Preparation of Temperature-Sensitive Poly(*N*-isopropylacrylamide)/β-Cyclodextrin-Grafted Polyethylenimine Hydrogels for Drug Delivery

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ABSTRACT: In this study, novel thermally sensitive semi-interpenetrating polymeric network hydrogels composed of poly(*N*-isopropylacrylamide) (PNIPAAm) and β-cyclodextrin-grafted polyethylenimine were prepared by radical polymerization. Compared to normal PNIPAAm hydrogels, the semi-interpenetrating network hydrogels had a higher swelling ratio at room temperature and exhibited faster shrinking kinetics when the temperature was elevated to above the lower critical solution temperature. Propranolol as a model drug was loaded into the gels, and the release results show that compared to that of the normal PNIPAAm hydrogel, the release time of propranolol from the cyclodextrin-containing gel was prolonged. These improved drug-release properties may have been due to the formation of inclusion complexes between the drug molecules and cyclodextrin groups. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3031–3037, 2008

Key words: drug delivery systems; hydrogels; interpenetrating networks (IPN); stimuli-sensitive polymers

INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAAm) has attracted the attention of many researchers in the past 3 decades due to its particular phase transition properties.[1–6] PNIPAAm dissolves well in water at a temperature below the lower critical solution temperature (LCST; $\sim 32^{\circ}C$), whereas PNIPAAm chains precipitate from water, and the solution becomes cloudy with increasing temperature above the LCST. Chemically crosslinked PNIPAAm is a typical temperature-sensitive hydrogel, which exhibits reversible swelling–deswelling behavior in water when the environmental temperature is changed oscillatorily. This special property has widely been used in many biomedical and biotechnological fields, including drug-delivery systems,[7–11] chemical separation,[12] and gene carriers.[13]

Stimuli-sensitive hydrogels, which exhibit controllable property changes in response to environmental stimuli, such as temperature and pH, are on the forefront of smart drug delivery systems. Actually, temperature-sensitive gels are perhaps the most widely investigated class. In general, the drug molecules are entrapped in the swollen networks through physical interaction forces, and their release kinetics are controlled by changes in the environmental temperature. For a swollen hydrogel containing a hydrophilic drug, it exhibits a Fickian release when the temperature is below the LCST, which depends on the swelling ratio (SR) of the gel.[14] When the temperature is increased above the LCST, the gel matrix will shrink and form a dense layer on the surface. Therefore, the whole process can be characterized as a burst release at the first stage followed by a release termination.[15,16] Drug release behavior from the dried glassy hydrogels has also been studied; in these systems, the loaded drug molecules diffuse out during the reswelling process, and the release rate is determined by the reswelling ratio and the rate of water absorption.[17] Okano et al.[10] achieved a thermal on–off switch for the application of a self-regulating drug release system. Positive controlled release patterns, that is, rapid drug release at increased temperature and slow drug release at decreased temperature, are also designed to satisfy some special needs.[9,11] However, because of the hydrophilic nature of PNIPAAm chains at room temperature, the release rate of small molecules from PNIPAAm gels is so fast that it restricts the practical applications of PNIPAAm hydrogels.

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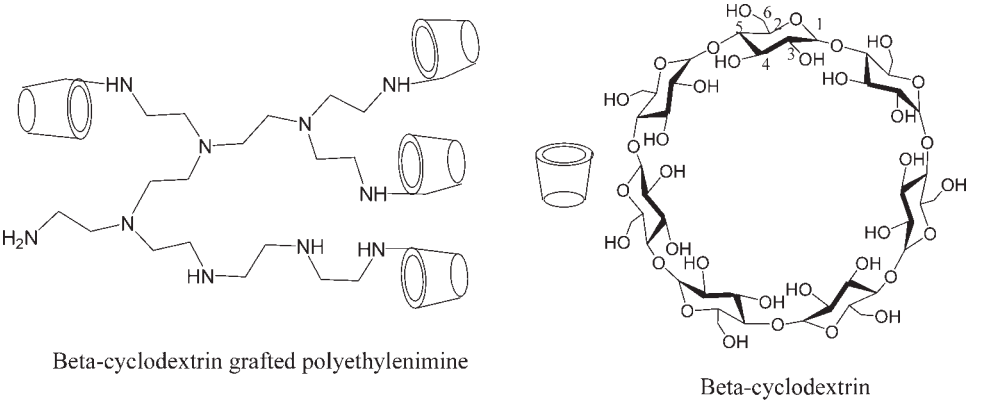

Scheme 1 Structure of PEI-*g*-CD.

Cyclodextrins (CDs), with a polar hydrophilic outer shell and a hydrophobic cavity, have been studied extensively as drug release carriers.[18,19] Because of their ability to form inclusion complexes with some drug molecules, CDs have been used to design advanced drug-delivery devices and improve pharmaceutical efficiency.[18–21] It has also been reported that PNIPAAm hydrogels with interpenetrating network (IPN) structures have some desirable properties, such as a fast response rate to temperature changes and improved mechanical strengths.[22,23] Polyethylenimine (PEI) is a highly branched polymer containing a large amount of primary amine groups, which can be easily modified by reaction with carboxyl groups or epoxy groups.[24,25] Because of the high density of amine groups, we wished to graft more CD onto PEI chains. Also, the branched structure decreases the steric hindrance among different CD groups. So PEI was chosen as the main chain to graft CD. In this study, temperature-sensitive PNIPAAm hydrogels containing β-cyclodextrin-grafted polyethylenimine (PEI-*g*-CD) were prepared by the formation of IPN structures. Propranolol, as a model drug, was loaded into the hydrogel to study the release properties. The results show that compared to that of a normal PNIPAAm hydrogel, the release time of the model drug from the PEI-*g*-CD incorporated gel was retarded, which was probably ascribed to the formation of inclusion complexes between the drug molecules and CD.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAAm; Aldrich Chemical Co., Inc., St. Louis, MO) was purified by recrystallization from a mixed solvent of benzene and *n*-hexane. The PEI solution (weight-average molecular weight = 50,000–100,000; 50 wt % in water) was purchased from ICN Co. (Osaka, Japan). β-Cyclodextrin (β-CD), *N,N′*-methylenebis(acrylamide) (MBAAm), ammonium persulfate (APS), and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were analytical grade and were used as supplied by Shanghai Chemical Co. (Shanghai, China).

Synthesis of PEI-*g*-CD

The structure of PEI-*g*-CD is shown in Scheme 1. First, 6-*O*-(*p*-tosyl)-β-cyclodextrin was prepared according to the references.[26,27] Then, PEI-*g*-CD was synthesized as follows: 4 g of PEI solution and 5 g 6-*O*-(*p*-tosyl)-β-cyclodextrin were dissolved in 100 mL of aqueous potassium hydroxide solution (pH = 11), and the mixture was stirred for 48 h at 60°C. After it was cooled to room temperature, the solution was dialyzed against water and was then freeze-dried. From the elemental analysis results (N: 13.24%, C: 45.03%, H: 9.46%), we calculated that the molar substitute ratio was 5.7% and the weight ratio of PEI to CD in the grafted polymer was 1.33. ^{1}H-NMR spectra were performed on a Mercury VX-300 spectrometer (Varian, Palo Alto, CA) with D_2O as a solvent.

^{1}H-NMR (D_2O, ppm, δ): 4.8 (m, H1), 4–2.95 (m, H2–6; H1–6 are labeled in Scheme 1), 2.95–2.2 (m, —NCH_2CH_2N—), 2.1 (m, —O*H*, —NH_2, —N*H*—).

The ^{1}H-NMR results indicate that the weight ratio of PEI to CD in the PEI-*g*-CD polymer was 1.225, which was close to the result obtained by elemental analysis.

Preparation of the PNIPAAm/PEI-*g*-CD semi-interpenetrating network (semi-IPN) hydrogels

PEI-*g*-CD, NIPAAm, and MBAAm were dissolved in distilled water at room temperature, and then, the initiator APS and the accelerator TEMED were added to the solution to initiate the polymerization/crosslinking. The reactions were carried out at room temperature for 24 h to obtain gel samples with a di-

TABLE I
Feed Composition for the Novel Semi-IPN Hydrogels

Component	Sample code				
	PCDH0	PCDH05	PCDH10	PCDH15	PCDH20
NIPAAm (mg)	100	100	100	100	100
PEI-*g*-CD (mg)	0	5	10	15	20
MBAAm (mg)	5	5	5	5	5
H_2O (mL)	0.95	0.95	0.95	0.95	0.95
33 wt % APS (μL)	20	20	20	20	20
TEMED (μL)	20	20	20	20	20
Yield (%)[a]	96.5	94.7	85.0	80.7	80.2

[a] The yields of the synthesized hydrogels were calculated by the division of the weight of dried gels by the weights of the monomer, crosslinker, and PEI-*g*-CD polymer.

ameter of 25 mm and a thickness of 3 mm. Then, the prepared gels were taken out and immersed in abundant distilled water for 3 days, during which the water was replaced repeatedly to wash out the monomers and other chemicals. The feed compositions of the hydrogels are listed in Table I.

Fourier transform infrared (FTIR) measurement

The FTIR analyses of hydrogel samples were measured on a Nicolet 170SX FTIR spectrophotometer (Madison, WI). The dried gel samples were scanned with wave numbers ranging from 4000 to 500 cm^{-1}.

SR

SR is defined as SR = W_s/W_d, where W_s is the weight of the water in the hydrogel networks and W_d is the weight of the dried hydrogels. At temperatures ranging from 18 to 39°C, the hydrogels were incubated at a particular temperature point for at least 24 h; then, after the excess water was wiped from the surface of the gels with moistened filter paper, W_d was determined gravimetrically to calculate SR. The temperature at which SR decreased sharply was regarded as the LCST of the hydrogels.

Shrinking kinetics

After the hydrogels swollen at room temperature were transferred into 37°C hot water, the weight of the hydrogels was recorded gravimetrically at designed time intervals to determine the water retention (WR). WR is defined as WR = W_t/W_s, where W_t is the weight of the water within the gels at different time points. The experiments were carried out two times for all of the samples, and the average values were used to draw the Figure 3.

Reswelling kinetics and drug release

PCDH20 was selected as a typical sample to study the reswelling properties and drug-release behavior. The gel swollen at room temperature was first dried at ambient atmosphere for several days and then further dried *in vacuo* completely. The dried gel was immersed in propranolol alcohol solution (2 mg/mL) for 3 days to reach equilibrium. The drug-loaded gel was placed at atmosphere for 3 days and then dried *in vacuo*. The loading efficiency was defined as W_{drug}/W_d, where W_{drug} is the weight of the drugs loaded in the gel network.

The dried drug-loaded hydrogels were put into 20 mL of a 0.1*M* phosphate buffer solution (PBS) prepared from Na_2HPO_4 and KH_2PO_4 (pH = 7.4) to carry out the release experiment. At fixed time intervals, the samples were taken out, and the concentrations of released propranolol in the solutions were measured and calculated by the absorption at 223 nm with a UV spectrometer (Lamda Bio40) (PerkinElmer Instruments); the drug solution was replaced by 20 mL of fresh PBS. At the same time, the weight of the gel was gravimetrically measured to obtain the reswelling kinetics and water uptake (WU) of the hydrogel, which is defined as follows: WU = W_t/W_s. The experiments were carried out two times for the PCDH20 and PNIPAAm gels, and the average values were used to plot the profiles.

RESULTS AND DISCUSSION

In this study, a pair of redox initiators composed of APS and TEMED were used to initiate the polymerization/crosslinking reactions. Here, the polymerization of NIPAAm and MBAAm occurred immediately after the addition of the initiators, and the gels formed within several minutes. PEI-*g*-CD was incorporated into the PNIPAAm networks to form semi-IPN structures. The hydrogels remained transparent during the polymerization reaction, which indicated that no phase separation appeared and that the gels were homogeneous. The yields of the synthesized hydrogels decreased with increasing CD polymer in the preparation (Table I). This was because elevating

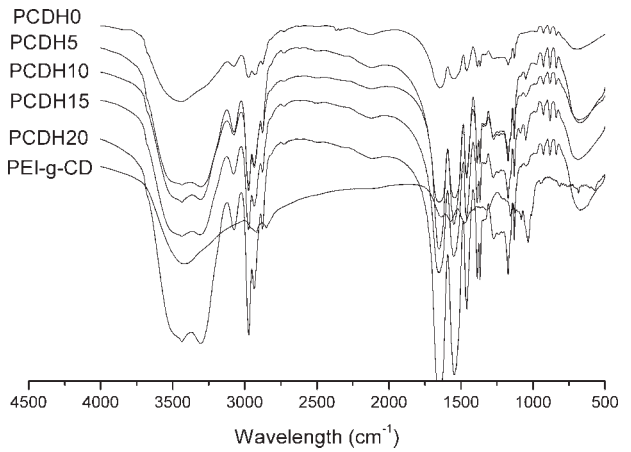

Figure 1 FTIR spectra of the semi-IPN hydrogels.

the content of PEI-*g*-CD resulted in an increase in the viscosity of the reaction solutions, in which the diffusion rate of the generated radicals decreased, which led to a lower conversion efficiency of the monomer and crosslinker. So, the yields decreased from PCDH0 to PCDH20. From the FTIR spectra of the dried gel samples (Fig. 1), we found the amide I band (1641 cm^{-1}) ascribed to the C—O stretch of PNIPAAm and the amide II band (1541 cm^{-1}) due to the N—H vibration in every spectrum. The broad peak at the range from 3200 to 3600 cm^{-1} belonged to the N—H or O—H vibration. The peaks at 1050 cm^{-1} in the spectra of CD-containing hydrogel samples were attributed to the C—O—C stretch of CD, whereas no peak at this position in the spectrum appeared in PCDH0; this suggests that β-CD was successfully introduced into the hydrogels by the formation of IPN structures.

SR is an important parameter for evaluating hydrogels and can illustrate the LCST behaviors of IPN hydrogels. The SR values of the PNIPAAm/PEI-*g*-CD semi-IPN hydrogels as a function of temperature are displayed in Figure 2, from which one can see that at room temperature, the SR values of the hydrogels increased with increasing content of PEI-*g*-CD and that SR of all of the gels decreased with further increasing temperature. SR decreased sharply at about 33°C, which was considered the LCST. When the gels swollen at room temperature were transferred into hot water, the gels began to shrink. The shrinking kinetics of the gel samples at 37°C are shown in Figure 3. From this figure, it is clear that the shrinking rate of the normal PNIPAAm hydrogel (PCDH0) was slower than those of the PEI-*g*-CD-containing hydrogels. Additionally, the shrinking kinetics became faster as the PEI-*g*-CD content in the gel matrix was elevated. PCDH20 lost at least 85% water within 10 min and arrived at an equilibrium state within 30 min. On the contrary, the normal PCDH0 hydrogel only lost about 20% water within 10 min and needed over 24 h to reach equilibrium.

It has been reported that there is a hydrophilic/hydrophobic balance existing in the PNIPAAm hydrogel network because of the existence of the hydrophilic —CONH— groups and the hydrophobic $—CH(CH_3)_2$ groups in the side chains.[28–30] At room temperature, water molecules interact with the side chains through the hydrogen bonds between the water molecules and the hydrophilic parts. These hydrogen bonding interactions allow water molecules to orientate neatly around isopropyl groups to form a cage structure, and the polymer chains swell well in water. As the temperature is increased, the balance structures become disrupted, and the hydrogen bonding interactions decrease. The hydrophobic groups become naked, and the interactions among the hydrophobic groups begin to play a dominant role so that the polymer chains aggregate together. As a result, the entrapped water is squeezed out,

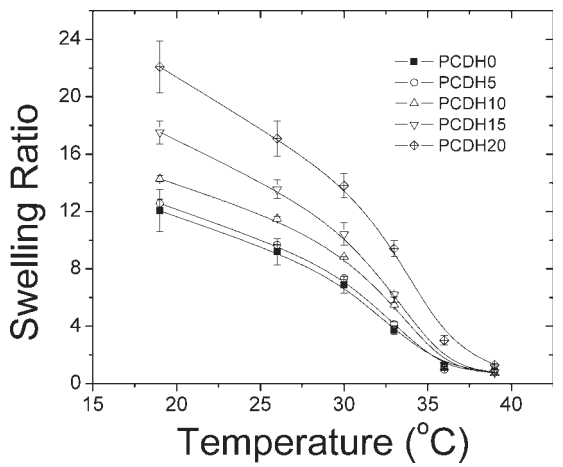

Figure 2 Temperature dependence of the equilibrium SR of the semi-IPN hydrogels at temperatures ranging from 18 to 39°C.

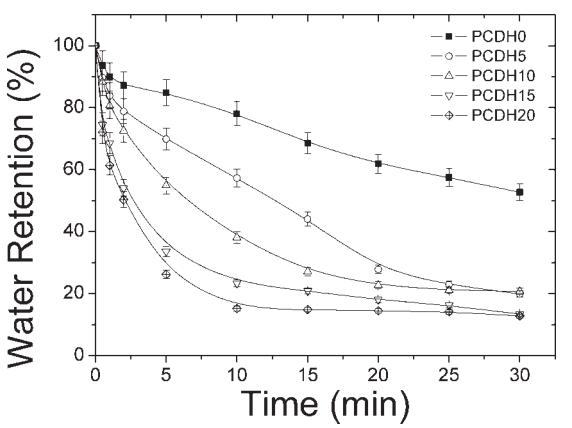

Figure 3 Shrinking kinetics of the hydrogels. The hydrogels were transferred from 12°C water to hot water (37°C) to carry out the measurement of the deswelling kinetics.

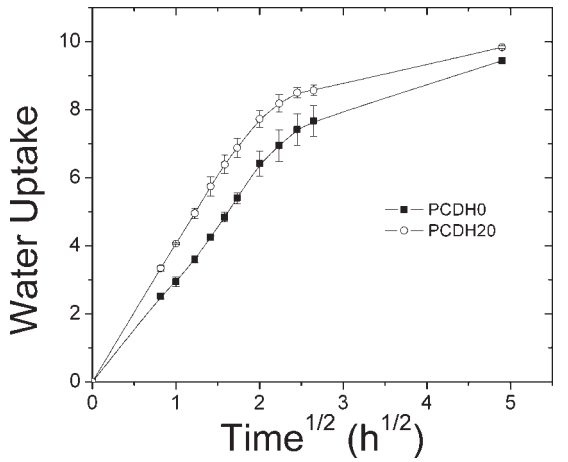

Figure 4 Reswelling kinetics of the hydrogels as a function of the square of time at 25°C.

and the hydrogel networks collapse. Because the PEI-*g*-CD was a hydrophilic polymer, the gel containing more PEI-*g*-CD may have been more hydrophilic and had a higher SR at room temperature. The PEI-*g*-CD chains were interpenetrated into the PNIPAAm network but were independent of the PNIPAAm chains, so the LCSTs of the IPN hydrogels were almost the same as that of the normal PNIPAAm gel. It is well known that a dense, thick layer forms on the surface of conventional PNIPAAm hydrogels during shrinking experiments, which prevents the water molecules from being squeezed out of the gel matrix. As a result, the increasing inner pressure blows up some parts of the dense skin layer, and a few bubbles appear on the surface of the PNIPAAm hydrogel.[31,32] In this study, many bubbles were observed on the surface of PCDH0 during the shrinking process, whereas no bubbles appeared on the surface of PCDH20, which might have been caused by the introduction of hydrophilic PEI-*g*-CD. The interpenetrated PEI-*g*-CD chains increased the hydrophilicity of the gel systems, which may have destroyed the dense layer by decreasing the hydrophobic interactions. Also, hydrophilic PEI-*g*-CD chains could have probably acted as water-release channels in the PNIPAAm networks and allowed water molecules to be diffused out easily during the process of shrinking. So the shrinking rates of the semi-IPN gels were faster than that of the normal PNIPAAm hydrogel. More PEI-*g*-CD chains in the network led to more water-release channels, and as a result, the shrinking kinetics of the gels increased with increasing PEI-*g*-CD.

Figure 4 illustrates the reswelling kinetics of the semi-IPN hydrogel, PCDH20, and the normal PNIPAAm hydrogel, PCDH0, in PBS at 25°C. We found that the reswelling rate of the PCDH20 was faster than that of PCDH0, and both of the gels reached equilibrium in 24 h. In the initial stage, WU was proportional to the square root of the time; then, the gels swelled at a slower rate. In the process of reswelling, three steps have been suggested to occur continuously[33]: water molecules diffuse into the polymer gel, the polymer chains become relaxed, and the hydrated network expands into the solution. If the first step determines the process, the amount of water absorption will be proportional to the square of the time, and if the second step is the dominant factor, the amount of WU will be proportional to the time. As shown in Figure 4, the reswelling of the hydrogels was controlled by the diffusion of the water molecules. The possible explanation is that both PNIPAAm and PEI-*g*-CD were hydrophilic polymers, and the diffusion rate of water molecules into the hydrogel networks was rapid, which determined the reswelling process. PCDH20 was more hydrophilic than PCDH0, so the reswelling rate of PCDH20 was faster.

Release profiles of propranolol in PBS at 25°C are shown in Figure 5. The drug-release rate from PCDH20 was slower than that of the normal PNIPAAm gel (PCDH0). When the dried drug-loaded gels were immersed in PBS, the drug located on the surfaces first diffused into the PBS, so there was a burst release at the initial stage for both gels. In the following process, the reswelling kinetics may have governed the release behavior, and drug molecules diffused into PBS by exchange with water molecules when the gel networks became swollen. Just as discussed previously, the reswelling rate; PCDH20 would be faster than that of PCDH0 if the drug release was completely controlled by reswelling; the release rate of propranolol from PCDH20 should have been faster than that of PCDH0. However, on the contrary, the release kinetics of PCDH20 was slower, which suggested that β-CD was able to form

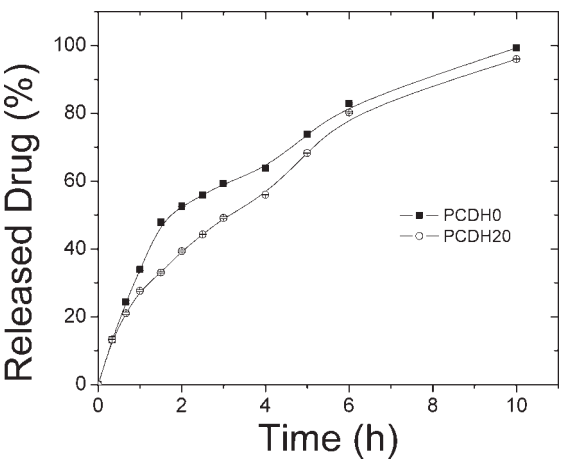

Figure 5 Cumulative release of propranolol from the normal PNIPAAm gel (PCDH0) and the β-CD-containing hydrogel (PCDH20) as a function of time in PBS at 25°C.

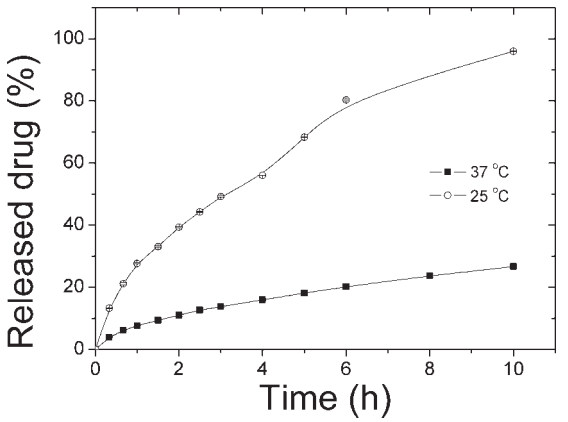

Figure 6 Cumulative release of propranolol from the β-CD-containing hydrogel (PCDH20) as a function of time in PBS at 37 and 25°C.

inclusion complexes with propranolol by host–guest interaction. It has also been reported that β-CDs are capable of forming inclusion complexes with propranolol molecules.[34–37] Therefore, the introduction of β-CD into the PNIPAAm network could have prolonged the drug-release time and improved the release properties of the normal PNIPAAm hydrogel through the formation of inclusion complexes of CD and drug molecules. The loading efficiencies of propranolol in PCDH0 and PCDH20 were 2.02 and 1.55%, respectively. Theoretically, the loading efficiency in PCDH20 should have been higher than that of PCDH0 due to the incorporation of the host CD molecules. However, the results were totally opposite, which may be explained by the hydrophilicity of the hydrogels. It has been discussed that the hydrophilicity of PCDH20 was higher than that of PCDH0 because of the hydrophilic nature of PEI-*g*-CD. The more hydrophilic network of PCDH20 was not a good environment for loading the hydrophobic drug propranolol. Therefore, the loading efficiency in PCDH20 was lower.

The release of propranolol from PCDH20 at 37°C in PBS as a function of time is displayed in Figure 6, from which one can see that only about 25% of propranolol was released within 10 h. This was because at 37°C (above LCST), the polymer chains aggregated together because of the strong hydrophobic interactions among the hydrophobic groups. Only the drug molecules on the surface could enter the PBS medium, and the inner propranolol could not diffuse out of the gel in the shrunken state. As a result, the release behaviors of propranolol from the PCDH20 hydrogel could be modulated by a change in the environmental temperature. The CD-incorporated hydrogels also have potential application in the controlled release of some other small-molecule hydrophobic drugs that can form inclusion complexes with CD.

CONCLUSIONS

A series of temperature-sensitive semi-IPN hydrogels composed of PNIPAAm and PEI-*g*-CD were prepared by the radical polymerization/crosslinking of NIPAAm and MBAAm in the presence of PEI-*g*-CD polymer. Swelling measurements displayed that these semi-IPN hydrogels exhibited the same LCST as the conventional PNIPAAm hydrogel. However, the SR semi-IPN gels were higher at room temperature, and the more PEI-*g*-CD polymer was incorporated, the higher the SR of the resultant hydrogels was. Compared to that of the PNIPAAm hydrogel, the shrinking rates of the semi-IPN hydrogels were faster with increasing temperature to above the LCST, which was due to the formation of water-releasing channels and the disruption of the dense skin layer by the incorporation of PEI-*g*-CD. The propranolol release rate from PCDH20 was retarded because of the formation of complexes between the drug and β-CD groups, and we could tune the release kinetics by controlling the environmental temperature.

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