Synthesis and Characterization of the Environmental-Sensitive Hyperbranched Polymers as Novel Carriers for Controlled Drug Release

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ABSTRACT: Novel hyperbranched polymers, which contain a hydrophobic branched poly(*p*-(chloromethy)styrene) (PCMS) core and poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMA) shell that exhibited environmental sensitivity, have been synthesized by atom transfer radical polymerization (ATRP). At first, a hyperbranched polymer (PCMS) core is obtained via ATRP of *p*-(chloromethy)styrene (CMS), which may act as an "inimer"-monomer and initiator. Then the modified hyperbranched polymers having different average arm length consisting of PCMS and PDMA are synthesized by ATRP using anterior PCMS as macroinitiators. Their macromolecular structures are characterized by FTIR and ^{1}H NMR. Using chlorambucil as a model drug, the behaviors of the controlled drug release from the environmental-sensitive hyperbranched polymers with different average chain length of PDMA and degree of branching are studied. The data demonstrate that the rate of the drug release can be effectively controlled by pH value, and these environmental-sensitive hyperbranched polymers have the potential to be used as novel carriers in some controlled drug release systems in the future. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 311–316, 2006

Key words: environmental sensitivity; hyperbranched polymer; poly(*N*,*N*-dimethylaminoethyl methacrylate); *p*-(chloromethy)styrene; controlled drug delivery system

INTRODUCTION

Hyperbranched polymers are highly branched, polydispersed, three-dimensional macromolecules, which, because of their unique structures and properties, have attracted increasing attention.[1–4] Compared to their linear analogs, hyperbranched polymers are expected to have different physical properties such as a huge number of modifiable surface functionality, lower viscosities, and better solubility.[5–7] Although hyperbranched polymers are of irregular shapes, not perfectly symmetrical as dendrimers, they can be prepared in a single, one pot reaction, which was the reason for a relatively high interest in the industry of hyperbranched polymers.[8–10] Since structural perfection is not a strict prerequisite for most biomedical applications, even the area of life science, which until recently seemed to be reserved for the perfectly structured dendrimers, appears to be a promising field for new applications of hyperbranched polymers.[11–19] Hyperbranched polymers can be used as nanomaterials for host–guest encapsulation.[20,21] Frey and coworkers have reported the synthesis of hyperbranched polyglycerols with a hydrophobic shell and hydrophilic core structure. Comparison of the hyperbranched architecture with the perfect linear analogue suggests that the hyperbranched topology plays a crucial role in the supramolecular encapsulation.[5] Moreover, as drug carriers, hyperbranched polymers can also offer their interior or peripheral functional groups to covalently fix drug molecule, or depending on their core–shell architecture, to sequester guest molecules.[1] However, hyperbranched polymers generally can not match the high demands for controlled and targeted delivery, because of their failure to respond to changes in environmental conditions.[13] Poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMA), which has the aliphatic tertiary amino group, is a typical combined pH- and temperature-sensitive polymer.[22–26] Controlled polymerization processes such as atom transfer radical polymerization (ATRP) readily allow the synthesis of PDMA with well-controlled molecular weights and defined topology.[27] Therefore, if such a PDMA segment can be introduced to a hyperbranched polymer surface, the obtained PDMA-shelled hyperbranched polymer would be expected to be an intelligent carrier in controlled drug release field.

In this article, we wish to report on the synthetic method of a novel intelligent hyperbranched polymer (PCMS-*g*-PDMA), containing a hydrophobic branched poly(*p*-(chloromethy)styrene) (PCMS) core and PDMA shell, and the resulting polymer could be hopefully used

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as novel carriers in some controlled drug release systems in the future. To fulfill this goal, we first synthesized hyperbranched PCMS with different degree of branching via ATRP. Then, the modified hyperbranched polymers having different average arm length consisting of PCMS core and PDMA shell are synthesized by ATRP using anterior PCMS as macroinitiators. Moreover, using chlorambucil (CLB) as a model drug, the behaviors of the controlled drug release from PCMS-*g*-PDMA with different average chain length of PDMA and degree of branching are studied.

EXPERIMENTAL

Materials

N,*N*-dimethylaminoethyl methacrylate (DMA) and *p*-(chloromethy)styrene (CMS) were purchased from ACROS Chemical Industries (Pittsburgh, PA, USA) and purified by distillation under reduced pressure. CLB was purchased from Fluka Chemika (Shanghai, China) (more than 98% purity), All other reagents, including phenolphthalein (PP) and cuprous chloride (CuCl) were of analytic grade made in China, and used as received without further purification.

Synthesis of hyperbranched poly(*p*-(chloromethy)styrene)

In a cylindrical reaction vessel equipped with magnetic stirring, CuCl and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiehylenetriamine (PMDETA) were dissolved in mixed solvent following which CMS was added under vigorous stirring (feed composition, see Table I). The mixed solution was bubbled with nitrogen gas for 15 min and vacuumized for 15 min, and the process was circulated thrice to remove oxygen. The reaction vessel was sealed under vacuum, and the polymerization was conducted under stirring at 80°C for 6 h. After the reaction was completed, 50 mL of THF was added, and the mixture was stirred at room temperature for several hours to complete the dissolution of the polymer and allow the catalyst to oxidize. The resulting solution was filtered through filter paper to remove the insoluble salts, then was concentrated and precipitated into methanol/water-mixed solution (1/1, v/v). The resulting polymer was reprecipitated from THF in methanol/water-mixed solvent and dried under vacuum.

Synthesis of PDMA-shelled hyperbranched polymer (PCMS-*g*-PDMA)

In a cylindrical reaction vessel equipped with magnetic stirring, macroinitiator-hyperbranched PCMS, PMDETA, and CuCl were dissolved in mixed solvent following which DMA was added under vigorous stirring (feed composition, see Table II). The mixed solution was bubbled with nitrogen gas for 15 min and vacuumized for 15 min, the process circulated thrice to remove oxygen. The reaction vessel was sealed under vacuum. The polymerization was conducted under stirring at 80°C for 20 h. After the reaction was completed, the mixture was dialyzed in dialysis bag (molecular weight cut off: 8000–10,000) for 96 h against distilled water, which was exchanged at intervals of 3–6 h. The dialyzed product was lyophilized and used for further characterization.

Instrument analyses

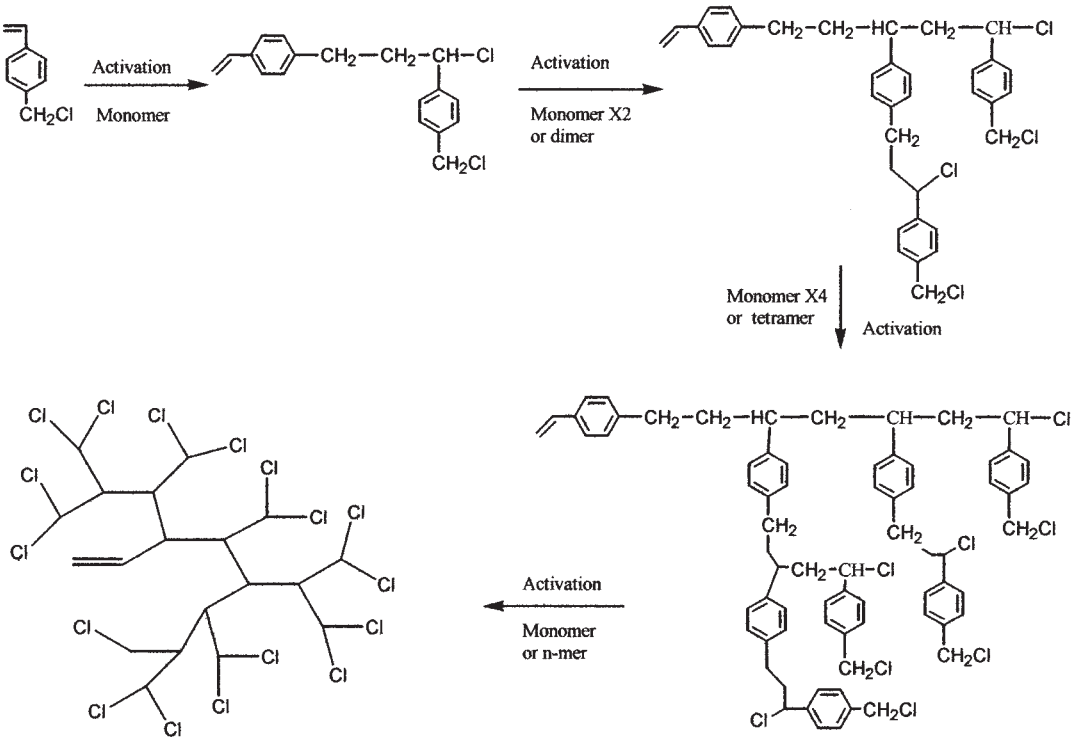
^{1}H NMR measurements were conducted on Varian INOVA-400 spectrometer (Palo Alto, CA, USA), at room temperature, using $CDCl_3$ as solvent. Infrared spectroscopy measurements were preformed on a Specode 75 model, Germany, using KBr as the sample holder. UV spectroscopy measurements were recorded on shimadzu UV-2550 model, Japan. GPC was made on waters Breeze model chromatography, USA, in THF at 25°C, PSt calibration standards, refractive index detector.

CLB loading into the PCMS-*g*-PDMA

CLB and PCMS-*g*-PDMA (5/100, w/w) were dissolved in appropriate tetrahydrofuran under vigorous stirring. The solvent was evaporated at room temperature. Then the membranes with a thickness of 0.2 mm, which consist of the PCMS-*g*-PDMA loaded CLB, were prepared. The products were dried under ambient conditions for 1 day, and at room temperature for 4 days in a vacuum oven.

CLB release studies

The CLB-loaded membranes and pure CLB were sealed with dialysis bag of 4 mm length, respectively. The dialysis bag was used because the PCMS-*g*-PDMA has a significantly higher molecular weight, so that they would stay inside the dialysis membrane, whereas the small molecular weight drug would readily diffuse out of the dialysis bag. At 37°C, they were immersed in 40.0 mL of a pH 10.0 buffer solution with an ionic strength of 0.1 mol l^{-1}. In a special interval, 5.0 mL buffer solution was removed and replaced by 5.0 mL of fresh buffer solution. When a sample's cumulative release time in the solution of pH 10.0 reached 4.5 h, the old solution was replaced by a fresh buffer solution of pH 1.4. The experiments were conducted in the same way as earlier. After 2 h, the buffer solution was replaced with the solution of pH 10.0, following pH 1.4 buffer solution after 2 h. The concentrations of CLB released were analyzed by spectrophotometry at 211.5 nm (pH 1.4) and 242.0 nm



Scheme 1 Release profile of CLB from CLB, PC10D50, and PC200D50 under the conditions of pHs 1.4 and 10.0 at 37°C.

(pH = 10.0), respectively. All the resulting solutions were kept at 37°C for 48 h prior to measurements. All release measurements were carried out in triplicate for each sample and the average values were plotted.

RESULTS AND DISCUSSION

Synthesis and characterization of hyperbranched poly(*p*-(chloromethy)styrene)

The ATRP of CMS was first reported by Matyjaszewski[28] and later discussed in detail by Weimer et al.[29] The action of the ATRP mechanism in the case of this monomer should lead to a branched product, as CMS may act as an "inimer"-monomer (styrenic double bond) and initiator (abstraction of the chlorine and generation of the CH_2 radical). Scheme 1 shows the synthetic routes of hyperbranched polymer PCMS. The chlorine atom at the benzylic position is abstracted by Cu(I) homolytically, forming Cu(II)Cl and a benzyl radical capable of initiating the polymerization of monomer through the double bonds. The propagating chain is reversibly deactivated by Cu(II)Cl. This results in the formation of a polymer chain with pendent groups consisting of *p*-benzyl chloride, with a double bond at one chain end and a chlorine atom at the other. Both of these chlorine atoms can be abstracted to form radicals, which can reinitiate the polymerization. The double bond at the chain end can be incorporated into a growing polymer chain, resulting in a branch point. That is, each of the polymer chains can react with monomers, dimers, trimers, etc. According to the report by Weimer et al.,[29] the amount of catalyst is an important variable to get a polymer of different degree of branching (DB). Therefore, in this research the catalyst (CuCl) ratio to CMS were 0.5, 1, and 10 mol %. By comparison of the integration of the signals from the double bond to those of the aromatic region, the degrees of polymerization of our polymers

TABLE I
Feed Ratios and Parameters of Hyperbranched Polymer PCMS

	Feed ratio						Polymer parameters	
Sample	CuCl (g)	PMDETA (g)	Water (g)	Ethanol (g)	Cyclohexanone (g)	CMS (g)	Degree of polymerization	Minimum linearity (%)
PCMS10	0.263	0.933	4.0	2.0		4.03	9.2	33
PCMS100	0.026	0.099	0.5		3.98	3.96	10.3	74
PCMS200	0.013	0.047	0.5		4.03	3.99	11.2	100

TABLE II
Feed Composition and Parameters of Hyperbranched Polymers PCMS-*g*-PDMA

Sample	Feed ratio								Polymer parameters	
	PCMS (g)		CuCl (g)	PMDETA (g)	Water (g)	Ethanol (g)	DMF (g)	DMA (g)	$M_{n,\,GPC}$	M_w/M_n
	PCMS10	PCMS200								
PC10D20	0.194		0.144	0.486	2.8	2.5		4.04	2.57×10^4	2.75
PC10D50	0.114		0.084	0.302	2.5	2.8		6.01	6.21×10^4	2.41
PC10D100	0.062		0.049	0.140	2.0	2.0		6.30	1.12×10^5	2.38
PC200D20		0.194	0.129	0.446	4.0		5.0	3.99	3.25×10^4	2.10
PC200D50		0.100	0.065	0.223	4.0		4.0	5.00	6.52×10^4	2.05
PC200D100		0.061	0.040	0.149	4.3		4.0	6.29	1.51×10^5	2.22

are 9.2, 10.3, and 11.2, respectively. According to the method developed by Weimer, the 'minimal percent of linearity' in the polymers are 33, 74, and 100%, respectively. The detailes of synthesis parameters and polymer parameters are listed in Table I.

Synthesis of the hyperbranched poly(*p*-(chloromethy)styrene) with PDMA grafts on the surface (PCMS-*g*-PDMA)

Using PCMS having different degree of branching (DB) as macroinitiators, CuCl as catalyst and PMDETA as ligand, the copolymers PCMS-*g*-PDMA with a hyperbranched PCMS core and linear PDMA arms were synthesized by ATRP continually. The chain length of PDMA arms were controlled by varying ratio of [DMA]/[PCMS]. Table II showed synthesis parameters of PCMS-*g*-PDMA.

FT-IR analysis

IR spectra of hyperbranched polymers PCMS-*g*-PDMA derived from macroinitiator PCMS10 and PCMS200 are shown in Figures 1 and 2. The bands occurring between 2800 and 2950 cm^{-1} are associated with the symmetric and asymmetric C—H stretching vibrations of the aliphatic CH_2 and CH_3 groups. Other FT-IR spectral features including the C═O stretching vibration at 1728 cm^{-1} and C—N stretching vibration at 1270 cm^{-1} are the characteristic of the segments of PDMA.

^{1}H NMR analysis

Figure 3 showed ^{1}H NMR spectrum for PC10D50 in $CDCl_3$. The resonance signals were attributed to the protons originated from PDMA chain unit as shown in Figure 3. The signals occurring between 1.1 and 2.0 ppm are associated with the —CH_2—CH— protons adjacent to the aromatic atoms.[30] The disappearance of the peak at 4.6 ppm correspond to the Ph—CH_2—Cl protons indicates that ATRP took place.

CLB release from hyperbranched polymer PAMAM-DMA

To investigate the potential of PCMS-*g*-PDMA to control drug release, the behaviors of the drug release from the PCMS-*g*-PDMA with different DB and arms chain length are studied by using CLB, a anticancer

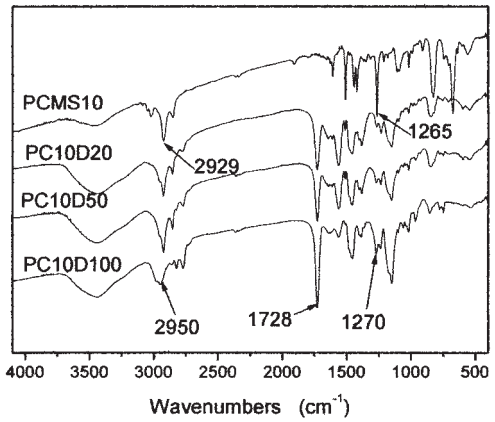


Figure 1 IR spectra of PCMS10, PC10D20, PC10D50, and PC10D100.

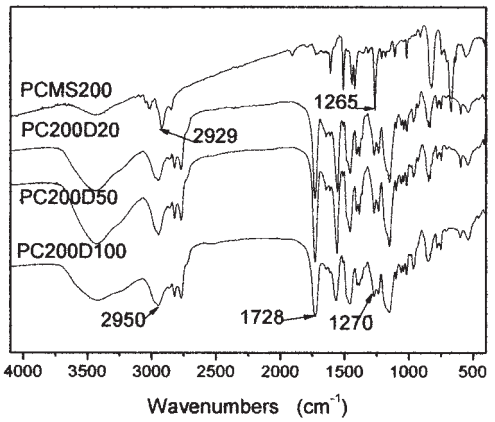


Figure 2 IR spectra of PCMS200, PC200D20, PC200D50, and PC2000D100.

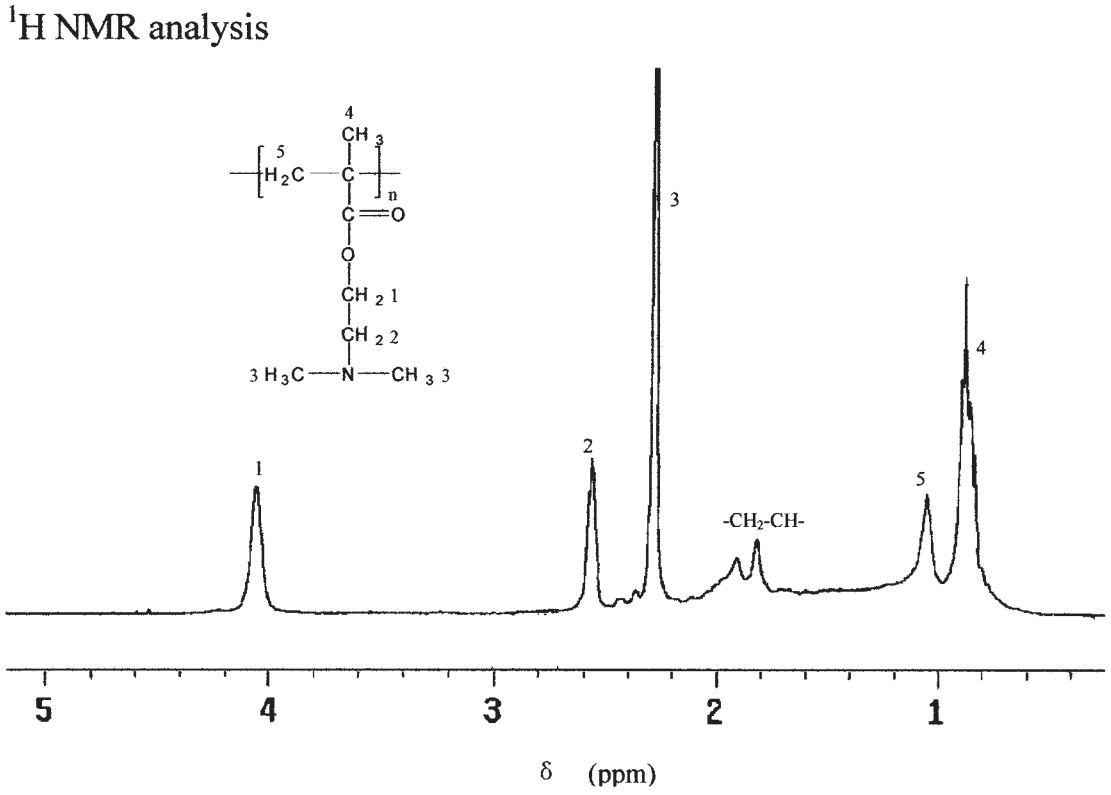


Figure 3 ^{1}H NMR spectrum for PC10D50 in $CDCl_3$.

drug, as a model drug. All release experiments were carried out under the conditions of pHs 1.4 and 10.0, and 37°C. Figure 4 exhibits the cumulative amounts of CLB released from pure CLB and the loaded polymers with different chain length of arms. As can be seen from Figure 4, the release rates of CLB from pure CLB are faster at pH 10.0 than at pH 1.4, which due to different solubility at diffident pH buffer solution,[31]

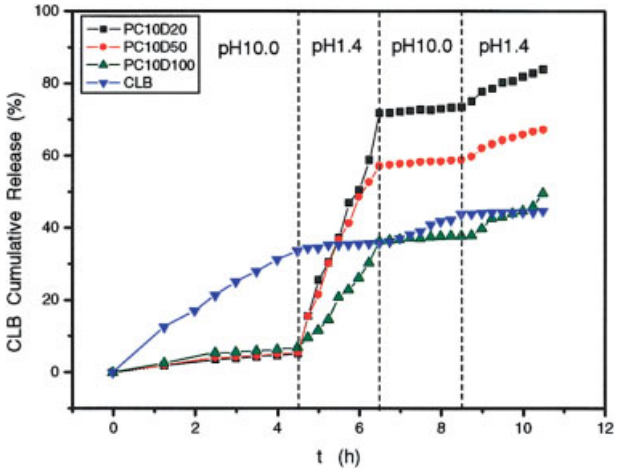


Figure 4 Release profile of CLB from CLB, PC10D20, PC10D50, and PC10D100 under the conditions of pHs 1.4 and 10.0 at 37°C.

whereas the release rates of CLB from the PCMS-*g*-PDMA are faster at pH 1.4 than at pH 10.0. This suggests that the release behavior of CLB from drug/hyperbranched polymer complex is significantly different from pure CLB, and the release rate of CLB from PCMS-*g*-PDMA can be controlled by varying pHs. It can also be seen from Figure 4 that the longer the chain length of PDMA arms, the slower the release rate of CLB. This can be explained in terms of the polymer structure. PCMS-*g*-PDMA has the characteristic molecular shape, in which the PDMA segments are radially aligned on the three-dimensional core. PDMA shows a conformational transition from expanded structure to compact coil on elevation of the surrounding pH, reflecting the ionization state of the PDMA segment. It is therefore expected that the release of CLB will also be varied due to conformational change of the PDMA segment. At higher pH (i.e., pH 10.0), PDMA segment takes a hydrophobic, compact coil conformation which prevents CLB releasing from drug/hyperbranched polymer complex. When the pH is reduced to pH 1.4, PDMA segments show completely expanded conformation and CLB can readily diffuse out of the drug/hyperbranched polymer complex. These aspects can explain why the surrounding pHs can control the release rates of CLB from the PCMS-*g*-PDMA and why the longer segment length of PDMA led to slower release rate of CLB.

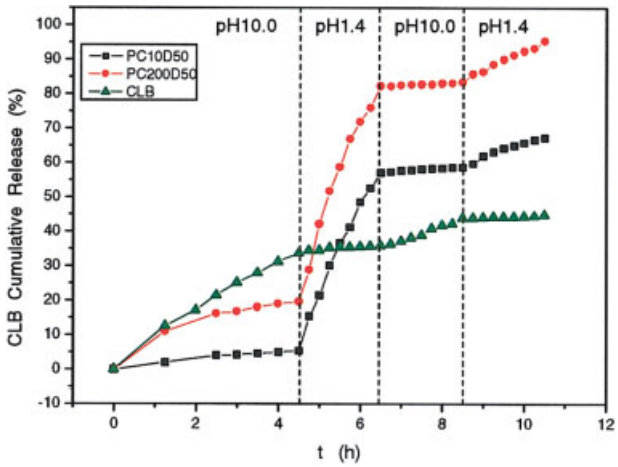


Figure 5 Release profile of CLB from CLB, PC10D50, and PC200D50 under the conditions of pHs 1.4 and 10.0 at 37°C.

Figure 5 exhibits the cumulative amounts of CLB released from pure CLB and the loaded samples with different DB. Because of structural differences, especially the DB, the differences in the release process of CLB were expected. Indeed, during the release of CLB, the release rate of CLB from the loaded sample PC10D50 was slower than PC200D50. This is because the sample PC10D50 derived from PCMS10, has a hyperbranched structure, whereas the sample PC200D50 derived from PCMS200 has a comb-shaped structure. It is understandable that CLB diffuse from the hyperbranched polymer more difficultly than from the comb-shaped polymer.

CONCLUSIONS

The hyperbranched PCMS with different DB were synthesized via ATRP of CMS, which may act as an "inimer"-monomer and initiator, by controlling the ratio of CuCl to CMS. Hyperbranched copolymers PCMS-*g*-PDMA, containing hydrophobic branched PCMS core and PDMA shell, are synthesized by ATRP, using anterior PCMS with different DB as macroinitiators. Their macromolecular structures are characterized by FTIR and ^{1}H NMR. Using CLB as a model drug, the behaviors of the controlled drug release from the environmental-sensitive hyperbranched polymers with different average chain length of PDMA arms and degree of branching are studied. The results demonstrate that the drug release rate can be effectively controlled by pH value.

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