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Supramolecular Hydrogels Induced Rapidly by Inclusion Complexation of Poly(ϵ-caprolactone)−Poly(Ethylene Glycol)−Poly(ϵ-caprolactone) Block Copolymers with α-Cyclodextrin in Aqueous Solutions

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On the basis of the synthesis of water-soluble poly(ϵ-caprolactone)−poly(ethylene glycol)−poly(ϵ-caprolactone) (PCL-PEG-PCL) block copolymers, the supramolecular hydrogels were fabricated rapidly in aqueous solutions by their inclusion complexation with α-cyclodextrin. X-ray diffraction (XRD) analyses confirmed the supramolecular self-assemblies of α-cyclodextrin threaded onto amphiphilic PCL−PEG−PCL block copolymers. The resulting hydrogels display a high degree of elasticity, with the storage modulus (G') greater than the loss modulus (G'') over the entire range of frequency. Moreover, their viscosity greatly diminished as they were sheared. By controlling the molecular weight of the PEG component in the block copolymers and the content of the block copolymer, their rheological properties could be modulated. Such hydrogel materials have the potential to be used as tissue engineered scaffolds, biosensors in the human body, and carriers for controlled drug delivery.

Introduction

Cyclodextrins (CDs) are a series of cyclic oligosaccharides consisting of six to eight glucose units linked by α-1,4-linkages and named α-, β-, and γ-CD, respectively. They are water-soluble but have hydrophobic internal cavities, with the diameters in the range of 0.42−0.53 nm for α-CD, 0.56−0.65 nm for β-CD, and 0.68−0.83 nm for γ-CD.[1,2] Such cavities are available for including various small compounds and polymers.[3−11] For this reason, various CDs have been widely used in pharmaceutical application for increasing solubility and stability to low-molecular-weight drugs via supramolecular associations.[12−15] The ability of cyclodextrins to form inclusion complexes can be also utilized to modify the surface of the cyclodextrin-based, DNA-containing particles without interfering with polycation/DNA interactions and particle morphology[16−18] and to reduce the toxicity of some amphiphilic drug molecules.[19]

Recently, the supramolecular hydrogels resulting from the inclusion complexation of CD molecules with other polymer molecules have attracted much attention due to their potential applications in the field of biomedical engineering, as delivery matrixes for drugs or cells. Li et al.[20,21] prepared such hydrogel materials by using linear polymers, such as poly(ethylene glycol) (PEG) and /or poly(propylene glycol) (PPG), to penetrate the inner cavity of α- or β-CD; Huh et al.[22,23] used the graft copolymers consisting of dextran as a hydrophilic backbone and PEG or PPG as a linear side chain for the fabrication of supramolecular hydrogels, in which the PEG or PPG side chains were used for the inclusion complexation with CDs and the dextran was used to strengthen the hydrogel structure. However, in these cases, only high-molecular-weight PEG or PPG could lead to stable hydrogels with CDs, and a high concentration was also needed in aqueous solutions. Moreover, relatively long gel-induction times were required for the hydrogel formation.

In this work, water-soluble poly(ϵ-caprolactone) (PCL)−poly(ethylene glycol) (PEG)−poly(ϵ-caprolactone) (PCL) triblock copolymers with good biodegradability were synthesized and used as a building block for constructing supramolecular hydrogels together with α-CD. This strategy resulted in rapidly forming stable supramolecular hydrogels in aqueous solutions. Although the crystalline inclusion complexes between α-CD and polyester or its block copolymer with PEG could be fabricated in the presence of organic solvents,[7,24] the formation of the supramolecular hydrogels by their inclusion complexation in aqueous solutions has not been explored. This is the first report that biodegradable PCL−PEG−PCL block copolymers form supramolecular hydrogels with α-CD in aqueous solutions.

Experimental Section

Materials. ϵ-Caprolactone (ACROS, USA) was purified by vacuum distillation over CaH_2. PEG (Japan, M_n = 6000, 10 000, 20 000) was used after drying under vacuum at 95 °C for 48 h. Stannous 2-ethyl hexanoate (Sigma, USA) was used as received. α-CD (Sigma, USA) was used after drying in a vacuum at 65 °C for 24 h. All other chemicals used were analytical grade and used without further purification.

Preparation of PCL−PEG−PCL Block Copolymers. Weighted amounts of distilled PEG and ϵ-caprolactone monomer were added in a 100 mL round-bottomed flask. An amount of 0.2 wt % stannous 2-ethyl hexanoate was added to the flask, which was degassed by connecting a vacuum pump for 20 min

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TABLE 1: Molecular Characteristics of the PCL−PEG−PCL Triblock Copolymers and Preparation of Supramolecular Hydrogels in This Study

	PCL−PEG−PCL block copolymer					gel composition		
hydrogels code	composition[a]	M_{nPEG}[b]	DP_{PEG}[c]	DP_{PCL}[d]	M_w/M_n[e]	copolymer (wt %)	α-CD (wt %)	gelation time (s)
A	CL_7-EO_{136}−CL_7	6000	136	14	1.10	5	12	55
A′	CL_7-EO_{136}−CL_7	6000	136	14	1.10	10	12	35
B	CL_9-EO_{227}−CL_9	10,000	227	17	1.12	5	12	40
B′	CL_9-EO_{227}−CL_9	10,000	227	17	1.12	10	12	24
C	CL_{11}-EO_{454}−CL_{11}	20,000	454	20	1.14	5	12	15

[a] Theoretical composition based on feed composition. [b] Theoretical molecular weight of PEG in copolymers. [c] $DP_{PEG} = M_{nPEG}/44$. [d] Number of CL in copolymers calculated from ^{1}H NMR integral ratios. [e] Molecular weight distribution measured by GPC.

and purged with argon gas. The purging process was repeated three times. The mixture was reacted at 125 °C with magnetic stirring in argon atmosphere. After 20 h, the resulting product was cooled to room temperature, the obtained copolymer was dissolved in trichloromethane and precipitated in anhydrous ethyl ether, then filtered and dried at 40 °C under vacuum for 48 h. The block copolymer was obtained with a 94% yield.

Formation of Supramolecular Hydrogels. A required amount of aqueous α-CD solution was added to a required amount of aqueous solution of PCL−PEG−PCL triblock copolymer at room temperature, and the molar ratio ([CL]/[CD]) of the PCL repeating unit to α-CD was kept at 1. The resulting mixture was stirred vigorously. A gelation occurred rapidly to result in a physical network due to the supramolecular self-assembly between α-CD and the triblock copolymer.

Characterization. For the PCL−PEG−PCL block copolymer, an ^{1}H NMR spectrum was recorded at room temperature on a BRUKER DRX-400 spectrometer at 400 MHz with tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as the solvent. Its polydispersity (M_w/M_n), where M_n is the number-average molecular weight and M_w is weight-average molecular weight, was determined by gel permeation chromatography (GPC) (Water Associates model PL-GPC210) at room temperature in THF using polystyrene as a standard.

Wide-angle X-ray diffraction (WAXD) measurements were performed on hydrogel samples using a Rigaku D/max-2200 type X-ray diffractometer. The radiation source used was Ni-filtered, Cu Kα radiation with a wavelength of 0.154 nm. The voltage was set to 40 kV, and the current was set to 40 mA.The proportional counter detector collected data at a rate of $2\theta = 1°\ min^{-1}$ over the range $2\theta = 5-35°$.

Rheological behaviors of supramolecular hydrogels were investigated by a strain-controlled ARES rheometer (Advanced Rheometric Expansion System-TA Instruments, New Castle, DE) using 25-mm parallel plate geometry. Storage modulus (G') and loss modulus (G'') were measured as a function of the frequency under oscillatory shear at a strain of 2%, which is within the linear viscoelastic region, as determined by dynamic strain sweep experiments. The viscosity was measured as a function of shear rate in steady mode. All tests are performed at 25.0 °C.

Results and Discussion

Synthesis and Characterization of PCL−PEG−PCL Block Copolymers. The PCL−PEG−PCL block copolymers were synthesized by the ring-opening polymerization of the monomer ϵ-caprolactone (CL) using various molecular weights of PEG as the initiator and stannous 2-ethyl hexanoate ($Sn(Oct)_2$) as the catalyst. The mechanism of the reaction is that of coordination polymerization.[25] Of the many catalysts available, $Sn(Oct)_2$ appears to have the advantage of producing a polymer with better yield.[26] It is important to note that the amount of $Sn(Oct)_2$ used has a significant effect on the polymer produced. At low concentration, the polymer obtained has a narrower polydispersity index. However, an insufficient amount of $Sn(Oct)_2$ would result in failure in the polymerization. In this study, an optimum amount of $Sn(Oct)_2$ was found to be 0.2 wt % of the monomer.

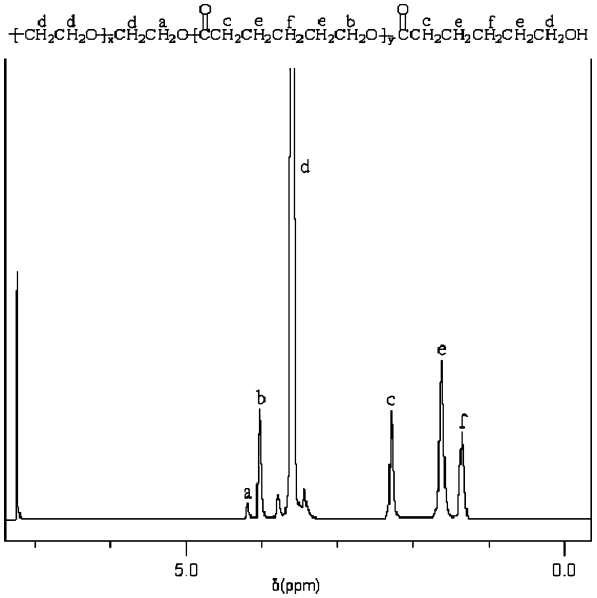

Figure 1. ^{1}H NMR spectrum of the $CL_7-EO_{136}-CL_7$ block copolymer in $CDCl_3$.

By modulating the feed ratio of CL to PEG during the polymerization, three samples of water-soluble PCL−PEG−PCL block copolymers, namely, $CL_7-EO_{136}-CL_7$, $CL_9-EO_{227}-CL_9$, and $CL_{11}-EO_{454}-CL_{11}$, were obtained, as shown in Table 1. Their composition, structure, and molecular weight were characterized by NMR and GPC techniques. Figure 1 shows a ^{1}H NMR spectrum of a typical PCL−PEG−PCL block copolymer in $CDCl_3$. The small peak at about 4.30 ppm belongs to methylene protons of the PCL−CO−OCH_2−CH_2−O−PEG segment, indicating the successful synthesis of the PCL−PEG−PCL block copolymer.[27] The absence of a peak at 4.9−5.0 ppm suggests that there was negligible or no PCL homopolymer in the PCL−PEG−PCL block copolymer. The composition of the block copolymers was determined from the peak intensity ratio of the $-CH_2CH_2-$ group in the PEG block (~3.63 ppm) and the $-CH_2-$ group in the PCL block (~4.10 ppm). Table 1 gives the structural characteristics of these samples. From Table 1, the copolymer composition determined by ^{1}H NMR analysis nearly coincided with the feed composition, indicating that the reaction is a controlled polymerization one. From GPC measurements, the M_w/M_n ratios were found to be in the range of 1.10 to 1.14 (shown in Table 1), indicating that the copolymers

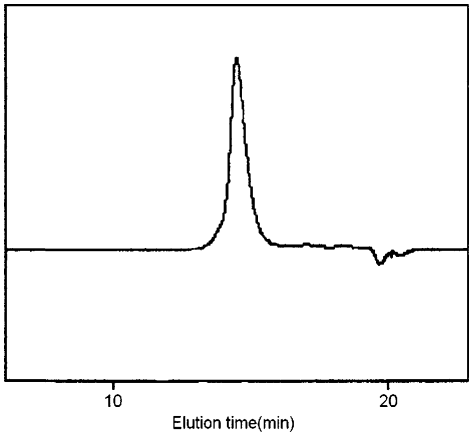

Figure 2. GPC curve for the $CL_7-EO_{136}-CL_7$ block copolymer.

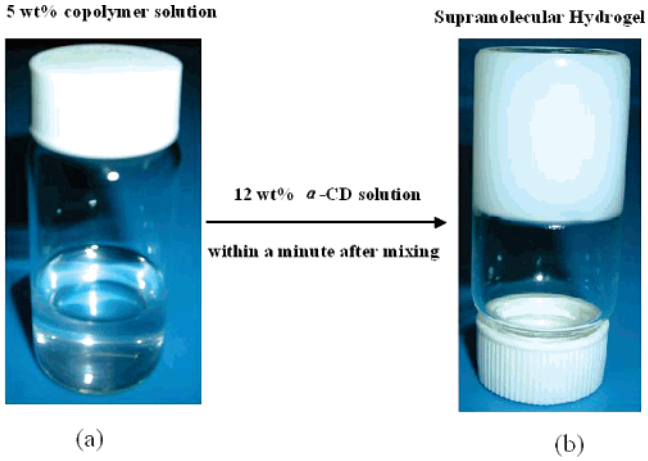

Figure 3. Photographs of the transparent solution of 5 wt % $CL_9-EO_{227}-CL_9$ block copolymer (a), and the inverted glass vial of the supramolecular hydrogel formed from the solution containing 5 wt % $CL_9-EO_{227}-CL_9$ and 12 wt % α-CD (b).

synthesized had a narrow polydispersity index. Figure 2 gives a GPC curve of a typical PCL−PEG−PCL block copolymer.

Gelation during Supramolecular Self-assembly of PCL−PEG−PCL Block Copolymers with α-CD. When aqueous α-CD solution with the concentration of 12 wt % was mixed with aqueous PCL−PEG−PCL block copolymer solution with the concentration of 5 or 10 wt % at room temperature, it is interesting to note that a rapid physical gelation occurred instantaneously for all systems shown in Table 1. It was reported [20] that aqueous solutions of some PEG−polyester block copolymers could form gels at high concentrations. We tested the gel formation of above-mentioned three block copolymers at concentrations of 5, 10 and 35 wt %, respectively, and found that only 35 wt % aqueous solution could be gelled at room temperature. It seems that α-CD can act as a powerful gelator and aid the gel formation at room temperature for aqueous solution of PCL−PEG−PCL block copolymer even at a low polymer concentration. Figure 3 shows the solution states of 5 wt % aqueous $CL_9-EO_{227}-CL_9$ solution before and after mixing with 12 wt % aqueous α-CD solution. As seen, the solution changed from a transparent sol (Figure 3a) to a white gel (Figure 3b) immediately after the mixing. Moreover, the vial containing the hydrogel could be inverted but exhibited no flow under the influence of gravity (Figure 3b), showing the formation of a stable physical hydrogel. Obviously, this rapid gelation can provide a greater advantage for the application of the supramolecular hydrogels.

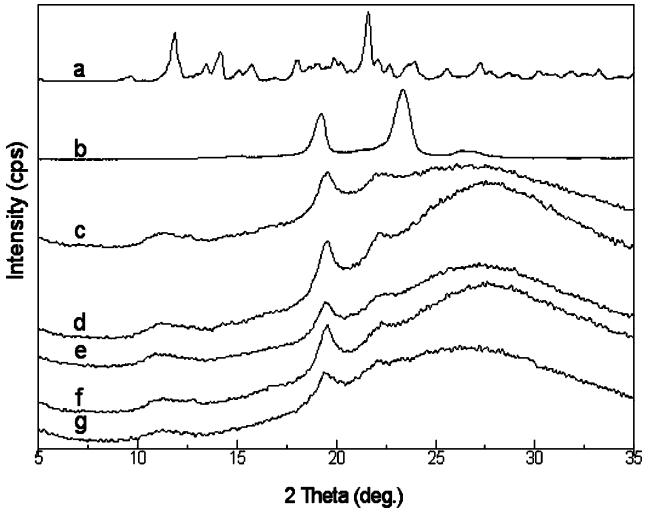

Figure 4. Wide-angle X-ray diffraction patterns of α-CD (a), $CL_9-EO_{227}-CL_9$ copolymer (b), hydrogel A (c), hydrogel A′ (d), hydrogel B (e), hydrogel B′ (f), and hydrogel C (g).

It is known[3,28] that α-CD will form crystalline inclusion complexes with low-molecular-weight PEG or poly(ethylene oxide) (PEO) in aqueous solution. The inclusion complexes formed by α-CD and the PEG blocks of PCL−PEG−PCL block copolymer may be thought to aggregate into microcrystals, which act as physical cross-links and induce the formation of the supramolecular polymer network. To confirm the existence of the inclusion complex of α-CD and the PEG blocks of PCL−PEG−PCL block copolymer, X-ray diffraction studies were carried out. Figure 4 shows that the X-ray diffractograms for the α-CD as the host molecule, the $CL_9-EO_{227}-CL_9$ as the guest molecule, and the supramolecular hydrogels. As seen, the α-CD is characteristic of multiple diffraction peaks corresponding to the crystalline form (Figure 4a), and the $CL_9-EO_{227}-CL_9$ block copolymer is characteristic of two main strong peaks at 19.17 ° and 23.27 ° (Figure 4b). For the supramolecular hydrogels investigated, however, all patterns (parts c−g of Figure 4) exhibit a number of sharp reflections and the main one at 19.5°, which are different from that of either α-CD or $CL_9-EO_{227}-CL_9$ block copolymer. Very similar patterns were also obtained by Harada et al.[3,28] for the channel-type structure of a crystalline-necklace-like complex of α-CD and PEG or PEO.

In addition, the hydrophobic association of PCL−PEG−PCL block copolymer in aqueous solution may also be important during the gelation due to the amphiphilic property of the block copolymer.[29] We carried out the comparison experiment without the PCL blocks by investigating the gelation process of the aqueous system containing 5 wt % aqueous PEG (M_n = 10 000) solution and 12 wt % aqueous α-CD solution and found that about 600 min were required for its gelation, which is much longer than the gelation time (within a minute) in the case of the PCL−PEG−PCL block copolymer (Table 1). It seems that a combination of the inclusion complexation between α-CD and the PCL−PEG−PCL block copolymer and the hydrophobic interaction of the PCL−PEG−PCL block copolymer provides the driving force for rapid formation of the supramolecular hydrogel. Figure 5 shows the graphical description about this plausible gelation mechanism.

Rheological Properties of Supramolecular Hydrogels. Supramolecular hydrogel samples were characterized by dynamic mechanical rheology. Figure 6 presents the storage modulus (G') and loss modulus (G'') evolutions of the hydrogels formed from 5 wt % of the block copolymers and 12 wt % of

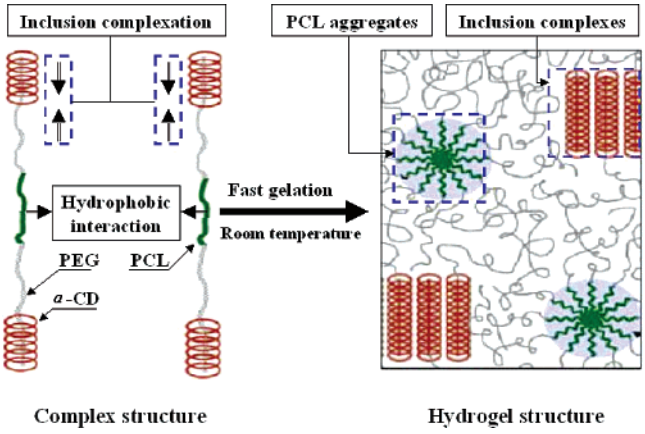

Figure 5. Graphical description about the gelation mechanism for the formation of the supramolecular hydrogel.

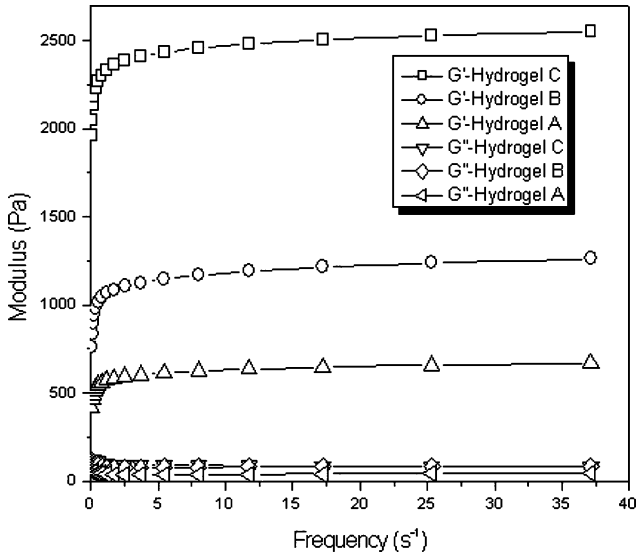

Figure 6. Storage modulus (G') and loss modulus (G'') evolutions as a function of frequency for the supramolecular hydrogels.

α-CD as a function of frequency. The G' value of all hydrogels exhibits a substantial elastic response and is greater than the loss modulus G'' over the entire range of frequency. With the increase of molecular weight of the PEG component (PEG6000, PEG10000, PEG20000) in the block copolymers, the G' value increases while the G'' value increases slightly. Figure 7 compares the storage moduli of the hydrogels containing 5 wt % of the copolymers with the hydrogels containing 10 wt % of the copolymers. It was found that the G' values of the hydrogels containing 10 wt % of the copolymers are greater than those of the hydrogels containing 5 wt % of the copolymers, which may be attributed to the increase of interaction sites with the increase of copolymer concentration.

Figure 8 shows the viscosity of the hydrogel systems as a function of shear rate. The viscosity of the hydrogels greatly diminished as they were sheared. Moreover, it was observed that a disrupted sol phase could be turned reversibly into a gel after shearing and then standing for a particular period of time. This property is important for injectable drug delivery matrix through fine needles. In addition, the viscosity of the hydrogels is sensitive to the molecular weight of the PEG component in the block copolymers and the content of the block copolymer. Under the fixed shear rate, the viscosity increases with the molecular weight of the PEG component and the content of the block copolymer.

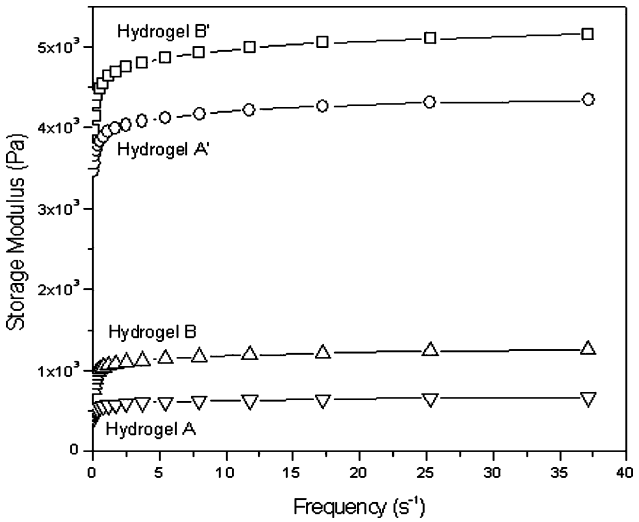

Figure 7. Comparison of the storage moduli for various supramolecular hydrogels.

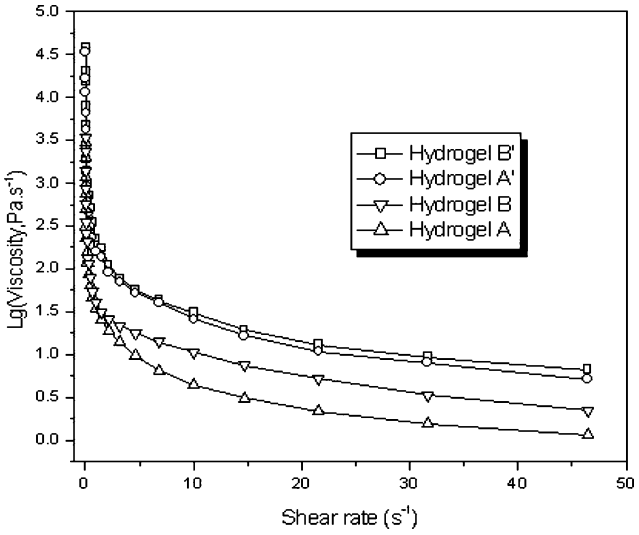

Figure 8. Relationship between apparent viscosity and shear rate for the supramolecular hydrogels.

Conclusions

Supramolecular hydrogels were fabricated in aqueous solutions by blending an aqueous α-CD solution with aqueous solution of PCL−PEG−PCL block copolymer at room temperature. For this purpose, water-soluble PCL−PEG−PCL block copolymers were synthesized and characterized. The gelation was found to occur within a minute after mixing. The fast hydrogelation might result from a combination of the inclusion complexation between α-CD and PCL−PEG−PCL block copolymer and the hydrophobic association of PCL−PEG−PCL block copolymer in aqueous solution. The storage modulus of these hydrogels is greater than the loss modulus over the entire range of frequency, and their apparent viscosity decreases with the increase of shear rate. In addition, their rheological properties could be tailored by changing the molecular weight of the PEG component in the block copolymers, the concentration of the block copolymer, and the actual molar ratio of the block copolymer and α-CD, which offers a means of controlling the mechanical properties for particular biomedical applications. Further study on the detailed mechanism of the hydrogel formation and the application as injectable hydrogels for drug delivery systems are in progress.

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