Synthesis and Drug-Release Properties of Hyperbranched Polyesters Grafted with Biocompatible Poly(ε-caprolactone)

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Received 5 July 2007; accepted 19 November 2007
DOI 10.1002/app.28233
Published online 2 May 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A hyperbranched, functional, and biocompatible polymer, poly(ε-caprolactone)-grafted hyperbranched polyester (HPCL), was synthesized via a facile two-step routine as a prospective nontoxic, biodegradable, and biocompatible drug delivery system. ^{1}H-NMR measurements provided direct evidence that ε-caprolactone was grafted by a reaction with the peripheral functional hydroxyl groups of hyperbranched polyesters. pH-responsive HPCL in different synthetic body fluids (SBFs) could controllably incubate aspirin. In the different SBFs, the release rates were diverse. In SBF with a pH of 7.4, the release time was nearly 110 h, whereas in SBF with a pH of 6.4 and SBF with a pH of 7.8, it was 185 and 71 h, respectively. In comparison with previously reported systems, our system had a longer release life. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2089–2094, 2008

Key words: drug delivery systems; hyperbranched; synthesis

INTRODUCTION

Biomaterials, which are widely used in medical applications, are attracting increasing attention in the field of drug delivery systems. Choosing an excellent system for drug delivery is a major challenge because of the uncontrollable release rate of medicine in the inner body. An attractive solution to this problem involves the development of new drug delivery systems in which drug molecules can be encapsulated so that they can be selectively concentrated in malignant tissues, especially in tumor tissues, because of the enhanced permeability and retention effect.[1]

It is well known that polymeric micelles prepared from linear polymers can be used as carriers for the delivery of promising biomolecular drugs such as therapeutic peptides, proteins, genes, and anticancer drugs. Various amphiphilic linear block copolymers are known to be able to self-assemble into polymeric micelles in selective solvents above the critical micelle concentration. However, the formed micelles are not stable and suffer from dissociation into free chains when environmental parameters, such as the temperature and concentration, change.[2]

Hyperbranched polymers are highly branched, polydisperse, three-dimensional macromolecules that, because of their unique structures and properties, have attracted increasing attention.[3–7] Compared to their linear analogues, hyperbranched polymers are expected to have different physical properties, such as a huge number of modifiable surface functionalities, lower viscosities, and better solubility.[8–11] Although hyperbranched polymers are irregularly shaped and not perfectly symmetrical like dendrimers, they can be prepared in a single, one-pot reaction, and this is the reason for relatively high interest in the industry of hyperbranched polymers.[3–11] Moreover, as drug carriers, hyperbranched polymers can also offer their interior or peripheral functional groups to covalently fix drug molecules or, depending on their core–shell architecture, sequester guest molecules. Therefore, hyperbranched polymers are promising candidates for drug delivery.

So far, there have been two main methodologies that use polymers as drug carriers. The first route involves polymers as matrices in which the drug is embedded; the drug is then released by physical and

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Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20604024.
Contract grant sponsor: Natural Science Foundation of Zhejiang Province; contract grant number: Y406008.
Contract grant sponsor: Program for Changjiang Scholars and Innovative Research Team in University; contract grant number: 0654.

chemical modifications such as swelling of the polymer, diffusion, or chemical erosion of the polymeric matrix. The main advantage of this method is the preservation of the chemical integrity and pharmacological properties.[12,13] The second method involves drug conjugation to an appropriate polymeric carrier. In this case, the drug is covalently bound to the polymer, and its release occurs via chemical or enzymatic cleavage of hydrolytically labile bonds.[14–16] The main advantage of this method is that the drug–polymer conjugate diffuses more slowly than the free drug and can be absorbed at specific interfaces, and this allows tissue targeting and controlled delivery. Hyperbranched polymers have a huge number of modifiable surface functional groups that can be placed in contact with environmentally sensitive segments. The resultant hyperbranched polymers can respond to changes in environmental conditions and match the high demands for controlled and targeted delivery. Hyperbranched polymeric systems are perfect delivery system candidates because their low polydispersity can ensure reproducibility of the pharmacokinetic behaviors and the presence of modifiable end groups allows improved water solubility and environmentally sensitive and nonstatistical attachment of drug molecules. The covalent scaffold also provides a stable structure for the internal encapsulation of a drug that is not based on thermodynamics or physical factors.[17,18] In this study, we designed a novel and effective drug carrier, poly(ε-caprolactone)-grafted hyperbranched polyester (HPCL) with a hyperbranched polyester (HPE) core and many poly(ε-caprolactone) (PCL) arms, that is nontoxic, biocompatible, and easily prepared in only two steps. Aspirin can be encapsulated into HPCL, and it also can be released from HPCL at different rates in synthetic body fluids (SBFs) of different pH values.

EXPERIMENTAL

Materials

Tetrahydrofuran (analysis-grade), chloroform (analysis-grade), acetone (analysis-grade), *n*-hexane (analysis-grade), and dimethyl chloride (analysis-grade) were purchased from East China Chem Ltd. Co. (Shanghai, China) and used as received. 2-Ethyl-2-hydroxymethyl-1,3-propanediol (TMP), 2,2-bis(hydroxymethyl) propanic acid (bis-MPA), *p*-toluene sulfonic acid (*p*-TSA), ε-caprolactone (CL), and tin(II) 2-ethylhexanoate [$Sn(Oct)_2$] were used as received from Acros (Shanghai, China). *o*-Acetylsalicylic acid was used as received from Alfa Aesar (Ward Hill, MA). Tris(hydroxymethyl)aminomethane was purchased from Sinopharm Chemical Reagent Ltd. Co. (Shanghai, China). All SBFs were prepared with a revised method.[19]

Synthesis of HPEs

All synthetic procedures were performed under a dry nitrogen atmosphere. HPEs (polyols with TMP as a core) were prepared by a procedure described in the literature.[20,21] Esterification was carried out at 140°C with *p*-TSA as an acid catalyst. The chosen molar ratio of TMP to bis-MPA was 1 : 21, corresponding to the theoretical molecular weight of 2573 g/mol and an HPE with 24 terminal hydroxyl groups. The crude polymer with a generation 3 structure in the theoretical calculation (HP3) was precipitated from acetone/*n*-hexane (1 : 1) and dried *in vacuo*. Fourier transform infrared (FTIR) showed no remaining carboxylic acid.

^{1}H-NMR [$(CD_3)_2CO$, δ, ppm]: 1.10–1.31 (—CH_3 and CH_3CH_2C), 2.04–2.18 (—CH_2OH), 3.63–3.73 (—CH_2OH), 4.10–4.32 (—$COOCH_2$).

Synthesis of HPCL

HPCL was synthesized by ring-opening polymerization, which was initiated with the hydroxyl of hyperbranched molecules with a catalytic amount of $Sn(Oct)_2$.[22] A mixture of 3.25 g of HPE and 6.78 g of CL was heated to 100°C to obtain a homogeneous melt of both monomers. This mixture was then polymerized in bulk by the direct addition of 0.9 mmol of $Sn(Oct)_2$. The mixing was carried out in the melt at 110°C for 20 h. The crude product was dissolved in dimethyl chloride, precipitated into *n*-hexane, and then dried *in vacuo*. The resultant compound was a low-melting material that was soluble in most organic solvents. FTIR notably showed no remaining hydrogen-bond association.

^{1}H-NMR ($CDCl_3$, δ, ppm): 3.90–4.32 (—$COOCH_2$—), 3.63–3.73 (—CH_2OH), 1.10–1.65 (—CH_3, CH_3CH_2C, CH_2CH_2COO, —$CH_2CH_2CH_2COO$, —$CH_2CH_2CH_2CH_2COO$), 2.20–2.31 (—CH_2COO).

Preparation of drug-loaded complexes

Drug-loaded complexes containing aspirin were prepared by a direct dissolution process. Briefly, 167 mg of HPCL was first dissolved in 10 mL of dimethyl chloride. Then, 490 mg of aspirin was added with vigorous stirring at room temperature. After 12 h of stirring to remove the organic solvent completely, the solution was dialyzed against distilled water for 24 h with a dialysis membrane (molecular weight cutoff = 1000) to remove the free drug. The water was replaced every 2 h for the first 8 h. After dialysis, the solution in the dialysis bag was collected and freeze-dried to obtain drug-loaded complexes.

Release study of aspirin-loaded HPCL

Aspirin-loaded HPCL (66 mg) was immersed in 35 mL of a phosphate buffer solution with different pH

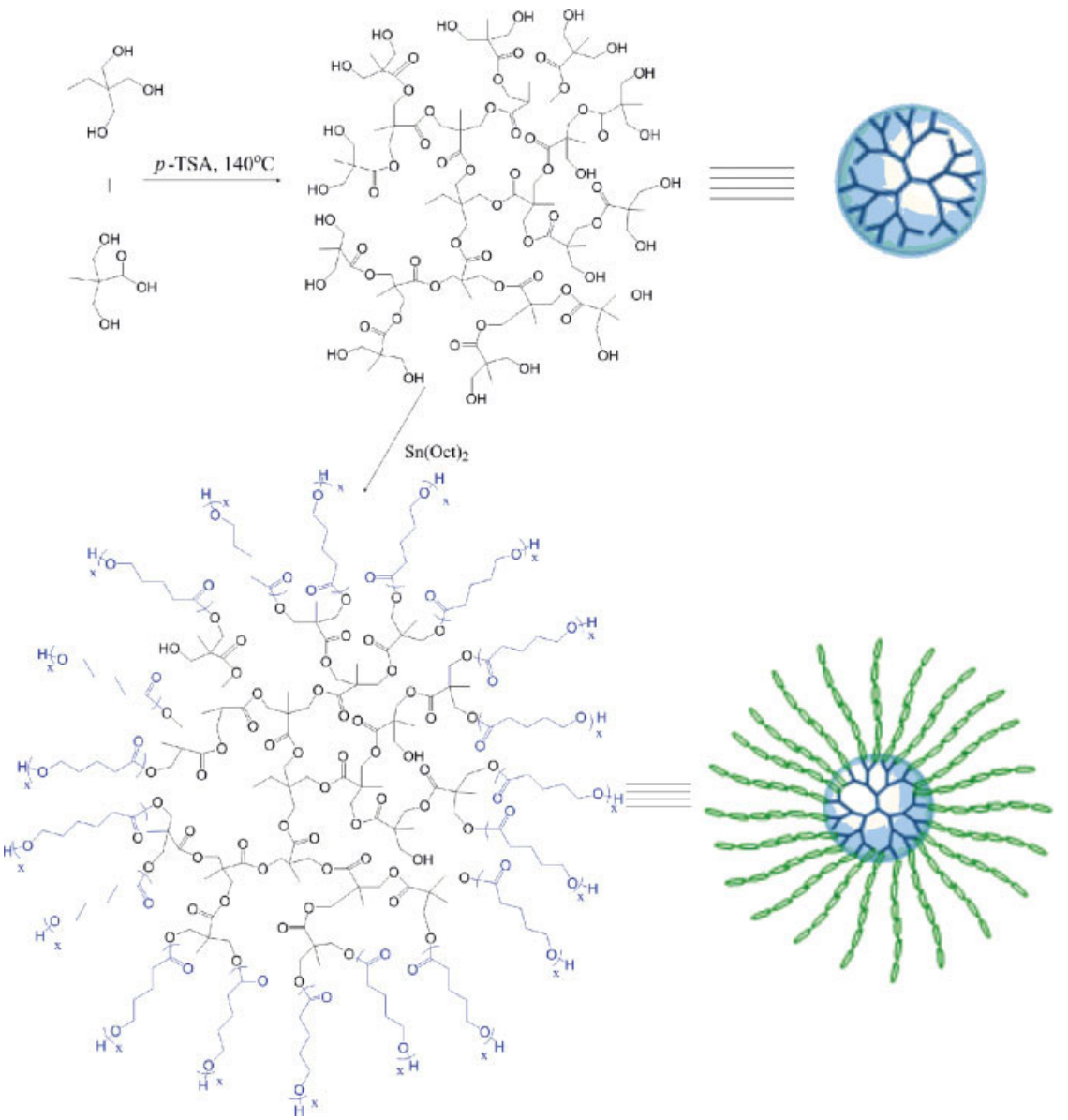
values (6.4, 7.4, and 7.8) and incubated at 37°C with continuous shaking at a speed of 100 rpm. At specific time intervals, 1-mL solutions were withdrawn from the release medium. The aspirin content of the samples was determined at 296 nm with an ultraviolet–visible spectrophotometer.

Analytical equipment

^{1}H-NMR spectra were recorded with an Avance AV 400 NMR spectrometer (Billerica, MA) with tetramethylsilane (TMS) as an internal standard at room temperature. FTIR measurements were carried out on a Nicolet 5700 spectrometer (Madison, WI). UV measurements of the resultant capsulated hyperbranched polymers were performed on a Lambda 900 spectrometer (Waltham, MA) with SBF as the solvent at 37°C. A Proteomics 4700 matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analyzer (Micro Mass, UK) was used. The sample was dissolved in a 30:70 mixture of CH_3CN and H_2O. The molecular weight and molecular weight distribution of HPCL were determined by gel permeation chromatography (GPC) (Milford, MA) with a Waters 1500 apparatus (with tetrahydrofuran as an eluent at a flow rate of 0.5 mL/min).

RESULTS AND DISCUSSION

The synthetic procedure for various polymers is outlined in Scheme 1. An aliphatic hyperbranched polymer (HP3) was first synthesized by the melt poly-



Scheme 1 Reaction scheme for the synthesis of HPCL. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

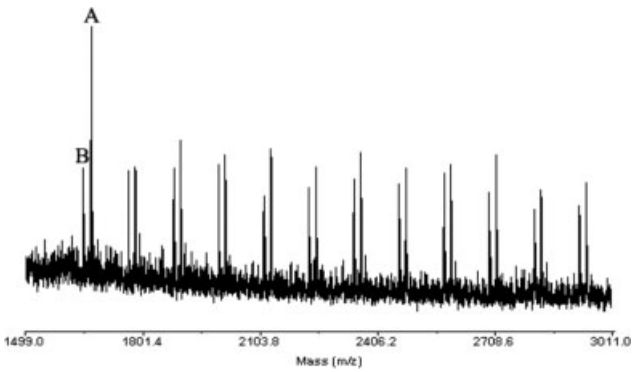


Figure 1 MALDI-TOF mass spectrum of an HPE sample.

condensation of bis-MPA and TMP at a molar ratio of 21/1 with *p*-TSA as a catalyst by a pseudo-one-step synthesis method.[20] Although MALDI-TOF may give incorrect data for the average molecular weight, it offers an opportunity to directly determine the molar mass of individual polymer chains and, consequently, their exact composition. The MALDI-TOF mass spectrum of HP3 (Fig. 1) contains two peak series corresponding to macromolecules with different end-group structures. The mass increment between peaks with identical end groups was 116.1 (the mass of the bis-MPA repeating monomer unit). Peak series A at $m/z = n \times 116.1 + 134.2 + 23\ (Na^+) + 1$ (n is the number of monomer units; 134.2 is the mass of TMP) corresponds to HPE initiated by TMP and terminated by a proton from bis-MPA. Peak series B at $m/z = n \times 116.1 + 134.2 + 23(Na^+) + 1 - 18$ (the molar mass of H_2O is 18) shows that two hydroxyls of bis-MPA reacted to form an ether group by eliminating a molecule of H_2O. The molecular characteristics of HP3 and HPCL were determined by GPC. The number-average molecular weight of HP3 was 2573 by the theoretical calculation. After the grafting of PCL, the number-average molecular weight of HPCL increased to 24,000.

Typical ^{1}H-NMR spectra of HP3 and HPCL are given in Figure 2. By the reaction between hydroxy end groups of HP3 and CL, HP3 was further transferred into HPCL with a considerable amount of PCL. ^{1}H-NMR data confirmed the chemical composition of the compound. Compared with the ^{1}H-NMR spectrum of HP3, the ^{1}H-NMR spectrum of HPCL exhibits two distinct primary groups of peaks (3.90–4.32 and 2.20–2.31). The distinct peaks at 3.90–4.32 ppm, which originated from methylene units adjacent to ester bonds in HPCL, were notably higher. Remarkably, peaks at 2.20–2.31 ppm, which originated from methylene units neighboring the ester bonds in HPCL, were observed, whereas peaks at 2.04–2.18 ppm, which originated from the hydroxyl of HP3, completely disappeared. In addition, peaks at 1.10–1.65 ppm, assigned to long chains of PCL, can be clearly observed in the ^{1}H-NMR spectrum of HPCL.

Knowing how to trigger the release of useful encapsulated medicine is crucial for the application of dendrimers and hyperbranched polymers as drug carriers. Specifically, the controllable release of the active ingredient from the carriers (hyperbranched polymers in this case) when it reaches a target site enhances its efficacy and reduces the toxicity of the drug. Aspirin has a number of properties that make it the most often recommended drug. It is also an

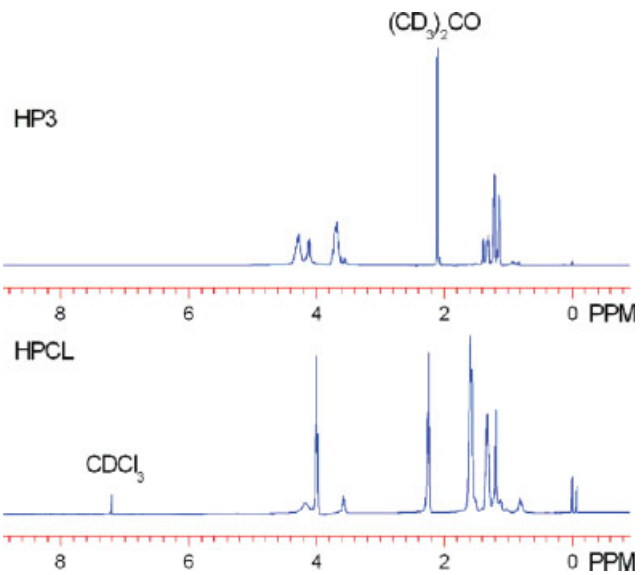


Figure 2 ^{1}H-NMR spectra of HPE and HPCL. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

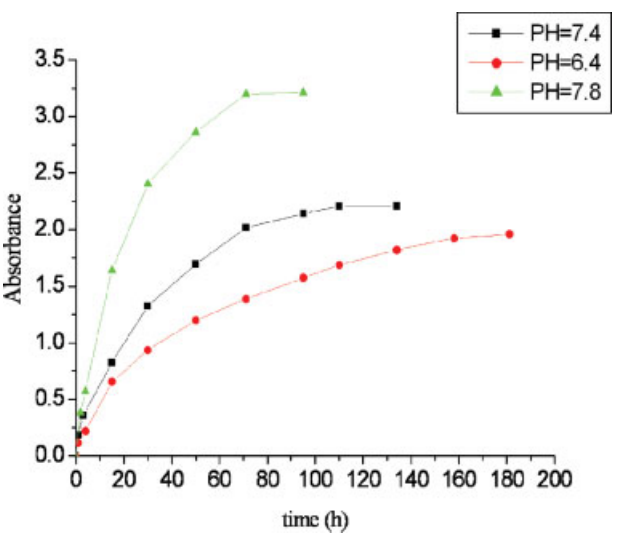
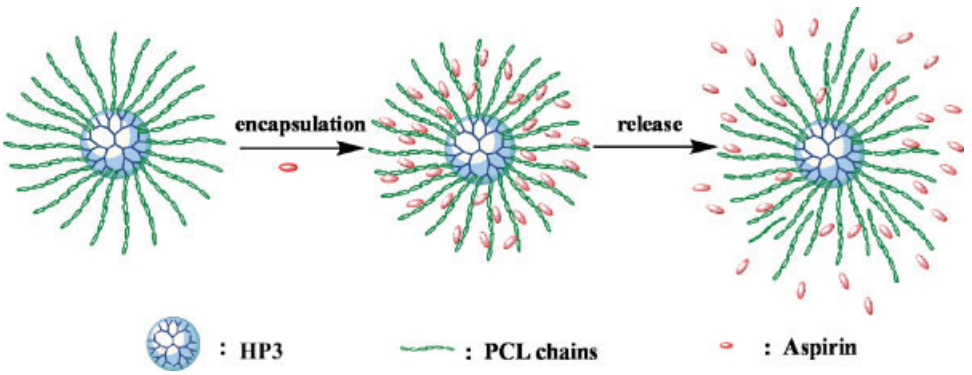


Figure 3 Time dependence of the release of aspirin-loaded HPCL in SBFs with different pHs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Scheme 2 Schematic illustration of the encapsulation and release of aspirin from HPCL. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

anti-inflammatory agent, providing some relief from the swelling associated with arthritis and minor injuries.[23] Therefore, we used this classic drug for our release study. Additionally, PCL possessing nontoxic and biocompatibility properties is able to encapsulate small compounds, so we released aspirin in SBFs with different pH values to evaluate the possible applications of HPCL for drug delivery. Aspirin-loaded HPCL was added to SBFs of different pH values, and the resultant solutions were shaken at 37°C for specific time intervals. The amount of aspirin released from aspirin-loaded HPCL was measured with UV spectroscopy at 296 nm. Because of the effect of H^+ and OH^- in the release environment, the rate and concentration of the critical release of aspirin should have been different.

Figure 3 provides direct proof by monitoring the aspirin concentration in the *in vitro* solution by UV spectroscopy. In slightly acidic SBF (pH = 6.4), the release rate of aspirin was considerably slower than that in neutral and slightly basic SBF (pH = 7.4 and pH = 7.8), in which the release of aspirin was restrained in SBF via the breaking of the adjacent hydrogen bond, and the complete release time was nearly 185 h. However, in the SBFs with pH values of 7.4 and 7.8, the rate of incubation was adverse, and the final release times were about 110 and 71 h, respectively, because of the weak hydrogen-bond reactions around the drug in the carriers. The overall encapsulation and release mechanism is depicted in Scheme 2. Because of the large arms at the periphery of HPCL, there was enough space to encapsulate small medical molecules. In addition, the largest amounts of aspirin moved to the room in the matrix, driven by the large ether and ester groups, for the hydrogen-bond interactions. However, as the drug-loaded complexes were placed in the different SBFs, the weak hydrogen-bond interactions could not stably catch hold of aspirin and incubated the drug at different rates when the environment changed.

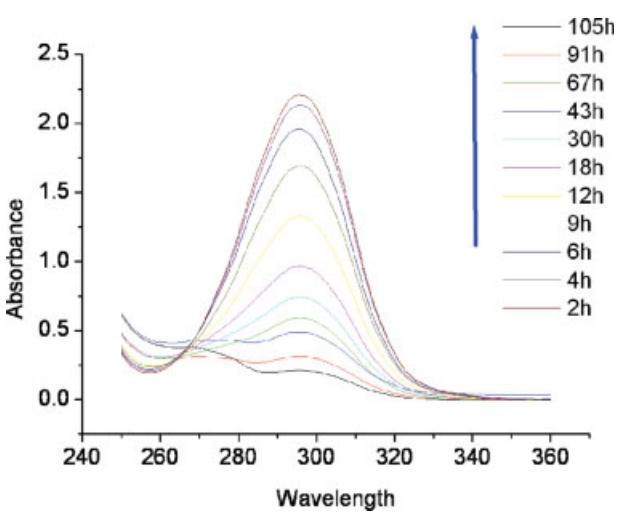


Figure 4 Typical absorbance spectra of the release of aspirin-loaded HPCL in SBF (pH = 7.4). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4 shows the aspirin-release results in SBF (pH = 7.4). The entrapped aspirin molecules were slowly released from HPCL, the drug delivery carrier. Remarkably, in the first 30 h, the release rate was much faster than for the remaining release time, and the amount of release was nearly 75% of the critical release concentration. The release time of most drug delivery systems is less than 48 h,[24,25] so our system is more effective and bioavailable. It is noteworthy that pH-sensitive HPCL in different SBFs can controllably incubate aspirin. Hence, the possibility of drug release with HPCL in SBF should be considered for designing a targeted drug release system.

CONCLUSIONS

In this study, a hyperbranched, functional, and biocompatible polymer, HPCL, was synthesized via a facile two-step routine as a prospective drug delivery system. ^{1}H-NMR measurements provided direct evidence that various CLs were grafted by a reaction

with the peripheral functional hydroxyl groups of HPEs (HP3). The release rate of aspirin depended on the pH value of the SBF solution. In SBF with a pH of 7.4, the release time was nearly 110 h, whereas in SBFs with pHs of 6.4 and 7.8, the times were 185 and 71 h, respectively. Compared with previously reported systems, our system has a longer release life. It is noteworthy that pH-responsive HPCL in different SBFs can controllably incubate aspirin. Hence, the possibility of drug release with HPCL in SBFs should be considered for designing a targeted drug release system.

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