NMR spectrum of PβCD. The signals found at 102.8 ppm, 82.1 ppm, 74.2 ppm, 73.5 ppm, 71.7 ppm and 69.9
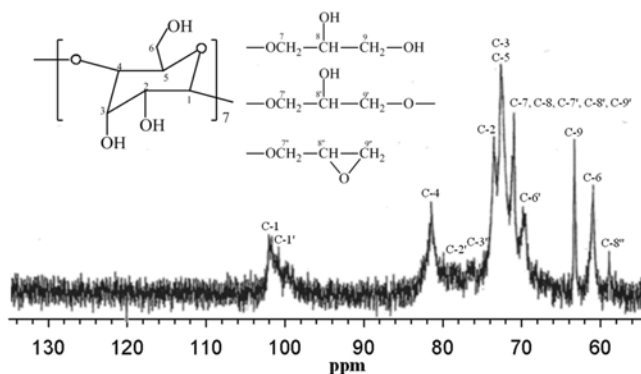

Fig. 2. ^{13}C NMR spectrum of PβCD.

ppm are ascribed to the carbons of β-CD residue. The signal at 69.9 ppm is assigned to carbons on the primary face of β-CD residue. The signals at 73.8 ppm and 71.7 ppm are from carbons on the secondary face. The signal of substituted carbon on the secondary face was not found, indicating that the hydroxy groups on the primary surface participated in the reaction with EPI. The signal at 60.7 ppm is from carbons of EPI residues. The results described above are in a good agreement with those reported in previous works [9,14].

The calibration curve for the determination of βCD content of PβCD was Y=−0.746 X−1.30 (R^2=0.9949), where X is the logarithmic value of absorbance at 550 nm and Y is the logarithmic value of βCD concentration in mg/ml. The equation for calibration curve is slightly different from that reported in a previous work [19], because the absorbance depends on the intensity of UV light. The content of βCD residues of PβCD was calculated to be about 37.4%, which corresponds to the molar ratio of βCD to EPI residue which is 1/20.5.

2. Characterization of Photo-sensitive Surfactant

Fig. 3 shows the ^{1}H NMR spectrum of TCC dissolved in CDCl$_3$. The signals in 7.63 ppm and 6.25 ppm are from the CH groups of the pyranone of coumarin residue, the signals in 7.36 ppm, 6.90 ppm, and 6.82 ppm are ascribed to the aromatic protons of coumarin residue, the signals in 4.19 ppm and 2.31 ppm originate from the CH$_2$ group of sorbitan moiety of Tween 20, the signals in 3.89 ppm are from the CH groups of the sorbitan moiety, the signals in 3.64 ppm are attributed to the CH$_2$ groups of PEO segments of Tween 20, the signals in 1.28 ppm are from the CH$_2$ groups of the hydrocarbon chain of lauric acid residue of Tween 20, and the signals in 0.88 ppm are from the CH$_3$ group of the lauric acid residue. The peak area of aromatic ring and CH groups in coumarin residue was 4.979 and the peak area of CH$_3$ group of Tween 20 was 4.559. Accordingly, the molar ratio of coumarin residues to Tween 20 was calculated to be about 1 : 1. A conjugate of which the molar ratio of coumarin residue to Tween 20 was greater than 1 could hardly
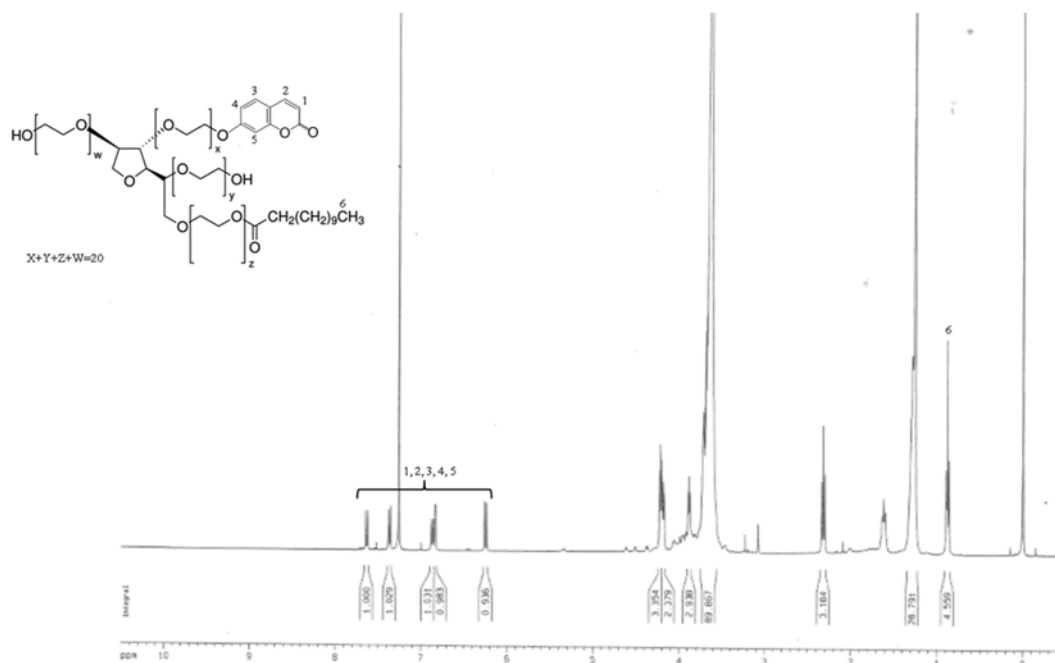

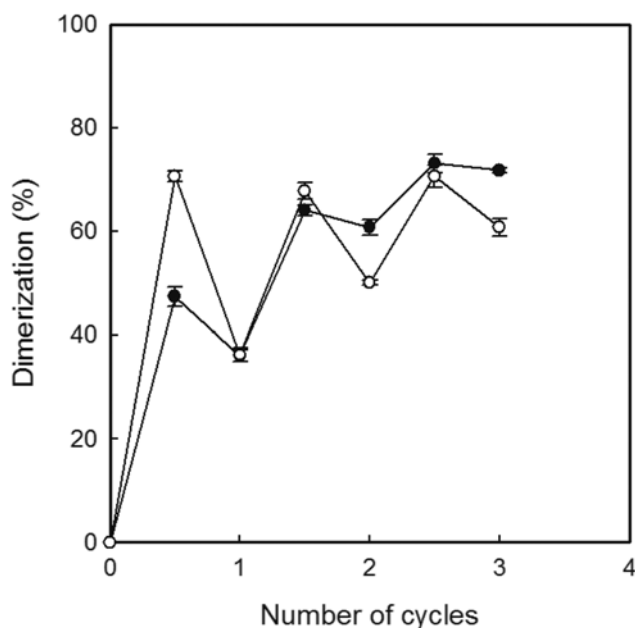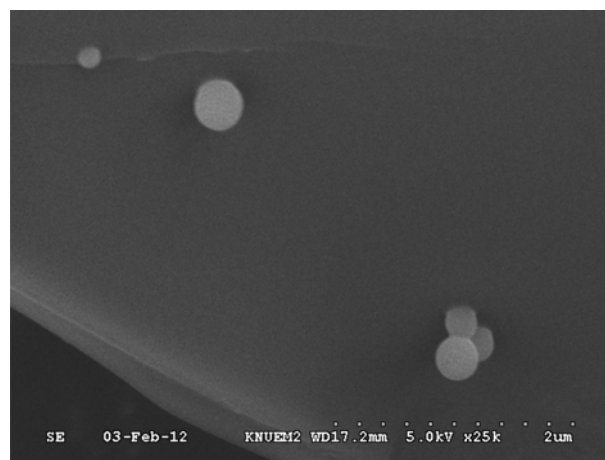
Fig. 3. ^{1}H NMR spectrum of TCC.

Fig. 4. Dimerization degrees of coumarin residue of TCC with (●) and without (○) PβCD under cyclic irradiation of UV light (λ=365 nm (400 W) for 10 min and then λ=254 nm (6 W) for 20 min) (n=3).

be prepared despite the feed ratio of reactive coumarin (7-methyl-sulfonylcoumarin) to Tween 20 was 5 : 1. An increase in the feed ratio and an extension in the reaction time could not raise the ratio [17]. Once one molecule of the coumarin derivative has been attached to the PEO segment of Tween 20, another molecule would hardly react with the other PEO segments because PEO segment is short (the number of EO units in one PEO segment is less than 20) and the coumarin derivative is bulky.
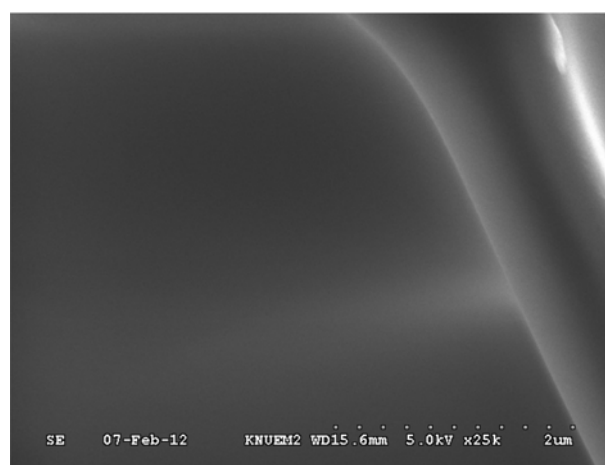
Fig. 4 shows the dimerization degrees of coumarin residue of TCC with and without PβCD under cyclic UV irradiation. The dimerization degree of coumarin residue of TCC without PβCD increased to 71% upon the 10 min-irradiation of 365 nm, and then it decreased to 35% upon the subsequent 20 min-irradiation of 254 nm. Coumarins are dimerized under UV irradiation (e.g., λ>310 nm) through the break-down of pyranone double bond and the formation of cyclobutane bridge, and de-dimerization takes place upon the irradiation of a UV light having a shorter wave (e.g., λ<260 nm) [20,21]. Cyclic dimerization/de-dimerization was observed in further cyclic irradiation. Coumarin residues of TCC in the presence of PβCD also exhibited a cyclic increase and decrease in the dimerization degree. However, the dimerization degree and the de-dimerization degree in each cycle were less than those of coumarin residue of TCC without PβCD. It was reported that coumarins can be included in the cavity of βCD [12]. Therefore, the coumarin residues of TCC would be included in the cavities of the βCD residues of PβCDs, and they are likely to be less susceptible to photo dimerization and photo de-dimerization due to the sequestering effect of βCD residues.

3. Characterization of Photo-responsive Microgels

Fig. 5 shows the SEM photos of freeze-dried PβCD/TCC solution (a) and freeze-dried PβCD solution (b). On the SEM photo of freeze-dried PβCD/TCC solution, spherical particles are observed and the diameter is hundreds of nm. TCC was prepared by covalently

(a)

(b)

Fig. 5. SEM photos of freeze-dried PβCD/TCC solution (a) and freeze-dried PβCD solution (b). The magnification was 25,000× and the bar represents 2 μm.

attaching coumarin to the head groups (PEO segments) of Tween 20. The coumarin moiety attached to the head (PEO segment) of TCC can interact with the βCD cavities of PβCD. Coumarins were reported to interact with the βCD cavities [12]. PEG segment, the head of the conjugate, is also known to interact with the βCD cavity [13]. The hydrocarbon chain of the conjugate is believed to hydrophobically interact with the βCD cavities of PβCD. As a result, TCC can act as a physical cross-linker for PβCD. In this circumstance, microgels can be formed as shown in Fig. 5(a). On the SEM photo of freeze-dried PβCD solution, no particle was found as shown in Fig. 5(b).

Fig. 6 shows the optical microscopic photos of PβCD/TCC solution (a) and PβCD solution (b). On the photo of PβCD/TCC solution, spherical particles were observed and they were fluorescent. Coumarins emit fluorescence light when excited with visible light [22]. Accordingly, the coumarin residue attached to the head of Tween 20 is responsible for the fluorescence. On the photo of PβCD solution, no particle was found as shown in Fig. 6(b).

Fig. 7 shows the size distributions of microgels before UV irradiation (a), after UV irradiation of λ=365 nm for 3 hr (b), and after

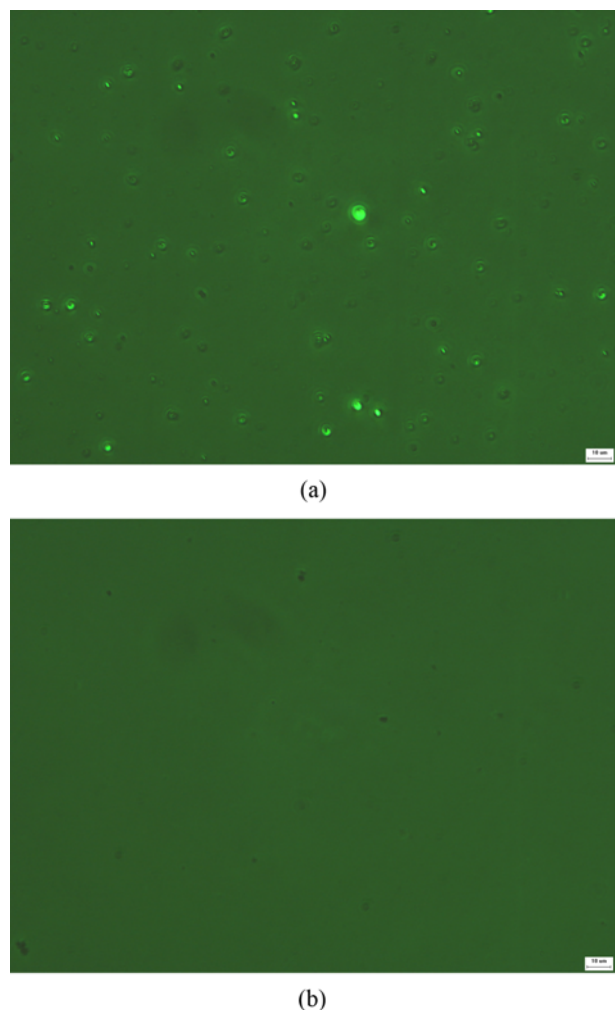Fig. 6. Optical microscopic photos of PβCD/TCC solution (a) and PβCD solution (b).

UV irradiation of λ=365 nm for 3 hr and then λ=254 nm for 30 min (c). Without UV irradiation, the diameter was in the range of 1 μm-6 μm and mean size was 2680.7±192.7 nm. Upon the UV irradiation of λ=365 nm, the diameter of microgel decreased to less than 1 μm and mean size was 935.02±48.7 nm. The coumarin residue of TCC was readily dimerized under the UV irradiation of λ=365 nm, even in the presence of PβCD (Fig. 4). The photo dimerization of the coumarin residues of TCC will increase the cross-linking density of the microgel and, thus, decrease the size. Upon the UV irradiation of λ=254 nm, the diameter of the photo cross-linked microgel increased to 7 μm-10 μm and mean size was 3334.6±155.8 nm. The dimerized coumarin residue of TCC was readily de-dimerized under the UV irradiation of λ=254 nm even in the presence of PβCD (Fig. 4). Photo de-dimerization will decrease the cross-linking density of the microgel and, thus, increase the size. On the other hand, whether UV was irradiated or not, no trace of particle was detected with PβCD solution containing no TCC.

CONCLUSIONS

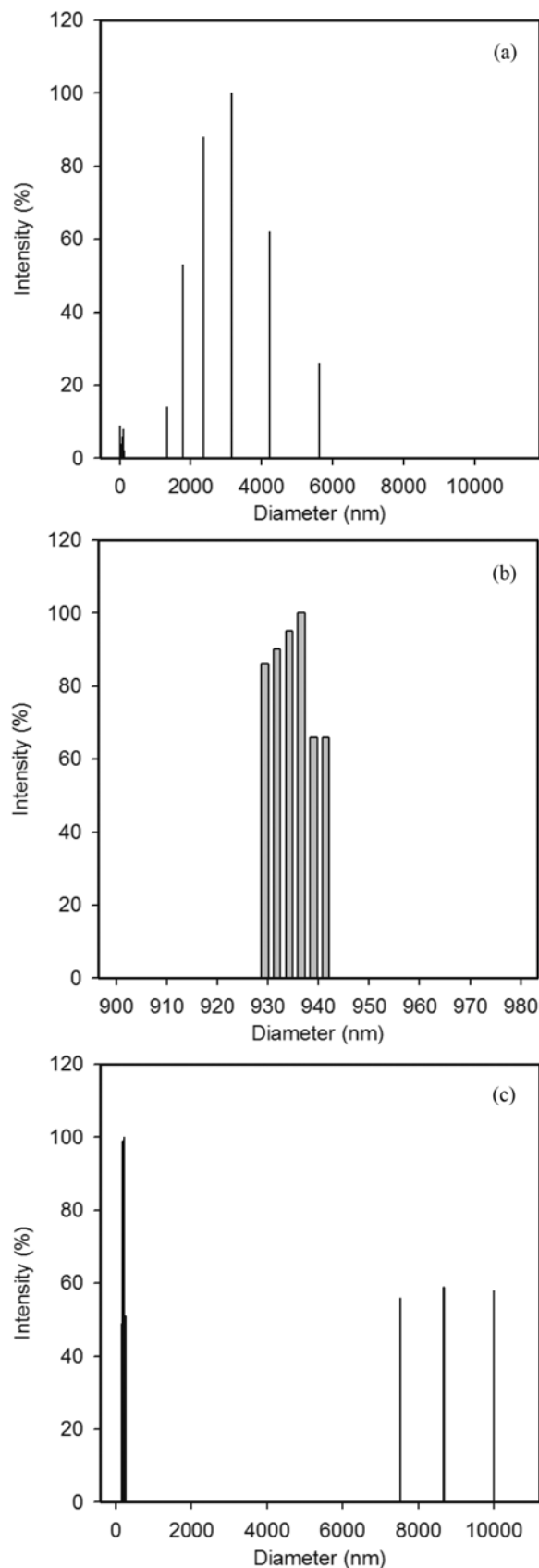PβCD was prepared by reacting EPI and βCD in the ratio of 10 : 1,

Fig. 7. Size distributions of microgels before UV irradiation (a), after UV irradiation of λ=365 nm for 3 hr (b) and after UV irradiation of λ=365 nm for 3 hr and then λ=254 nm for 30 min (c).

and the content of βCD residue was 37.4% (w/v). TCC was prepared by reacting with a coumarin derivative (methylsulfonylcoumarin) and Tween 20 in the molar ratio of 5 : 1, and the coumarin residue/Tween 20 molar ratio of TCC was 1 : 1. The coumarin residues of TCC in the presence of PβCD were somewhat less susceptible to photo dimerization and photo de-dimerization than that of TCC in the absence of PβCD was, possibly due to the sequestering effect of βCD residue. However, photo dimerization and photo de-dimerization readily took place even in the presence of PβCD. The addition of TCC to an aqueous solution of PβCD led to the formation of microgels. The head (coumarin residues and PEO segments) and the tail of TCC, both of them, are believed to interact with the βCD cavity of PβCD so TCC is thought to act as a physical cross-linker for PβCD. The microgels decreased their size in response to the irradiation of λ=365 nm and the photo-treated microgels increased their size in response to the irradiations of λ=254 nm. This is possibly because photo dimerization of the coumarin residues of TCC under the irradiation of λ=365 nm increases the cross-linking density of the microgels and photo de-dimerization under the irradiation of λ=254 nm decreases the cross-linking density. The microgel developed in the present work could be used as a drug carrier which releases its content in a controlled manner in response to the light irradiation. Such as during the surgery, the photo responsive microgel could be applied on the tissue or cells, then just give UV irradiation on the diseased part; the drug contained in the microgel can be released without hurting the normal cell, because the drug release part can be controlled by UV irradiation.

ACKNOWLEDGEMENTS

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