

Construction of Photoresponsive Supramolecular Micelles Based on Ethyl Cellulose Graft Copolymer^{*}

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Abstract In this work, a UV-Visible light controlled supramolecular system based on ethyl cellulose (EC) was constructed, combining the host-guest interaction of β -cyclodextrin (β -CD) group and trans-isomer of azobenzene (*t*Azo) group. To link β -CD to the hydrophobic section, renewable EC was used as macroinitiator to initiate the polymerization of ϵ -caprolactone (ϵ -CL) to form biocompatible and biodegradable comb copolymer EC-*g*-PCL, and β -CD was attached to the end of PCL side chain *via* click reaction. Meanwhile, hydrophilic PEG-*t*Azo was obtained by *N,N'*-dicyclohexylcarbodiimide (DCC) coupling. Then, the structures of the products were characterized by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). Subsequently, with the formation of inclusion complexes by β -CD and *t*Azo groups, the obtained EC-*g*-PCL- β -CD/PEG-*t*Azo supramolecular system self-assembled in water with hydrophobic EC-*g*-PCL- β -CD as core and hydrophilic PEG-*t*Azo as shell. Furthermore, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were utilized to investigate the particle size and size distribution, while NMR and UV-Vis spectra were applied to explore the UV-Visible light stimuli-responsiveness of the micelles.

Keywords: Ethyl cellulose; Supramolecular system; Self-assembly; Photoresponse.

INTRODUCTION

Cellulose as one of the most abundant natural materials has drawn much attention with the increasing demand for new and recycled materials, due to its biocompatibility, biodegradability, mechanical strength and low cost^[1–5]. Nowadays, research on cellulose as polymeric materials mainly focuses on two aspects^[6]: one is the effective and green solvent for cellulose to resolve its poor solubility in most solvent resulted from the existence of intermolecular hydrogen bonds, while the other is the further development of multifunctional cellulose and cellulose derivatives. Ethyl cellulose (EC) as one of the most common cellulose derivatives has been widely used as dispersants, stabilizers, water-retaining agents and adhesives in the food, coatings and pharmaceutical industry. Moreover, EC is soluble in most organic solvent, possesses many active sites and can be modified through diverse chemical reactions, such as graft polymerization, coupling reaction and oxidation reaction. The diversified modification of EC provided more extensive applications including porous materials^[7] and drug delivery carriers^[8–10]. So far, our research group has reported some studies about the functionalization of cellulose and cellulose derivatives^[11–15]. However, more detailed functions and applications of these materials are expected.

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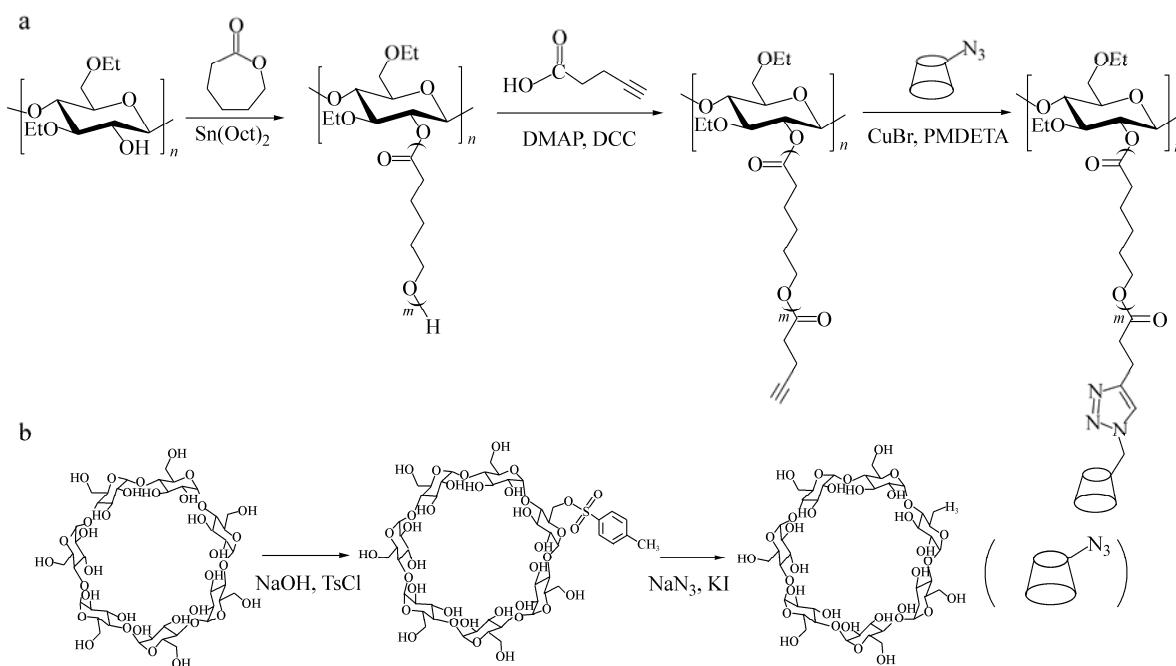
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Meanwhile, host-guest interactions^[16–18] stand out in supramolecular chemistry due to their specific recognitions^[19], which are very attractive and challenging in the field of biostimulation and biomedicine. The most common host molecules, such as cyclodextrin (CD)^[20, 21], cucurbituril (CB)^[22, 23] and crown ether^[24], always act as receptors for suitable organic or inorganic guest molecules with their well-established host properties. Amongst them, cyclodextrins [*n*] (*n* = 6–12), a family of macrocyclic compounds comprising *n* 1,4-linked D(+)-glucose units, possess a hydrophobic cavity that can form inclusion complexes with organic or organometallic guest molecules^[25]. Additionally, CDs have hydrophilic exteriors, resulting in good water solubility. Moreover, the modification of CDs is easier and more diversified than other host molecules investigated in the previous work^[26–28]. Thus, CDs are preferred in many fields such as the combination of CDs and polymers, which can be employed for the design of supramolecular architectures and the exploration of diverse applications^[29–33].

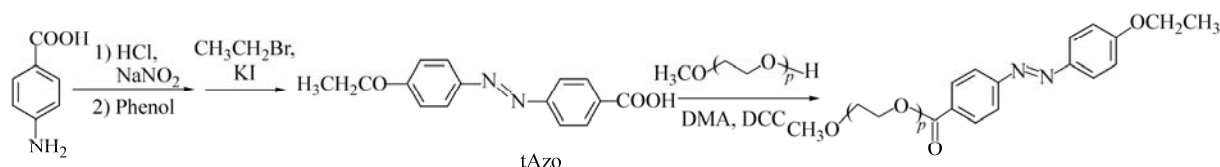
Furthermore, β -CD can form inclusion complex with trans-azobenzene (*t*Azo) selectively. In fact, *t*Azo moieties can be captured into the cavity of β -CD, whereas the cis-isomers of azobenzene (*c*Azo) cannot fit into it^[20]. Moreover, the photoisomerization of *t/c*-Azo isomers can be induced by external photostimulation^[34]. Hence, the light control recognition of β -CD and Azo has been served as the switch in the construction of supramolecular systems extensively^[35].

Taking these aspects into consideration, the work here constructed a unique supramolecular system combining the hydrophobic comb copolymer, EC-*g*-PCL- β -CD, and hydrophilic PEG-*t*Azo *via* the host-guest interaction between β -CDs and *t*Azo groups. In the hydrophobic segment, EC was used as macroinitiator in the polymerization of ε -caprolactone (ε -CL) to form biocompatible and biodegradable comb copolymer EC-*g*-PCL. β -CD was subsequently attached to the end of PCL side chain *via* click reaction (Scheme 1). Meanwhile, the hydrophilic PEG-*t*Azo was obtained from dehydration reaction with *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrant (Scheme 2). Then, the molecular structures of all intermediates and target products were confirmed by NMR and GPC. Afterwards, the supramolecular system EC-*g*-PCL- β -CD/PEG-*t*Azo self-assembled into micelles with hydrophobic EC-*g*-PCL- β -CD as core and hydrophilic PEG-*t*Azo as shell after the recognition of β -CD and *t*Azo groups. Subsequently, fluorescence spectra was used to investigate the critical micelle concentration (CMC) of the fabricated supramolecular system with pyrene as the probe, while DLS and TEM were utilized to explore



Scheme 1 Synthetic route of comb copolymer EC-*g*-PCL- β -CD

the particle size and size distribution of the micelles. Additionally, the UV-Visible light responsiveness of EC-*g*-PCL- β -CD/PEG-*t*Azo supramolecular system was demonstrated by the chemical shifts of the corresponding resonance of *t*Azo in ^1H -NMR spectra as well as the intensity changes of the characteristic absorption peaks of *t*Azo in UV-Visible absorption spectra.



Scheme 2 Synthetic route of PEG-*t*Azo

EXPERIMENTAL

Materials

EC ($M_n = 2.3 \times 10^4$ g·mol $^{-1}$, the degree of ethyl substitution was 2.3) was purchased from Luzhou North Chemical Industry Co., Ltd. and dried at 80 °C for 24 h under vacuum. Poly(ethylene glycol) (PEG, $M_n = 2.0 \times 10^3$ g·mol $^{-1}$) was dried by azeotropic distillation in the presence of toluene. ε -Caprolactone (ε -CL, Acros Organics, 98%), stannous octoate ($\text{Sn}(\text{Oct})_2$, Aldrich, 98%) and pentamethyldiethylenetriamine (PMDETA, Acros Organic, 98%) were purified with CaH_2 by vacuum distillation before use. β -CD was obtained from Tianjin Bodi Chemical Industry and recrystallized from water before use. Copper(I) bromide (CuBr , Acros Organics, 97%) was treated by stirring in acetic acid and washing with ethanol. *p*-Toluenesulfonyl chloride (TsCl , J&K, 99%), sodium azide (NaN_3 , Tianjin Nankai Share Compounds Co., Ltd., 99%), potassium iodide (KI , Beijing Chemical Works, 98.5%), 4-pentynoic acid (J&K, 95%), *N,N'*-dicyclohexylcarbodiimide (DCC, J&K, 99%), 4-dimethylaminopyridine (DMAP, J&K, 99%), acetonitrile, acetone and dimethyl formamide (DMF) were used as received. Xylene and chloroform were dried over CaH_2 for 24 h and distilled prior to use.

Characterization

^1H - and ^{13}C -NMR spectra were determined from a JEOL JNM-ECA 300 NMR spectrometer with tetramethylsilane as internal standard. The Viscotek TDA 302 gel permeation chromatograph equipped with two columns (GMHHR-H and M Mixed Bed) was performed with DMF as eluent at a flow rate of 1 mL·min $^{-1}$ at 25 °C and polystyrene as standard. UV-Vis absorption spectra and fluorescence spectra were recorded on a UV 2100 UV-Vis spectrophotometer (Shimadzu, Japan) and a Jasco F-6500 fluorescence spectrometer (Shimadzu, Japan) respectively with thermostatically controlled cuvettes. TEM observations were performed on a JEOL JEM-2010 electron microscope operated at an acceleration voltage of 120 kV and the DLS measurements were performed using the Zetasize 3000Hsa laser light scattering measurement system.

Synthesis of Mono-6-(azido)- β -cyclodextrin (mono- β -CD-6- N_3):

The synthesis of mono-6-(azido)- β -CD was performed according to the previous literature^[19, 36]. A representative synthetic process has two steps. First, the intermediate mono- β -CD-6-OTs was prepared: β -CD (40 g, 35.2 mmol) was dissolved in 350 mL of water, followed by the addition of 14 mL of sodium hydroxide solution (2.19 g, 54.7 mmol) within 5 min under magnetic stirring. Then, the flask was immersed into an ice-water bath and 20 mL of acetonitrile solution containing TsCl (6.72 g, 35.2 mmol) was dropwise added to the mixture. 30 min later, the flask was maintained at 25 °C and left to react for another 2 h. Afterwards, the crude product precipitated out of the reaction solution and was further purified by recrystallization. After dried in a vacuum oven at 60 °C for 24 h, the purified mono- β -CD-6-OTs was collected.

Next, mono- β -CD-6- N_3 was synthesized as follows: mono- β -CD-6-OTs (3 g, 2.34 mmol) was completely dissolved in 30 mL of water. Subsequently, NaN_3 (0.31 g, 4.68 mmol) and KI (1.92 g, 1.17 mmol) were added to the flask, which was immersed into an oil bath at 80 °C later. After reaction for 24 h, the crude product was

precipitated in a mixture of acetone and water ($V_{\text{acetone}}:V_{\text{water}} = 4:1$) to yield the purified product. Finally, after dried in vacuum at 60 °C for 24 h, the purified mono- β -CD-6- N_3 was obtained.

$^1\text{H-NMR}$ ($\text{DMSO-}d_6$, δ) of mono- β -CD-6-OTs: 7.77 (d, H, CH on aromatic ring), 7.42 (d, H, CH on aromatic ring), 5.76 (s, H, $-\text{OH-2,3}$ on β -CD), 4.86 (d, H, $-\text{H-1}$ on β -CD), 4.31 (s, H, $-\text{OH-6}$ on β -CD), 3.65 (m, H, $-\text{H-3,5,6}$ on β -CD), 3.38 (d, H, $-\text{H-2,4}$ on β -CD), 2.41 (s, 3H, CH_3).

$^1\text{H-NMR}$ (CDCl_3 , δ) of mono- β -CD-6- N_3 : 5.76 (s, H, $-\text{OH-2,3}$ on β -CD), 4.86 (d, H, $-\text{H-1}$ on β -CD), 4.31 (s, H, $-\text{OH-6}$ on β -CD), 3.65 (m, H, $-\text{H-3,5,6}$ on β -CD), 3.38 (d, H, $-\text{H-2,4}$ on β -CD).

Synthesis of Comb Copolymer EC-g-PCL₁₀

A typical synthetic procedure of EC-g-PCL₁₀ was performed as follows: EC (0.61 g, 26 μmol), ε -CL (2.15 g, 18.85 mmol, the molar amount was 10 times that of the hydroxyl of EC) and catalyst $\text{Sn}(\text{Oct})_2$ (77.0 mg, 0.19 mmol, 1% of the molar amount of ε -CL) were dissolved in 15 mL of anhydrous xylene. Then, the reaction flask was degassed via three freeze-pump-thaw cycles and immersed into an oil bath at 120 °C, followed by appropriate magnetic stirring under argon atmosphere. After 18 h, the reaction solution was exposed to atmospheric air and cooled rapidly in liquid nitrogen. Subsequently, the mixture was precipitated in methanol three times to get the purified product. After dried in a vacuum oven for 24 h, the comb copolymer EC-g-PCL₁₀ was obtained.

$M_{n,\text{GPC}} = 1.09 \times 10^5$, $M_w/M_n = 1.27$; $^1\text{H-NMR}$ (CDCl_3 , δ): 4.05 (t, 2H, CH_2O in PCL), 3.02–3.85 (protons on EC), 3.64 (t, 2H, terminal CH_2OH in PCL), 2.29 (t, 2H, COCH_2 in PCL), 1.64 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.38 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.16 (m, 3H, CH_3 in EC); $^{13}\text{C-NMR}$ (CDCl_3 , δ): 173.8 (C=O in PCL), 64.3 (CH_2O in PCL), 34.5 ($\text{OC=OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 28.6 ($\text{OC=OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 25.7 ($\text{OC=OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 24.7 ($\text{OC=OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL).

Synthesis of Alkynyl-appended EC-g-PCL₁₀ (EC-g-PCL₁₀-alkynyl)

As a typical synthesis, the hydroxyl-terminated EC-g-PCL₁₀ (1.968 g, 28.3 μmol), 4-pentynoic acid (0.4 g, 4.08 mmol) and catalyst DMAP (49.82 mg, 4.08 mmol, 50 % of the molar amount of the hydroxyl segments) were dissolved in 20 mL of anhydrous chloroform. Then, the flask was immersed into an ice-water bath. Afterwards, 5 mL of chloroform solution containing DCC (0.8416 g, 4.08 mmol, the molar amount of DCC was the same as that of the hydroxyl groups on PCL side chains.) was dropwise added to the mixture within 30 min under magnetic stirring. After reaction for 24 h at room temperature, the crude product was filtrated to remove the produced *N,N*-dicyclohexylurea (DCU). Subsequently, the filtrate was precipitated in methanol to eliminate the excessive 4-pentynoic acid, DCC and DCU. After dried in vacuum for 24 h, the purified EC-g-PCL₁₀-alkynyl was collected.

$^1\text{H-NMR}$ (CDCl_3 , δ): 4.05 (t, 2H, CH_2O in PCL), 3.02–3.85 (protons on EC), 3.64 (t, 2H, terminal CH_2OH in PCL), 2.53 (t, 2H, $\text{CH}_2\text{CH}_2\text{C}\equiv\text{CH}$), 2.29 (t, 2H, COCH_2 in PCL), 1.98 (s, H, $\text{CH}_2\text{CH}_2\text{C}\equiv\text{CH}$), 1.64 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.38 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.16 (m, 3H, CH_3 in EC).

Synthesis of β -CD-functionalized EC-g-PCL₁₀ (EC-g-PCL₁₀- β -CD)

After obtaining the purified EC-g-PCL₁₀-alkynyl and β -CD-6- N_3 , the hydrophobic EC-g-PCL₁₀- β -CD segment was prepared: EC-g-PCL₁₀-alkynyl (0.25 g, 0.26 mmol side chains) and β -CD-6- N_3 (0.3 g, 0.26 mmol) were dissolved in 25 mL of anhydrous DMF. Then the reaction flask was degassed via freeze-pump-thaw before CuBr (40 mg, 0.28 mmol) and PMDETA (48.5 μL , 0.28 mmol) were added into the solution, followed by two more freeze-pump-thaw cycles. The reaction was carried out at 35 °C with vigorous stirring for 30 h under N_2 atmosphere. Afterwards, the reaction mixture was exposed to atmospheric air and passed through a neutral alumina column to remove the copper catalyst. Further purification of the EC-g-PCL₁₀- β -CD copolymer was conducted by sequential dialysis against DMF and water to remove the excessive β -CD-6- N_3 and implement the exchange of solvent. After dialysis, the solvent was removed by lyophilization and the solid EC-g-PCL₁₀- β -CD was collected.

$^1\text{H-NMR}$ (CDCl_3 or DMSO-d_6 , δ): 7.92 (d, 2H, $\text{N}=\text{N}-\text{N}-\text{CH}_2$), 5.67 (s, 2H, $2,3-\text{OH}$ on $\beta\text{-CD}$), 4.80 (m, H, $1-\text{H}$ on $\beta\text{-CD}$), 4.42 (s, H, $6-\text{OH}$ on $\beta\text{-CD}$), 4.05 (t, 2H, CH_2O in PCL), 3.02–3.85 (protons on EC), 3.64 (t, 2H, terminal CH_2OH in PCL), 3.53 (m, 5H, $2\sim6-\text{H}$ on $\beta\text{-CD}$), 2.86 (m, 2H, $\text{C}=\text{OCH}_2$ at the end of PCL), 2.70 (m, 2H, $\text{C}=\text{OCH}_2\text{CH}_2$ at the end of PCL), 2.29 (t, 2H, COCH_2 in PCL), 1.64 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.38 (m, 2H, $\text{OCOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ in PCL), 1.16 (m, 3H, CH_3 in EC).

Synthesis of 4-Ethoxy-azobenzyl Carboxylic Acid (Azo-COOH)

4-ethoxy-azobenzyl carboxylic acid was synthesized in accordance with the previous literature^[37] and the synthetic process was performed in two steps. The intermediate product 4-hydroxy-azobenzyl carboxylic acid was synthesized in the first step: *p*-aminobenzoate (2.74 g, 0.02 mol) was dissolved in 6 mL of ethanol, before hydrochloric acid (4.14 mL, 37%) was dropwise added to the solution. Then the flask was immersed into an ice-salt bath, followed by the addition of sodium nitrite solution (8 mL, $2.5\text{ mol}\cdot\text{L}^{-1}$) via a constant pressure funnel. Afterwards, the mixture was transferred to a frozen dropping funnel and was dropwise added into 20 mL of sodium hydroxide solution ($2\text{ mol}\cdot\text{L}^{-1}$) containing phenol (2.07 g, 0.022 mol) in an ice bath. After reacting for 12 h at 25°C , the reaction solution was precipitated in acid solution with $\text{pH} = 3.5$ to get the crude product. The purified product 4-hydroxy-azobenzyl carboxylic acid was obtained by washing with water to reach neutral level and drying successively.

Subsequently, 4-ethoxy-azobenzyl carboxylic acid was synthesized as follows: 4-hydroxy-azobenzyl carboxylic acid (0.968 g, 4.0 mmol) was dissolved in 20 mL of ethanol, and 4 mL of sodium hydroxide solution ($2.5\text{ mol}\cdot\text{L}^{-1}$) was added to the mixture with stirring for 30 min. This was followed by the addition of bromoethane (0.872 g, 8.0 mmol) and potassium iodide (6.6 mg, 0.04 mmol), before the flask was immersed into an oil bath at 70°C with stirring for 12 h. Then, orange-yellow solid precipitated out of the mixture after adding 10 mL of hydrochloric acid solution ($2\text{ mol}\cdot\text{L}^{-1}$). Lastly, the purified 4-ethoxy-azobenzyl carboxylic acid (Azo-COOH) was obtained via washing with water to reach neutral level, recrystallization in anhydrous ethanol and vacuum dehydration in sequence.

$^1\text{H-NMR}$ (DMSO-d_6 , δ): 8.13 (d, H, CH on aromatic ring), 7.94 (d, H, CH on aromatic ring), 7.91 (d, H, CH on aromatic ring), 7.14 (d, H, CH on aromatic ring), 4.16 (m, 2H, CH_2), 1.38 (t, 2H, CH_3).

Synthesis of Azobenzyl Functionalized PEG (PEG-tAzo)

A typical synthetic procedure of PEG-Azo was adopted: PEG ($M_n = 2.0 \times 10^3$, 2 g, 1.0 mmol) was dissolved in methylbenzene and azeotropic distillation was used to remove the traces of water. Then Azo-COOH (0.54 g, 2.0 mmol), DMAP (0.061 g, 0.50 mmol) and THF (50 mL) were added to the flask, which was later immersed into an ice-water bath. Subsequently, 5 mL of THF solution containing DCC (0.413 g, 2 mmol; the molar amount of DCC was the same as that of carboxyl.) was dropwise added to the mixture with magnetic stirring. After reacting for 12 h at room temperature, the precipitated white solid was filtered off and the filtrate was precipitated in diethyl ether to remove the excessive Azo-COOH, DCC and DCU. The purified PEG-Azo was obtained after dried in vacuum for 24 h.

$^1\text{H-NMR}$ (CDCl_3 , δ): 8.13 (d, H, CH on aromatic ring), 7.94 (d, H, CH on aromatic ring), 7.91 (d, H, CH on aromatic ring), 7.14 (d, H, CH on aromatic ring), 4.16 (m, 2H, CH_2), 3.63 (m, 2H, CH_2 on PEG), 1.38 (t, 2H, CH_3).

Assembly/Disassembly of EC-g-PCL₁₀- β -CD/PEG-tAzo System

After obtaining the hydrophobic segment (EC-g-PCL₁₀- β -CD) and the hydrophilic chain (PEG-tAzo), EC-g-PCL₁₀- β -CD/PEG-tAzo self-assembled in the selective solvent DMF/H₂O ($2\text{ mg}\cdot\text{mL}^{-1}$, 1:5, V/V). 11.0 mg EC-g-PCL₁₀- β -CD (74.7 μmol , $\beta\text{-CD}$ groups: 2.0 μmol) and 9.1 mg PEG-tAzo (4.0 μmol) were dissolved in 1 mL of DMF completely. After ultrasonic treatment for another 1 h, the mixed solution was added with 5 mL of deionized water via injection syringe at a rate of $1\text{ mL}\cdot\text{h}^{-1}$, accompanying with magnetic stirring. Subsequently, the obtained mixture was dialyzed against deionized water to remove the excessive PEG-tAzo and implement the exchange of solvent. After dialysis, the EC-g-PCL₁₀- β -CD/PEG-tAzo assembly system was collected.

Furthermore, 1 mL solution of the EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo assembly system was exposed to a UV-light of 365 nm in a quartz colorimetric utensil, and the disassembly of EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo system occurred after some time has passed.

RESULTS AND DISCUSSION

Preparation and Characterization of Mono-6-(azido)- β -cyclodextrin (mono- β -CD-6-N₃)

Mono- β -CD-6-N₃ was synthesized via two steps from β -CD. In the first step, TsCl was utilized to activate β -CD and the intermediate mono- β -CD-6-OTs was obtained. Then, mono- β -CD-6-N₃ was prepared from nucleophilic substitution with -N₃ as the nucleophile and -OTs as the excellent leaving group.

Shown on the ¹H-NMR spectra in Fig. 1, the characteristic peaks (a, b, c, e, d, f and H on -OH) of β -CD could be identified, and the peaks g, h and i were attributed to protons of the attached tosyl group. After the substitution of -N₃, it is clear to see the corresponding resonance of the tosyl group disappeared completely, indicating a high efficiency of the substitution reaction.

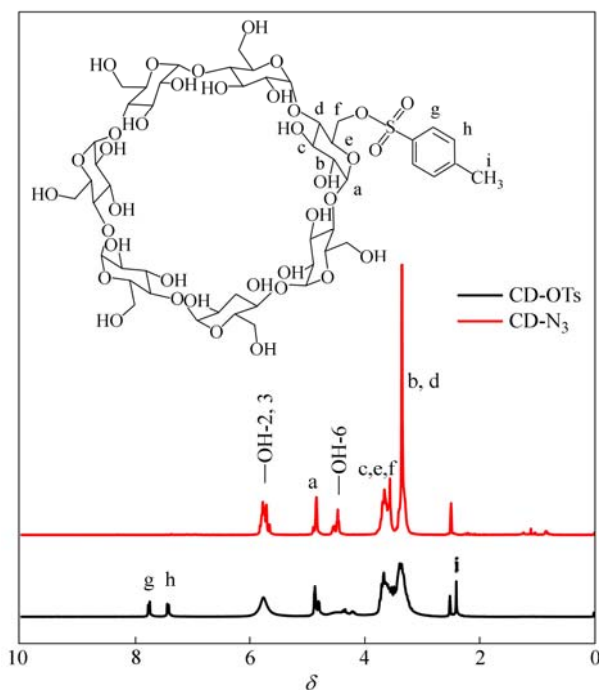


Fig. 1 ¹H-NMR spectra of mono- β -CD-6-OTs and β -CD-6-N₃

Preparation and Characterization of Comb Copolymer EC-*g*-PCL₁₀

EC was used as macroinitiator to initiate the polymerization of ϵ -CL via ring opening polymerization. The produced comb copolymer EC-*g*-PCL₁₀ has the properties of good biocompatibility and biodegradability with EC as the backbone and PCL as side chains. Different lengths of PCL side chains grafted onto the EC backbone could be controlled by the molar ratio of EC to ϵ -CL.

As depicted in Fig. 2(A), the corresponding resonance peaks of PCL were assigned as a, b, c and d (d'). Amongst them, d' was assigned to the CH₂ adjacent to terminal hydroxyls of PCL, while peak e was assigned to protons of methyl on the EC backbone. It is clear to see d' (δ = 3.64) shifted to higher field compared with peak d (δ = 4.05), which was resulted from the electron withdrawing effect. Furthermore, the average polymerization degree of ϵ -CL grafted on each glucose unit of the EC backbone was 9.5, which was calculated from the ratio of integral area of peak a (b, c or d) to peak e. In fact, this is almost identical with that calculated from the GPC data (10.5).

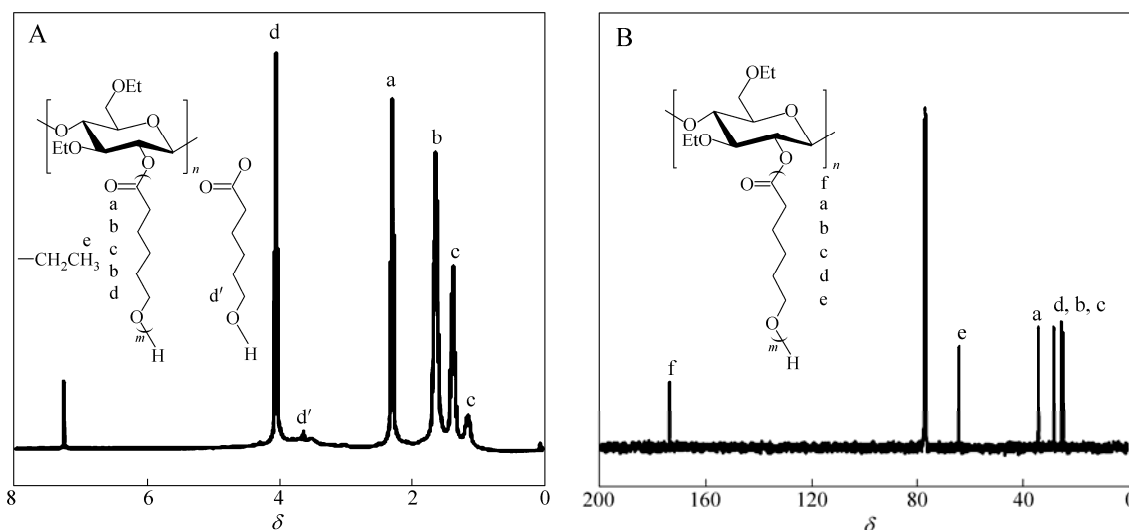


Fig. 2 (A) ^1H - and (B) ^{13}C -NMR spectra of EC- g -PCL $_{10}$

Preparation and characterization of Alkynyl-appended EC- g -PCL $_{10}$ (EC- g -PCL-alkynyl)

After the preparation of EC- g -PCL $_{10}$ copolymer, EC- g -PCL $_{10}$ -alkynyl was synthesized through DCC coupling with DMAP as catalyst. The peaks located at $\delta = 2.53$ and 1.98 in Fig. 3(A) indicated the successful connection of alkynyl to the end of PCL side chains. Moreover, according to the ratio of integral area of methyl proton peaks on EC at $\delta = 1.16$ to alkynyl proton peak at $\delta = 1.9$, nearly 70% of terminated hydroxyls have been modified by 4-pentynoic acid.

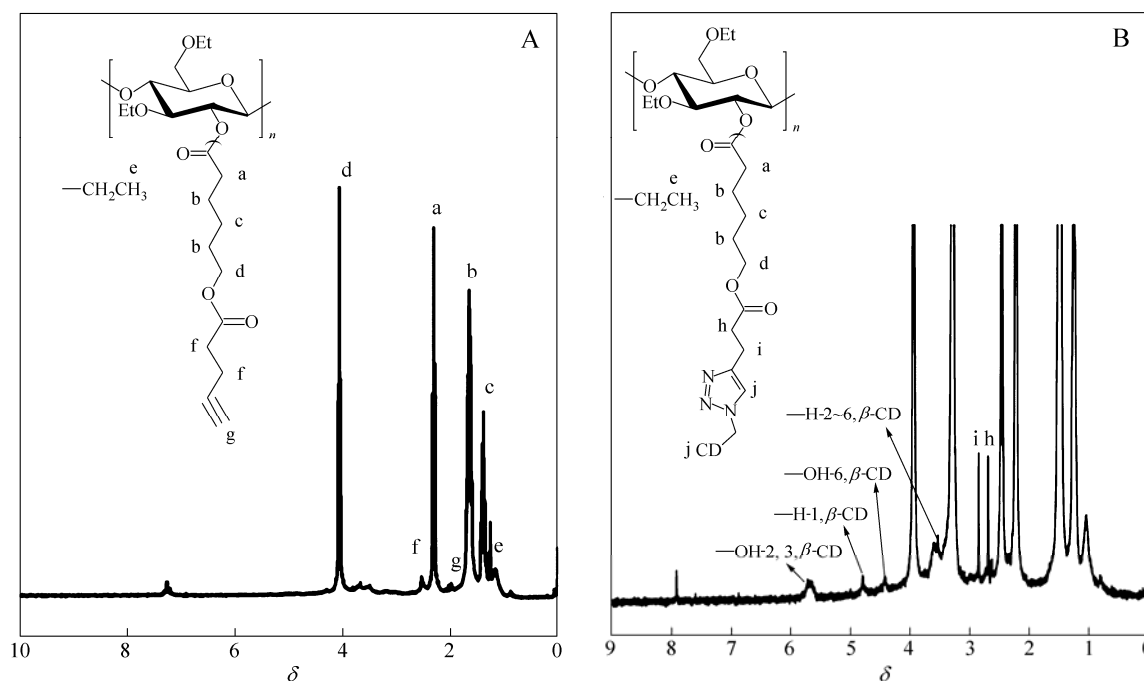


Fig. 3 ^1H -NMR spectra of (A) EC- g -PCL $_{10}$ -alkynyl and (B) EC- g -PCL $_{10}$ - β -CD

Preparation and characterization of β -CD-functionalized EC-g-PCL₁₀ (EC-g-PCL₁₀- β -CD)

Based on the prepared EC-g-PCL-alkynyl and mono- β -CD-6-N₃, EC-g-PCL₁₀- β -CD was synthesized via click reaction of alkynyl groups and diazo groups with Cu(I) as catalyst. According to the ¹H-NMR spectra (Fig. 3B), the typical proton resonance peaks of β -CD were clearly visible. Comparing the ¹H-NMR spectra of mono- β -CD-6-N₃ and EC-g-PCL₁₀- β -CD in Fig. 3, it is obvious that the characteristic peaks of alkynyl group disappeared, while the characteristic peaks of β -CD from $\delta = 6.00$ to $\delta = 3.00$ appeared.

Additionally, the peak located at $\delta = 7.92$ was attributed to the methylene protons of the triazole ring, which indicated the formation of the triazole ring. Meanwhile, the peak of methylene protons at $\delta = 2.5$ (peak f) in Fig. 3(A) has shifted to $\delta = 2.70$ and 2.86 (peak i and peak h) in Fig. 3(B). This was resulted from the negative inductive effect and was consistent with the formation of triazole ring. Furthermore, the GPC data in Table 1 presented the molecular weight and molecular weight distribution of EC-g-PCL₁₀- β -CD. Calculated from the GPC molecular weight of EC-g-PCL₁₀ and EC-g-PCL₁₀- β -CD, approximately 35% of PCL side chains have been attached with β -CD.

Table 1. Molecular weights of EC-g-PCL₁₀ and EC-g-PCL₁₀- β -CD

Samples	Degree of polymerization of side chain (theoretical value)	Degree of polymerization of side chain (actual value) ^a	$M_{n, GPC}^b$ (g·mol ⁻¹)	M_w/M_n^b
EC-g-PCL ₁₀	10	9.5	1.09×10^4	1.27
EC-g-PCL ₁₀ - β -CD	10	9.5	1.38×10^4	1.30

^a Degree of polymerization of side chain (actual value) was determined by ¹H-NMR spectra;

^b $M_{n, GPC}$ and M_w/M_n were determined by GPC analysis with polystyrene as standards and DMF as eluent.

Preparation and Characterization of 4-ethoxy-azobenzyl Carboxylic Acid (Azo-COOH)

The synthetic route of Azo-COOH was divided into two steps. One is the coupling reaction between diazotizing *p*-aminobenzoic acid and phenol, while the other is alkylation reaction to improve the stability of the product. Shown on the ¹H-NMR spectrum in Fig. 4(A), the peaks at $\delta = 8.13$, 7.94, 7.91 and 7.14 were assigned to methyne protons on the aromatic ring, while peaks at $\delta = 4.16$ and 1.38 were attributed to the methylene and methyl protons respectively.

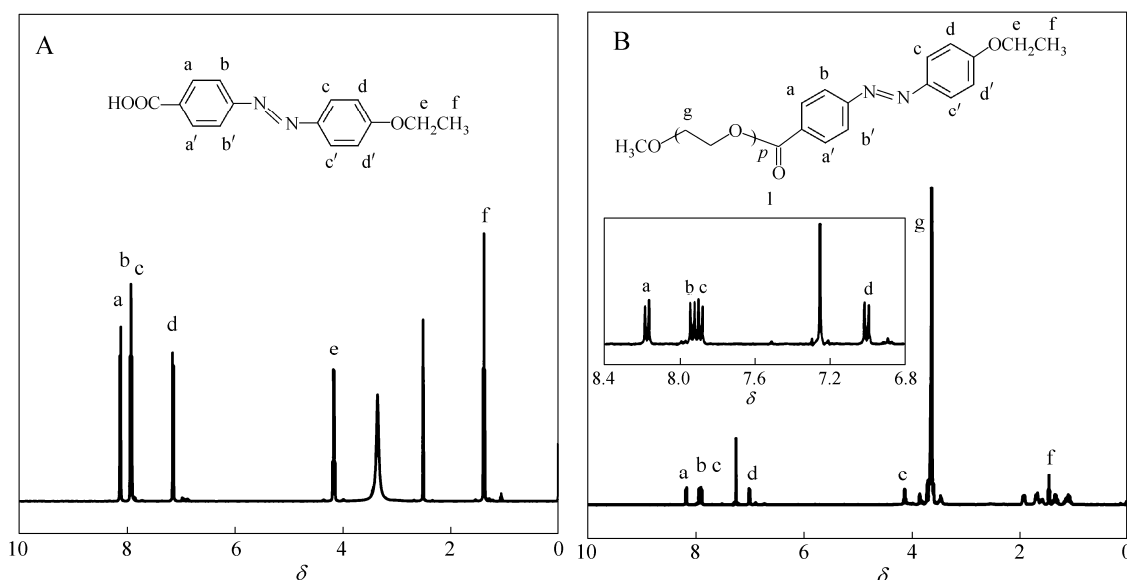


Fig. 4 ¹H-NMR spectra of (A) 4-ethoxy-azobenzyl carboxylic acid (Azo-COOH) and (B) PEG-tAzo

Preparation and Characterization of Azobenzyl Functionalized PEG (PEG-tAzo)

After synthesizing Azo-COOH, PEG-tAzo was obtained via dehydration reaction of PEG-OH and Azo-COOH. It is apparent to see the characteristic peaks (a, b, c, d, e and f) of azobenzyl group in Fig. 4(B) at $\delta = 8.13, 7.94, 7.91, 7.14, 4.16$ and 1.38 . By comparing the integrated area of peak a (or b, c and d, methyne on terminated aromatic ring) and peak g from the PEG backbone ($\delta = 3.63$), the azobenzyl functionality was determined to be 0.9, which was defined as the number of azobenzyl groups per PEG chain.

Self-assembly Behavior of EC-g-PCL₁₀- β -CD/ PEG-tAzo System

After the synthesis of the hydrophobic segment (EC-g-PCL₁₀- β -CD) and the hydrophilic chain (PEG-tAzo), the system EC-g-PCL₁₀- β -CD/PEG-tAzo self-assembled in the selective solvent DMF/H₂O (2 mg·mL⁻¹, 1:5, V/V) via the light controlled host-guest interaction of β -CD and tAzo groups. The formed micelles, composed of EC-g-PCL₁₀- β -CD as core and PEG-tAzo as shell, were confirmed by TEM micrographs shown in Figs. 5(a) and 5(a') with an average diameter of approximately 250 nm. Furthermore, the average hydrodynamic diameter (R_h) of these micelles in aqueous media was measured by DLS. It was observed from Fig. 7(a) that the average R_h of these micelles was approximately 295 nm, which is slightly larger than the result measured by TEM. Whether judging from the TEM or DLS results, it is apparent that the size distribution of micelles is relatively wide which is consistent with the wide molecular weight distribution of EC-g-PCL₁₀- β -CD. Thus, it was inferred that the random linkage of β -CDs contributes to the wide size distribution of micelles. Particularly, there are some relatively small micelles with an average radius of about 40 nm (Fig. 5), owing to the partial self-assembly of EC-g-PCL₁₀- β -CD by itself due to the hydrophilic character of its β -CD exterior^[19]. To further understand the small micelles, pure EC-g-PCL₁₀- β -CD was used to self-assemble in water, the DLS spectrum in Fig. 6(a) showed an average R_h of about 65 nm and the TEM image in Fig. 6(a) inset also indicated the average diameter of EC-g-PCL₁₀- β -CD micelles was about 40 nm. The results were consistent with the speculation made about the small micelles in Fig. 5.

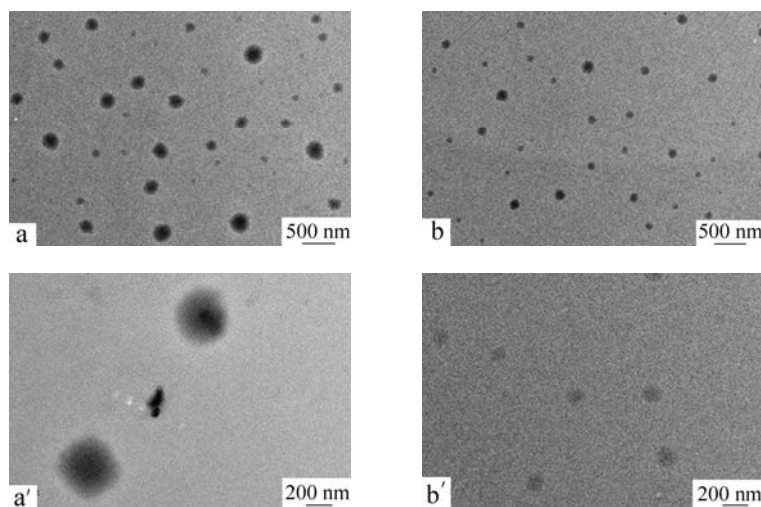


Fig. 5 TEM images of (a), (a') EC-g-PCL₁₀- β -CD/PEG-tAzo micelles and (b), (b') after UV irradiation at 365 nm

In addition, critical micelle concentration (CMC) of the EC-g-PCL₁₀- β -CD/PEG-tAzo self-assembled system was measured via fluorescence method with pyrene as the probe. It is well-known that the ratio of I_1/I_3 (I_1 : ~373 nm, I_3 : ~383 nm) of pyrene in fluorescence spectrum can reflect the varying polarity of microdomain. More specifically, the smaller the ratio, the more the nonpolar microdomain it becomes^[38]. Above the CMC, EC-g-PCL₁₀- β -CD/PEG-tAzo self-assembled into micelles to form hydrophobic microdomain to solubilize the hydrophobic pyrene, which led to a sharp decrease in I_1/I_3 value. Observed from Fig. 6(b), the CMC of EC-g-PCL₁₀- β -CD/PEG-Azo in aqueous solution is about 0.01 mg·mL⁻¹.

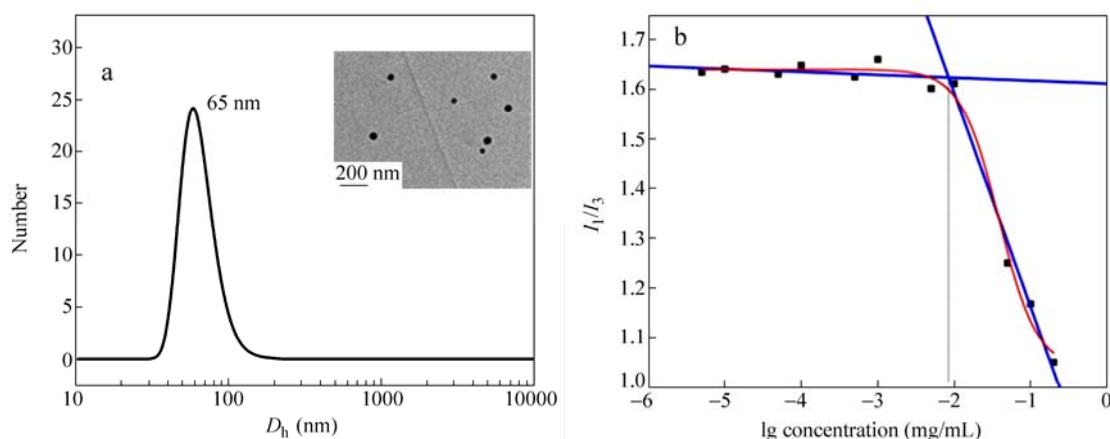


Fig. 6 (a) DLS spectrum of EC-*g*-PCL₁₀- β -CD micelles, inset: TEM image of EC-*g*-PCL₁₀- β -CD micelles and (b) Fluorescence spectrum of EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo self-assembled system in water

Stimuli-responsiveness of the System EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo

The EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo system was constructed via the β -CD/*t*Azo host-guest interaction. β -CD/*Azo* inclusion complex is well-known for its reversible association/disassociation under UV-Vis irradiation^[35]. Thus, the micelles are expected to show photoresponse upon external light exposure.

From the TEM images of EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo micelles in Fig. 5, an obvious decrease in diameter of the micelles was noticed after exposing to UV-light at 365 nm for 10 min. After the UV-light exposure, the *Azo* group underwent isomerization from *trans*- to *cis*-, leading to the disassociation of β -CD/*Azo* inclusion complex, which resulted in the disassembly of EC-*g*-PCL₁₀- β -CD/PEG-*t*Azo micelles. In addition, DLS was used to measure the size variation of the micelles before and after the radiation of UV-light. In agreement with the variation trend shown in TEM images, the average hydrodynamic diameter of the micelles was reduced from 295 nm to 224 nm after exposing to UV-light (Fig. 7a). Comparing the average R_h of the EC-*g*-PCL- β -CD/PEG-*Azo* micelles (224 nm) after the UV-light and EC-*g*-PCL- β -CD micelles (65 nm), the substantial difference between 224 nm and 65 nm could be attributed to two aspects. First is the partial existence of EC-*g*-PCL- β -CD/PEG-*Azo* micelles resulted from the incomplete transformation of *tAzo* to *cAzo*. Second, it is known that $\langle I \rangle \sim d^6$ in the DLS measurement^[39] ($\langle I \rangle$ is the average scattering light intensity, and d is diameter), thus the larger micelles have a stronger effect in the polydispersion system and the average R_h measured was closer to the larger micelles. Also, we monitored the size variation of the micelles with the extended time of exposing to UV-light, it was clear that the micelles underwent an evolution of slowly reducing size over time under UV-light (Fig. 7b).

To further elucidate the nature of the photoresponsive property of the micelles, ¹H-NMR spectra were performed to study the light-sensitive process. Figure 8 indicated the inclusion effect of β -CD/*Azo* groups and the conformational change of the *Azo* group under different lighting conditions^[20, 40]. Before the clathration of β -CD and *tAzo* groups, the characteristic peaks of aromatic protons on *tAzo* groups could be identified at $\delta = 7.92$, 7.60 and 6.81 (Fig. 8A). Then, these peaks shifted to lower field ($\delta = 8.13$, 7.81 and 7.16 in Fig. 8B) after the formation of β -CD/*tAzo* inclusion complex, which was resulted from the simultaneous cooperation of several weak interactions including hydrophobic interaction and steric effect. Furthermore, after the micelles solution was exposed to UV-light at 365 nm for 10 min, it can be seen that the peaks belonging to *tAzo* almost completely disappeared and *cAzo* became dominant ($\delta = 7.90$, 6.98 and 6.75 in Fig. 8C), which indicated PEG-*tAzo* has been transformed into PEG-*cAzo* and the micelles have disassembled. Subsequently, the micelles solution was irradiated by visible light at 450 nm for another 10 min, the *cis*-*Azo* groups then converted back to *trans*-conformation proved by the recovery of the corresponding chemical shifts (Fig. 8D), which indicated the recovery of EC-*g*-PCL₁₀- β -CD/PEG-*tAzo* micelles.

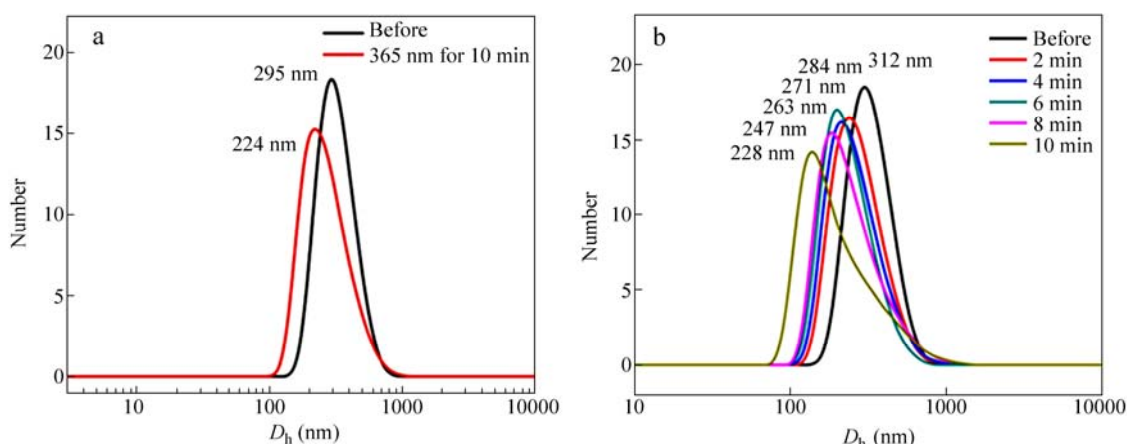


Fig. 7 DLS curves of EC-g-PCL₁₀-β-CD/PEG-tAzo micelles: (a) before and after UV irradiation at 365 nm and (b) upon UV irradiation at 365 nm over time

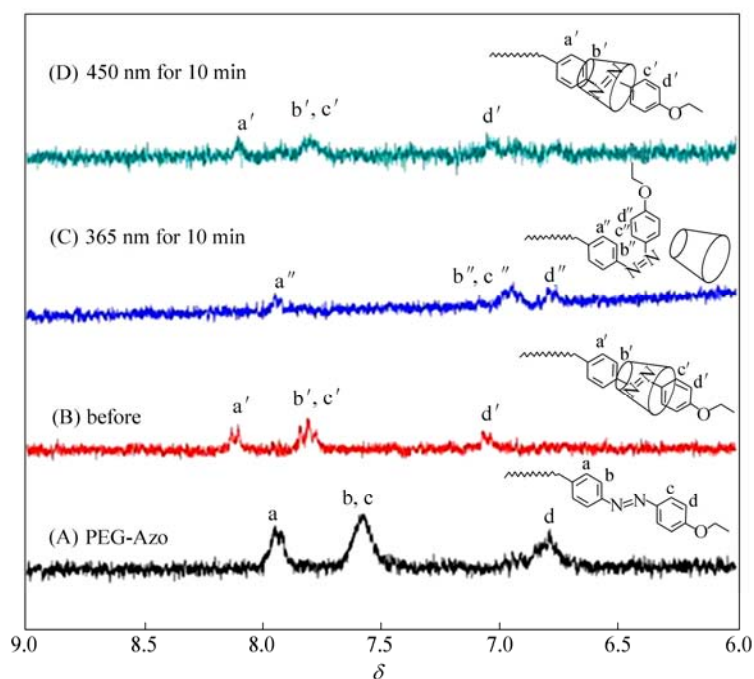


Fig. 8 ¹H-NMR spectra of (A) PEG-tAzo, (B) EC-g-PCL₁₀-β-CD/PEG-tAzo self-assembled system, (C) upon irradiation at 365 nm for 10 min and (D) upon irradiation at 450 nm for 30 min (The solvent is D₂O.)

Meanwhile, the conformation transition in real-time of *t/c*-Azo groups was further investigated via UV-Visible spectroscopy shown in Fig. 9. It is known that the maximum absorption wavelength of *t*Azo is 360 nm, while the maximum absorption peaks at 320 nm and 450 nm belong to *c*Azo^[20]. Additionally, the peaks of the UV-Visible absorption spectra correspond to the characteristic electron transitions. For the Azo group, the absorption peaks appeared at 360 nm and 450 nm can be ascribed to π - π^* and n - π^* transitions respectively. As shown in Fig. 9, it is evident that the strength of the maximum absorption bands (360 nm) of *t*Azo decreased substantially as the time of UV-radiation went on, but the strength of *c*Azo absorption bands (320 nm and 450 nm) increased at the same time. This demonstrated the conformation transition process of Azo from *trans*- to *cis*-, as well as the concomitant disassembly of EC-g-PCL₁₀-β-CD/PEG-Azo micelles.

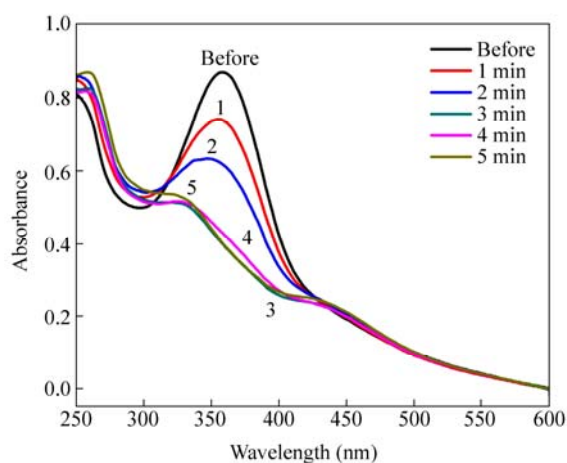


Fig. 9 UV-Vis spectra of EC-g-PCL- β -CD/PEG-Azo system before and after UV irradiation at 365 nm

CONCLUSIONS

In summary, a UV-Visible light controlled EC-g-PCL₁₀- β -CD/PEG-*t*Azo self-assembled system was constructed via the host-guest interactions of β -cyclodextrin (β -CD) group and *trans*-azobenzene (*t*Azo) group. More specifically, the hydrophobic segment, EC-g-PCL₁₀- β -CD, was synthesized by ring-opening polymerization and click reaction sequentially, while the hydrophilic chain, PEG-*t*Azo, was obtained by DCC coupling. Subsequently, the EC-g-PCL₁₀- β -CD/PEG-*t*Azo system self-assembled into micelles in aqueous solution with the comb copolymer, EC-g-PCL₁₀- β -CD, as core and PEG-*t*Azo as shell. From TEM and DLS analyses, the average radius of the micelles was determined to be approximately 250 nm and 295 nm respectively. Moreover, ¹H-NMR study has demonstrated the photoresponsive properties of the micelles through the shifting of the characteristic peaks of aromatic protons on the Azo groups. Additionally, UV-Visible spectroscopy was utilized to monitor the conformation transition of *trans/cis*-Azo groups upon exposure to UV-radiation in real-time. Overall, it is expected that these micelles based on EC may be used as a light-controlled drug release carrier due to their favorable light responsiveness as well as biocompatibility.

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