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Hyperbranched Polyether Surface Functionalized with Biomimetic Zwitterionic Polymers as Potential Drug Release Carriers

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Novel biomimetic hyperbranched copolymers were synthesized by polymerization of zwitterionic monomer (CBB) on the surface of a hyperbranched poly (3-ethyl-3-(hydroxymethyl)oxetane) (HBPO) core. The composition and morphology of resultant copolymers were investigated by ^{1}H NMR, TGA, DLS, and TEM. The biomimetic hyperbranched copolymers showed low toxicity, favorable protein resistant properties, and were ultra-stable in 100% fetal bovine serum (FBS) The conjugation with biomolecules (folate amine) can be easily achieved through N,N'-Dicyclohexycarbodiimide and N-hydroxysuccinimide (DCC/NHS) chemistry. The loading and release properties of a model drug, indomethacin (IND), using the resultant biomimetic hyperbranched copolymers as carriers were also investigated. The loading content was determined by UV-vis analysis to be 22.68 wt % and the drug release rate depends greatly on the pH and protein concentration of the solution which indicated the biomimetic copolymer could be as candidate carriers for biomedical applications.

[Supplementary materials are available for this article. Go to the publisher's online edition of Soft Materials for the following free supplemental resource(s): Synthesis of Zwitterionic Copolymers]

Keywords: Biomimetic hyperbranched copolymer, Protein resistant properties, Release properties

Introduction

Hyperbranched polymers have been extensively studied in the field of targeted drug delivery due to their great potential to work as carriers for both imaging and therapy (1–3). Hyperbranched polymers have three-dimensional structures and unique properties that are different from those of linear polymers (4, 5). For example, various types of hyperbranched polymers have high therapeutic drug loading property and are capable of releasing a payload at the target site (6, 7). In addition, hyperbranched polymers are usually prepared by facile one-pot synthesis from specific monomers with branching potential (8, 9).

Hyperbranched poly (3-ethyl-3-(hydroxymethyl)oxetane) (HBPO) is one kind of hyperbranched ether that has been widely investigated as a hydrophobic drug carrier. However, HBPO presents certain drawbacks that influence it in drug delivery performance. For instance, the hydrophilicity and stability of HBPO is poor and there is no stimuli-sensitivity and targeting release properties in HBPO (10, 11). Therefore, it is crucial to impart desired functionality to target HBPO with appropriate structures and properties. To address this issue, neutral and hydrophilic polymers are preferred as they can reduce opsonization and improve in vivo circulation half-life. Dextran and poly(ethylene glycol) (PEG) are the most widely studied polymers for this purpose (12, 13). However, both dextran and PEG present certain drawbacks that influence their in vivo performance (14–16). For instance, PEG is susceptible to oxidation damage and loses its function in biological media. Furthermore, there is only one functional group potentially available at the end of a long PEG chain (e.g., 2–5 KDa) to which biomolecules can be conjugated.

Poly(carboxybetaine) (PCB) is a kind of zwitterionic copolymer which showed excellent stability against complex media such as undiluted blood serum or plasma. (17). The structure of CB is similar to that of glycine-betaine, which is one of the compatible solutes vital to the osmotic regulation of living organisms. Estimates of glycine-betaine intake by humans range from 0.1 to 2.5 g per day; thus, it can be considered as biomimetic materials (10). What makes biomimetic PCB unique over other stealth materials is that each PCB chain has

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abundant carboxylate-anion groups as functionalizable groups for the immobilization of amine-containing biomolecules (15). Moreover, the biomimetic PCB functioned nanoparticles could be pH-sensitive due to the presence of the carboxylate-anion groups. These properties render biomimetic PCB an ideal material to construct multifunctional nanoparticles (NPs). For example, PCB coated Fe_3O_4 NPs can be ultra-stable in biological media and can evade the innate immune system. After being conjugated to a RGD (Arg-Gly-Asp-$_D$-Tyr-Lys) peptide, the uptake of these Fe_3O_4 NPs by HUVEC (human umbilical vein endothelial cell) cells was significantly enhanced (12). Unfortunately, because of the sharp hydrophilicity/hydrophobicity difference between the hyperbranched polyether (HBPO) core and the biomimetic PCB shell, it is very difficult to find a solvent or mixed solvent system to co-dissolve both hyperbranched polyether and biomimetic PCB homopolymers. Thus, to the best of our knowledge, no biomimetic hyperbranched polyether with zwitterionic shell has been synthesized as a drug carrier.

To solve this problem, we designed a new carboxybetaine monomer containing an isobutyryl bromide group (CBB, Scheme 1). The facile synthesized CBB monomer has good solubility in organic solvents, thus enabling the functionalized of HBPO with CBB polymers in a common solvent, such as DMF or DMSO. The biomimetic PCB polymers can be generated by hydrolysis of the isobutyryl bromide groups of the PCBB arms in deionized water (DI water) after covalent bonding with HBPO. The resultant biomimetic hyperbranched copolymers showed low toxicity, favorable protein resistant properties and were ultra-stable in 100% fetal bovine serum (FBS). To explore the potential application of the novel biomimetic hyperbranched copolymers, drug loading and in vitro release properties were also investigated.

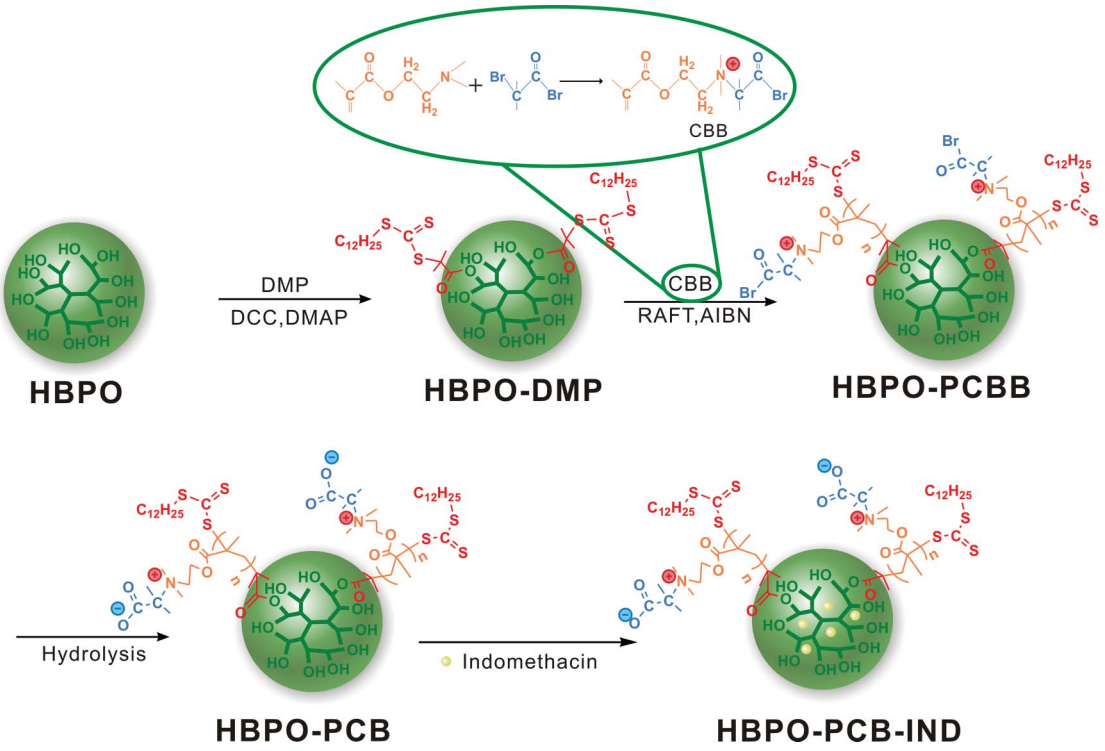
Experimental Section

Materials

HBPO and S-1-Dodecyl-S'-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate (DMP) were synthesized according to the previously published procedures (18, 19). Azobisisobutyronitrile (AIBN, 98 %) was recrystallized twice from ethanol and dried in vacuum prior to use. The AIBN was purchased from East China Chemical Co. (Shanghai, China). 4-dimethylaminopyridine (DMAP, 99%), Dicyclohexycarbodiimide (DCC, 99%), α-bromoisobutyryl bromide(98%), and 2-(dimethylamino)ethyl methacrylate (DMAEMA, 99 %) were purchased from Aladdin Reagent Co. (Shanghai, China) and used as received. All other reagents and solvents were of analytical grade and used as received without further purification.

Synthesis of CBB Monomer

As shown in Scheme 1, the N-(2-(methacryloyloxy)ethyl-N,N-dimethyl- 2-isbutyryl bromide (CBB monomer) was synthesized as follows. 11 g DMAEMA and 17.7 g α-bromoisobutyryl bromide were reacted in 50 ml anhydrous acetonitrile at 50°C under N_2 protection for 24 h. Upon addition of 250 ml ethyl ether to the reaction mixture, the formed yellow crystals were isolated and dried. The resulting CBB monomers were immediately stored in a desiccator at −20°C (yield 84%) (20–22). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.89 (s, 3H, CH_2=C(CH_3)COO-), 2.81(s, 6H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 3.47 (s, 6H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 3.74 (t, 2H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 4.51 (t, 2H, -$CH_2CH_2N(CH_3)_2C(CH_3)_2$-), 5.73 and 6.09 (s, 2H, $CH2$=C(CH3)COO-).



Scheme 1. Synthesis of HBPO-PCB copolymers and formation of HBPO-PCB-IND micelles (color figure available online).

Synthesis of HBPO-PCBB and HBPO-PCB

The HBPO-DMP RAFT chain-transfer agent (CTA) was synthesized as reported previously (23). The HBPO-PCBB was synthesized through a RAFT path by using HBPO-DMP as macro-CTAs, AIBN as catalyst. The general procedure was as follows. The macro-CTAs HBPO-DMP, CBB monomer, and AIBN were dissolved in DMF and the solution was transferred to the flask. After the mixture was added by syringe, the flask was immersed in liquid nitrogen followed by three cycles of freeze-pump-thaw procedures. Finally, the flask was flame-sealed under vacuum and placed in a pre-heated oil-bath at 80°C for 24 h. The HBPO-PCB copolymers can be generated by hydrolysis of the HBPO-PCBB in DI water and the final product was obtained by lyophilization. The feed ratio of CBB to the initiation site of macro-CTAs is controlled at 10, 20, 40, and 80 for HP-1 to HP-4, respectively. ^{1}H NMR (HP-4, D_2O) δ (ppm): 0.8-1.1 (3H, -$CH_2C(CH_3)(C)COO$-), 1.80-2.1 (2H, -$C(CH_3)_2CH_2$-), 2.55-2.82 (6H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 3.07-3.28 (6H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 3.65-3.74 (2H, -$CH_2N(CH_3)_2C(CH_3)_2$-), 4.15-4.39 (2H, -$CH_2CH_2N(CH_3)_2C(CH_3)_2$-).

Stability Study

To evaluate the stability and protein resistant properties of the resultant biomimetic hyperbranched copolymers, the HP-3 and HP-4 were mixed in 100% fetal bovine serum (FBS). The particle size was continuously monitored by DLS. Tests in serum were conducted at 37°C to mimic physiological conditions.

Drug Loading and Release

The indomethacin (IND) was loaded in HBPO-PCB copolymer according to the published procedure (see Supporting Information) (24). The release experiment was conducted in 10 wt% FBS in phosphate buffer solution (PBS) at pH 7.0 and in PBS at pH 6.0, 7.0, and 8.0. The temperature was maintained at 37°C. IND-loaded biomimetic hyperbranched polymers (IHP-1 to IHP-4) (50 mg) was dissolved in 5 ml PBS then the solution was put into a dialysis bag (MWCO 3.5 kDa) and immersed in a beaker containing 200 ml PBS. At predetermined time intervals, 5 ml of liquid was sampled from the outer solution, and then replaced with the same volume of release medium. The drug concentration was detected by measuring UV–vis absorbance at 320 nm.

Characterization

The ^{1}H NMR spectra were recorded on an AVANCE AV 400MHz Digital FT-NMR spectrometer operating at 400 MHz using deuterated DMSO-d6 and D_2O as the solvent. The sizes and morphologies of the resultant samples were characterized by JSM-2100 transmission electron microscopy (TEM) at an accelerating voltage of 200 kV, whereby a small drop of sample solution was deposited onto a carbon-coated copper EM grid (200 mesh) and dried at room temperature at atmospheric pressure. Dynamic light scattering (DLS) measurements were performed in aqueous solution using a HORIBA Zetasizer apparatus (LB-550 V) equipped with a 5.0 mW laserdiode operating at 650 nm at room temperature.

Results and Discussion

Synthesis of HBPO-PCB

The synthesis procedure of biomimetic HBPO-PCB is shown in Scheme 1. The terminal hydroxyl groups HBPO was reacted with DMP by esterification to produce the HBPO-DMP as the RAFT macro-CTAs. Then, the star copolymers of HBPO-PCBB were synthesized through a RAFT path by using CBB as monomer and AIBN as co-initiator. The resulting HBPO-PCBB was further hydrolysis in DI water to generate biomimetic HBPO-PCB (HP-1 to HP-4). The molecular weight (M_n) and yields of these copolymers were collected in Table 1.

The structure and composition of synthesized copolymers were investigated by ^{1}H NMR. As shown in Fig. 1, comparing the ^{1}H NMR spectra of HBPO and HP-4, a new peak at 2.20–2.30 ppm from methyl of (N-CH_3) groups can be observed, which indicates that HP-4 copolymer is successfully synthesized. The compositions and thermal properties of the resultant HBPO-PCBs were also studied by Fourier transform infrared (FT-IR) and thermogravimetric analysis (TGA) (see Supporting Information). The ^{1}H NMR measurements was further conducted to confirm the core–shell structure of the biomimetic HBPO-PCB copolymers (see Supporting Information).

TEM and DLS were further applied to investigate the morphology of the resultant biomimetic HBPO-PCBs. It is well-known that the star amphiphilic copolymers with hydrophilic arms will self-assemble into large compound micelles (25, 26). Figure 2 shows the TEM images of the HP-4 micelles in PBS at pH 7.0, which indicates that the self-assembled micelles are well-dispersed with spherical shape and the diameter of the HP-4 micelles is around 70 nm. For the DLS measurement, as shown in Fig. 3, the size of the HP-4 micelles is 201.5 ± 1.9 nm. The average micelle diameter determined by DLS is larger than that determined by TEM. This discrepancy is widely considered to be induced by the process of sample preparation and the difference of investigation method between DLS and TEM (27). In addition, DLS is sensitive to the hydrodynamic diameter that includes the highly solvated PCB chains.

Stability and Protein Resistant Properties of HBPO-PCB Micelles

The zwitterionic PCB is a new class of biomimetic materials which have been developed for protein resistant against complex media such as undiluted blood serum or plasma (10–13). As shown in Fig. 3, the hydrodynamic diameter of HP-4 in

Table 1. Structure parameters of HBPO-PCB copolymers and assemblies.

Copolymer	R_{feed}[a]	*Mn* (g/mol)[b]	Yield (%)	*D* (nm)[c]
HP-1	10	15245	75.8	373.1 ± 4.7
HP-2	20	23718	78.2	272.4 ± 2.9
HP-3	40	44975	80.7	252.1 ± 4.5
HP-4	80	56358	85.4	201.5 ± 1.9

[a] R_{feed} is the feed ratio of CBB to the initiation site of macro-CTAs.
[b] M_n values were determined by ^{1}H NMR spectra (see Supporting Information).
[c] *D* values were determined by DLS at pH 7.0 with a polymer concentration of 1 mg/mL (see Supporting Information).

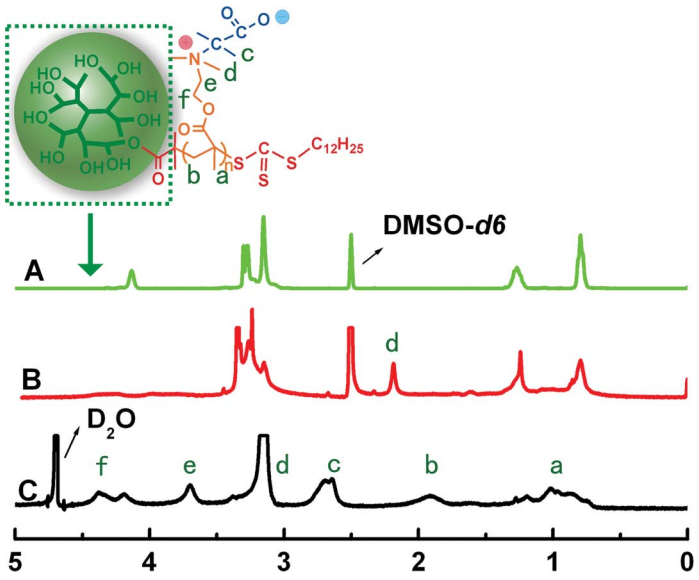


Fig. 1. ^{1}H NMR spectra of HBPO (A), HP-4 in DMSO-d_6 (B), and HP-4 in D_2O (C) (color figure available online).

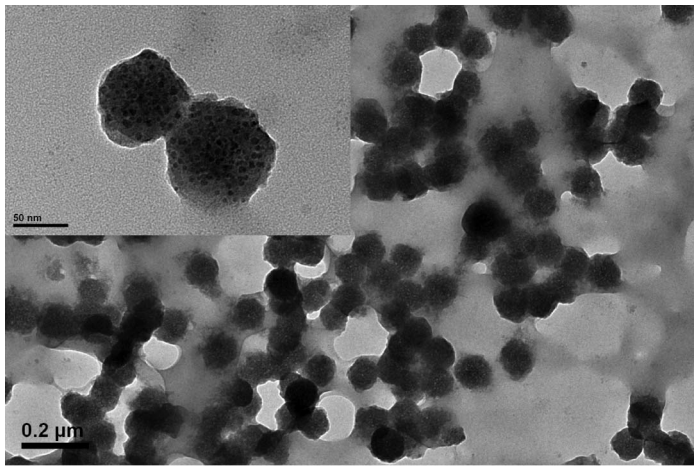


Fig. 2. TEM images of the HP-4 micelles in PBS at pH 7.0 with the concentration of 1 mg/mL.

PBS solution is much larger than that in 10 wt% FBS solution, changed from 201.5 ± 1.9 nm to 32.8 ± 1.7 nm. The distinct difference can be attributed to the protein resistant properties of the zwitterionic shell of the biomimetic HBPO-PCB. As shown in Scheme 2, when the protein is added to the solution of HP-4, the zwitterionic PCB shell of the HP-4 dehydrated and collapsed onto the surface of the micelles which lead to the distinct decrease of the hydrodynamic diameter. This phenomenon can also be confirmed by TEM, as show in Fig. 3, the size of HP-4 micelles changed from about 70 nm to 40 nm when 10 wt% FBS was added into the solution of the HP-4. The stability of HBPO-PCB in the complex medium was investigated (Fig. 4) by measuring the size change of the micelles as a function of time in 100% FBS. Both HP-3 and HP-4 micelles almost retained their original sizes after a 30 h incubation in 100% FBS indicating the excellent stability and ultra-low fouling ability of the biomimetic HBPO-PCB micelles in complex media.

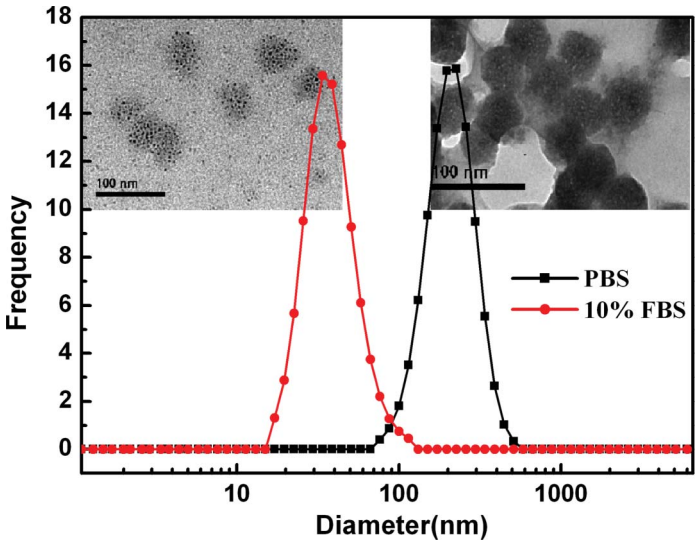
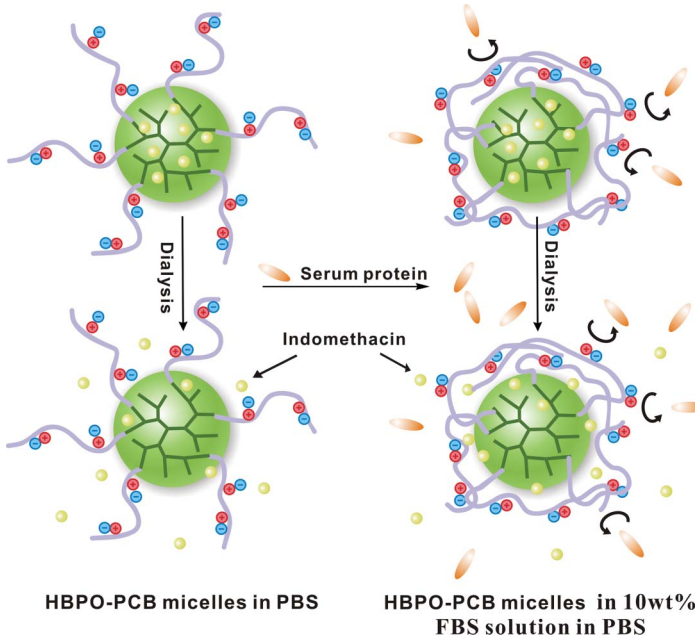


Fig. 3. DLS graphs and TEM images of the micelle size distribution of HP-4 in PBS and in 10% FBS at pH 7.0 (color figure available online).



Scheme 2. The illustration of the protein resistant and drug release properties of the HBPO-PCB (color figure available online).

Therefore, the biomimetic PCB-modified HBPO could evade the innate immune system and have long in vivo circulation half-life when it is applied in vivo drug delivery system (12). The potential toxicity of the biomimetic hyperbranched polymers was evaluated by the MTT method. The result of MTT showed the low toxicity and good compatibility of the biomimetic hyperbranched polymers to cells (see Support Information). Furthermore, the biomolecules (folate amine) can be conjugated to the surface of the biomimetic HBPO-PCB easily through DCC/NHS chemistry which make the HBPO-PCB candidate carriers for biomedical applications (see Support Information).

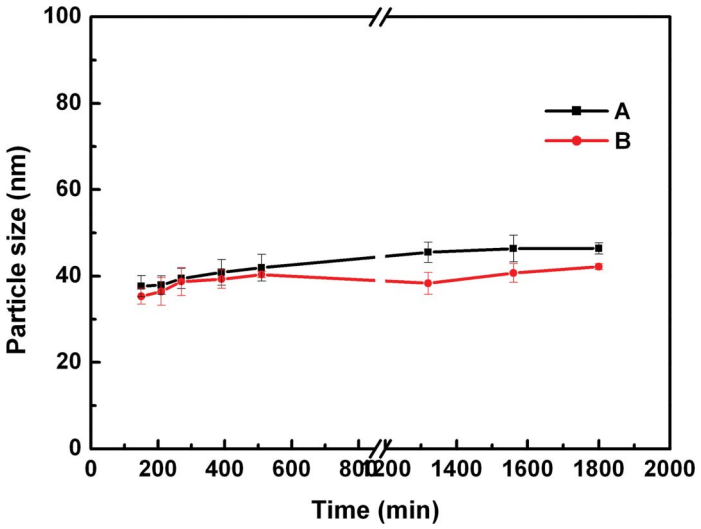


Fig. 4. Stability of HP-3 (A) and HP-4 (B) micelles in 100% FBS at 37°C as a function of time (color figure available online).

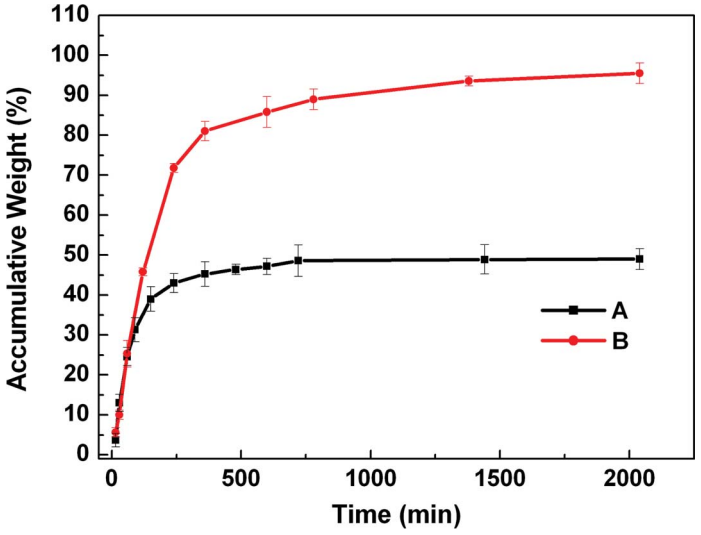


Fig. 5. Release of the IND from HP-4 micelles in 10 wt% FBS at pH 7.0 (A) and in PBS at pH 7.0 (B) (color figure available online).

In Vitro Drug Release

The HP-4 is selected to investigate the release properties of the biomimetic hyperbranched copolymers. The drug content in the HP-4 micelles is about 22.68% (w/w) determined by UV-vis spectrometry (see Supporting Information). Because of the protein resistant properties of the biomimetic HBPO-PCB copolymers, the release of the loaded IND from HP-4 micelles was also influenced by the concentration of protein in the solution. As shown in Fig. 5, when the release experiments were conducted in 10% FBS and PBS at pH 7.0, both of the curves show an initial burst release stage which is thought to be the release of drugs that are packed in the corona of micelles. After the initial burst release stage, the release rate for IND from the HP-4 in 10% FBS is much slower than that in PBS, and when the cumulative release of IND reached about 45%, there is almost no more IND release from the HP-4 micelles in 10% FBS. This phenomenon should be attributed to the conformational transition of PCB block from an expanding shape to a compact coil in accordance with the variation of the surrounding protein concentration. As illustrated in Scheme 2, when the protein is added into the solution of IHP-4, the biomimetic PCB shell of the HP-4 dehydrated and collapsed onto the surface of the micelles leading to slow down or even stop the release of the IND from the inner core of HBPO. Moreover, the IND-loaded micelles also showed pH-dependent release properties which were introduced by the abundant of the pH sensitive carboxyl groups of the biomimetic PCB shells (28, 29) (see Support Information).

Conclusions

A novel zwitterionic monomer was synthesized facilely and introduced to the surface of the HBPO to prepare biomimetic hyperbranched copolymers. The synthesized biomimetic hyperbranched copolymers showed low toxicity, good compatibility and favorable protein resistant property. The drug release rate depends greatly on the pH and protein concentration of the solution which indicated the copolymer is a candidate as a functional material for biomedical applications. Furthermore, because of the abundant of carboxyl group in PCB shells the resultant biomimetic hyperbranched copolymers can be modified by biomolecules easily. The investigation of targeted delivery of pharmaceuticals to folate-receptor-positive cancer cells is in progress.

Acknowledgments

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