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Acetals moiety contained pH-sensitive amphiphilic copolymer self-assembly used for drug carrier

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ABSTRACT

pH-sensitive nanoparticles were prepared from a novel amphiphilic copolymer poly(2-phenyl-1, 3-dioxan-5-yl methacrylate-*co*-2-hydroxyethyl acrylate), poly(PDM-*co*-HEA), which was synthesized from the pH-sensitive hydrophobic monomer 2-phenyl-1, 3-dioxan-5-yl methacrylate (PDM) and the hydrophilic monomer 2-hydroxyethyl acrylate (HEA) with unit ratio (4:6) via radical polymerization. The random amphiphilic polymer could form nanoparticles in aqueous media with sizes of about 167 nm (PDI = 0.03). The morphology of the nanoparticles was determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM). When the nanoparticles solution was adjusted to pH = 5.5, sizes of the nanoparticles increased from 167 nm to about 800 nm within 24 h, characterized by DLS. The critical aggregation concentration (CAC) of the copolymer was determined to be 5.3 mg/L (1.7×10^{-7} M). The insoluble Nile Red could be delivered into the Hep3B cells by the nanoparticles and released in cytoplasm determined by fluorescence microscopy.

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1. Introduction

During the past decades, amphiphilic polymers have attracted considerable attention due to their special self-assembly ability to form nano-scale aggregates, which have wide applications such as encapsulation for controlled release of drugs and enzymes, fillers, pigments, catalysts, adsorption materials and so on [1–4]. Significant progress has been made in the design and synthesis of variety amphiphilic polymers. However, most studies have focused on the self-assembly of well-defined copolymers such as amphiphilic block copolymers [5–8]. Only a few reports appeared in literature did concern the self-assembly of amphiphilic random copolymers which can be easily prepared and have potential applications in industry and biomedicine [9–13].

Amphiphilic copolymers responsive to stimuli have emerged as one of the most promising nano-carrier systems. In aqueous media, these polymers can self-assemble into various supramolecular structures and thus provide interiors able to noncovalently encapsulate guest molecules [14–16]; besides, the release of guest molecules can be triggered by external stimuli, such as pH [17–22], glutathione [23], enzyme [24], temperature [25] and so on. Since it is mildly acidic in tumor and inflammatory tissues as well as in the intracellular compartments such as endosomes and lysosomes [26], for the purpose of controlled release of drugs therein, it is more attractive to develop acid responsive drug carriers.

It is reasonable to insert cleavable linkers into amphiphilic copolymers to obtain acid-sensitive nanoparticles. So nanoparticles can be formed through self-assembly of copolymers and destroyed via cleavage of acid-sensitive bonds in acidic environment. So far, acid-labile covalent bonds such as hydrazone [27,28], orthoester [29] and acetal [30,31] were positioned into the main chain, side chain, or at the terminal of the core-forming blocks. For instance, Caixia Ding [32] and co-workers prepared acid-sensitive micelles by conjugating alkyl (C_{18}) to PEG via a novel benzoic-imine linker, which was assembled into pH-responsive micelles, used for drug delivery. Heller and co-workers [33] have prepared acid-sensitive micelles based on block copolymers of poly(ortho ester) and poly(ethylene glycol) (PEG), in which degradation of the poly(ortho ester) block under acidic conditions led to the release of loaded anticancer drugs. PH-sensitive micelle/nanoparticle used for drug carriers could release the drug molecules under endosomal pH, achieving high antitumor activity.

Many functional polymers as drug carriers have been prepared by physical methods from the natural macromolecules, which usually have much broad size distribution [34,35]. The polymer microspheres with uniform size are essential for drug delivery system (DDS) because the distribution of the microspheres in the body and the interaction with biological cells are greatly affected by the particle size [36,37]. Additionally, if monodisperse polymer microspheres are available, the drug release kinetics can be

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manipulated, therefore making it easier to formulate more sophisticated intelligent DDS.

In this paper, we synthesized a novel amphiphilic pH-sensitive copolymer poly(PDM-*co*-HEA), which could self-assemble into nanoparticles with a rather narrow PDI, and the sizes of nanoparticles increased from nano-scale to almost micro-scale in acidic environment, which was a very important property for drug release. Nile Red was employed as hydrophobic and fluorescence molecules to mimic the drug delivery and release.

2. Experimental section

2.1. Materials

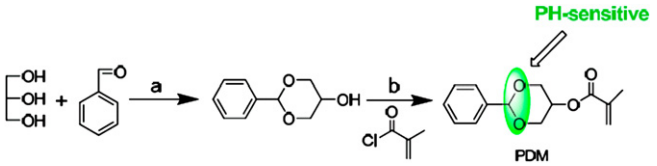
Benzaldehyde, hydroxyethylacrylate (HEA), glycerol, *p*-toulenesulfonic acid, triethylamine were all purchased from Shanghai chemical reagent Co. Ltd. as analytical regents and used without further purification. Phosphotungstic acid and Nile Red were purchased from Aldrich. Methacryloyl chloride was produced by Haimen Best Fine Chemical Industry Co. Ltd. and used after redistillation. Tetrahydrofuran (THF), cyclohexanone and *N*,*N*-dimethyl formamide (DMF) were purified by reduced pressure distillation. AIBN (98%) was recrystallized from ethanol. Other reagents such as methanol, ether, toluene, potassium carbonate (K_2CO_3) and magnesium sulfate were commercially available and used as received.

2.2. Instruments and methods

^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer, using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard at ambient temperature. Molecular weights (M_n) and polydispersity (M_w/M_n) relative to PMMA were measured on a gel permeation chromatography (GPC) utilizing Waters 515 pump and differential refractometer. DMF was used as a mobile phase at a flow rate of 1.0 mL/min. Conversions for monomer and polymer were determined by gravimetry. Room temperature emission and excitation spectra were carried out using Edinburgh-920 fluorescence spectra photometer. Melting point determinations were performed on a microscopic (20 × 10) melting point apparatus and were uncorrected. TEM observations were conducted on a Philips CM120 electron microscope at an acceleration voltage of 100 kV. The sample for TEM observations was prepared by placing a drop of nanoparticle solutions at a concentration of 0.2 mg/mL on copper grids, which were coated with thin films of Formvar and carbon successively. The size of nanoparticles was determined using dynamic light scattering (DLS). Measurements were carried out at 25 °C using the Zetasizer Nana-ZS from Malvern Instruments equipped with a 633 nm He–Ne laser using back-scattering detection. The single crystal X-ray diffraction data were recorded on a Rigaku Mercury CCD X-ray diffractometer. Steady-state fluorescence spectra were recorded with a FLS920 spectrofluorometer (Edinburgh Co., UK) with a slit of 1 nm for both excitation and emission. The excitation wavelength was 335 nm, and pyrene was used as the probe. The intensity ratio of the third band to the first band of the pyrene emission spectrum (I_3/I_1) was used to indicate the polarity of the pyrene environment.

2.3. Monomer synthesis

The monomer PDM was prepared by reaction of methacryloyl chloride with glycerol and benzaldehyde (Scheme 1), according to the modified procedure of previous literature [38].



Scheme 1. The synthetic route of PDM.

2.3.1. Preparation of 5-hydroxy-1, 3-benzylideneglycerol

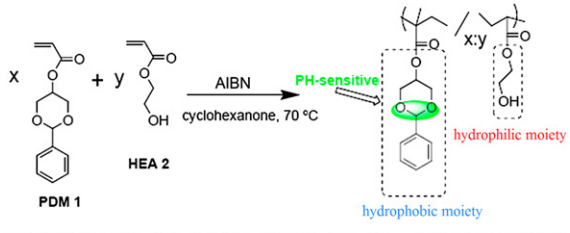
Benzaldehyde (106 g, 1 mol), glycerol (92 g, 1 mol), 200 mL of toluene, and 1 g of *p*-toluenesulfonic acid were placed into a 500 mL flask that was fitted with an oil–water separator. The mixture was refluxed with vigorous stirring for 12 h. The condensation product was diluted with half its volume of ether and then well shaken with 1% solution of potassium carbonate (K_2CO_3) (150 mL × 3) to remove the acid catalyst and any remaining glycerol. The organic layers were combined and dried over anhydrous magnesium sulfate, filtered and another 250 mL of ether were added. The mixture was then placed in a refrigerator overnight to obtain title compound. The crude product was recrystallized from ether to obtain a product of white, silky needles (yield: 31%); m.p. 80–81 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.08 (d, J = 11.23 Hz, 1H), 3.65 (d, J = 10.91 Hz, 1H), 4.17 (q, J = 11.87, 11.87, 11.66 Hz, 4H), 5.57 (s, 1H), 7.40 (d, J = 6.82 Hz, 3H), 7.51 (d, J = 6.17 Hz, 2H). Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.25; H, 6.66. TOF-MS (EI): calcd for $C_{10}H_{12}O_3$: 180.0786, found: 180.0787.

2.3.2. Preparation of 2-phenyl-1, 3-dioxan-5-yl methacrylate (PDM)

5-Hydroxy-1, 3-benzylideneglycerol (23.4 g, 0.1 mol) and triethylamine (10.1 g, 0.1 mol) were dissolved in 100 mL anhydrous tetrahydrofuran (THF) and cooled to 0 °C in a water–ice bath. Methacryloyl chloride (10.45 g, 0.1 mol) was added dropwisely. After the addition, the mixture was reacted at room temperature for another 12 h. The reaction mixture was filtered, concentrated, and purified by recrystallized from ethyl alcohol. White crystal was obtained (yield: 30%); m.p. 66–67 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.01 (s, 3H), 4.20 (d, J = 12.5 Hz, 2 H), 4.33 (d, J = 12.6 Hz, 2H), 4.77 (s, 1H), 5.58 (s, 1H), 5.65 (s, 1H), 6.29 (s, 1H), 7.38 (d, J = 6.6 Hz, 3H), 7.51 (d, J = 6.3 Hz, 2H). Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.10; H, 6.49. TOF-MS (EI): calcd for $C_{14}H_{16}O_4$: 248.1049, found: 248.1046.

2.4. Amphiphilic polymer synthesis

The copolymer poly(PDM-*co*-HEA) was prepared by reaction between 2-phenyl-1, 3-dioxan-5-yl methacrylate (PDM) 1 and Hydroxyethylacrylate (HEA) 2, according to the procedure shown in Scheme 2.



Scheme 2. The synthetic route of poly(PDM-*co*-HEA).

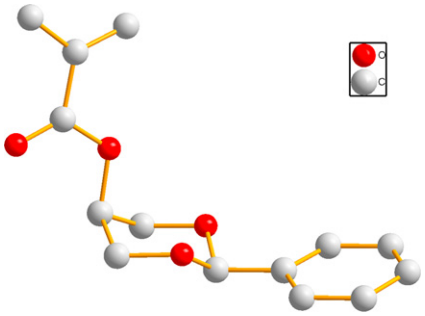


Fig. 1. The single crystal structure of cis-PDM.

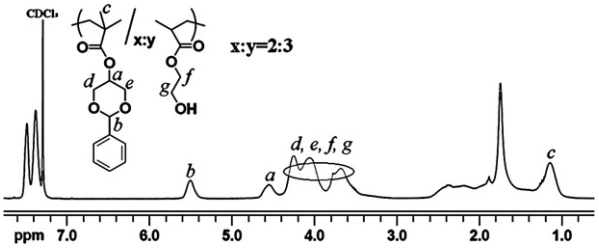


Fig. 2. ^{1}H NMR spectrum of polymer 3b in $CDCl_3$.

2.4.1. Synthesis of amphiphilic polymer poly(PDM-co-HEA) 3

Polymer 3 was synthesized by copolymerization of 1 and 2 with different feed ratios. A typical procedure for synthesizing polymer 3 with the feed ratio of 4:6 was described as below. To a solution of monomer 1 (297.6 mg, 1.2 mmol) and monomer 2 (208.8 mg, 1.8 mmol) in cyclohexanone (2.0 mL), AIBN (4.92 mg, 3.0 mol% to total amount of monomers) was added. The resulting solution was degassed by three freeze–evacuate–thaw cycles and heated at 70 °C for 3.5 h under argon. The mixture was cooled and diluted by THF, and then poured into a large amount of cooled diethyl ether (100 mL). The precipitate was filtered and dried under vacuum at ambient temperature for 24 h to obtain the polymer 3 (yield: 99%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.0–1.3 ($-CH_3$); 1.7–2.5 ($-CH_2-C(CH_3)-COO-$) and ($-CH_2-CH-COO-$); 3.5–4.4 ($-COO-CH_2-CH_2-$) and ($-O-CH(CH_2O-)_2-$); 4.5–4.65 ($-O-CH(CH_2O-)_2-$); 5.4–5.6 ($(-CH_2O)_2-CH-ph$); 7.3–7.56 ($-C_5H_6$).

2.5. Nanoparticle formation

Typically, the nanoparticle solution of the copolymer 3b was prepared by dropwise addition of 10 mL deionized water to copolymer 3b solution in THF (2 mg/mL) at room temperature, followed by stirring overnight to evaporate THF completely. The final concentration of the copolymer 3b was 0.2 mg/mL.

2.6. Encapsulation and release of Nile Red

Nile Red was loaded into micelles 3b by adding 20 μL of 1 mM Nile Red in acetone to 0.4 mL of copolymer solution in THF (5 mg/mL), followed by dropwise addition of 10 mL phosphate buffer (10 mM, pH = 7.4). To completely evaporate THF and acetone, the solution was stirred overnight and then vacuumed for 1 h. So the micelles have a concentration of 0.2 mg/mL and contain 2.0 μM Nile Red. The solution was divided into three 3 mL samples and the fluorescence intensity of each was measured as 100% intensity. Then the samples were adjusted to pH 4.0, 5.0, and 7.4, respectively, by addition of 75 μL of 0.4 M pH 4.0 and 5.0 acetate buffer or 7.4 phosphate buffer, while keeping the salt concentration the same. The samples were stirred at 37 °C and the fluorescence intensity was measured at the desired time points.

Table 1
Results of the Polymerization of poly(PDM-*co*-HEA).

Run	Initial feed ratio (*x*:*y*)	Polymer (unit ratio [*x*:*y*])[a]	M_n(M_w/M_n)[b]	Conversion [%][c]	Yield [%][d]
3a	2:8	3a (2:8)	42,200 (2.0)	98	99
3b	4:6	3b (4.2:5.8)	30,500 (2.1)	99	98
3c	6:4	3c (6.1:3.9)	72,000 (3.0)	97	96
3d	8:2	3d (7.9:2.1)	20,500 (2.3)	99	96

[a] Determined by ^{1}H NMR (400 MHz) spectrum.
[b] Estimated by estimated by GPC (DMF) using PMMA standard.
[c] Methanol-insoluble parts.
[d] Diethyl ether-insoluble parts.

2.7. Cell culture and fluorescence microscopic characterization

Hep3B cell, a human hepatocellular liver carcinoma cells line, was purchased from Hu'nan Wanbai Biotechnology Development Center, Changsha Province, China, and used for the cell uptake studies. The cells were cultured in complete Dulbecco's modified Eagles medium (DMEM, containing 10% Hyclone fetal bovine serum, 50 units ml^{-1} penicillin, and 50 units ml^{-1} streptomycin) at 37 °C and 5% CO_2 atmosphere.

The cellular uptake and intracellular release behaviors of Nile Red loaded 3b micelles were followed with fluorescence microscopy (OLYMPUS IX 51) using human hepatocellular liver carcinoma cells line (Hep3B). After hep3B cells were cultured in a disc to ~70% confluency (~8×10^4 cells/disc), 100 mL of PB solution of Nile Red loaded micelles (10 mg Nile Red per disc) was added. After incubation for 0.5 or 2 h, the culture medium was removed and the cells were rinsed two times with DMEM prior to the fluorescence assessment.

2.8. In vitro cytotoxicity of drug carrier poly(PDM-co-HEA) 3b

The sulforhodamine B (SRB) assay [39] is used to assess the cytotoxicity of drug carriers in different concentrations. Well-growing hepatoma Hep3B cells were placed in 96-well plates (8×10^3 cells per well). Six duplicate wells were set up in each sample. When the cells anchored to the plates, the culture medium was replaced with the medium containing various concentrations of the drug carrier (0, 7.844, 15.69, 31.38, 62.75, 125.5, 251 μg/mL)

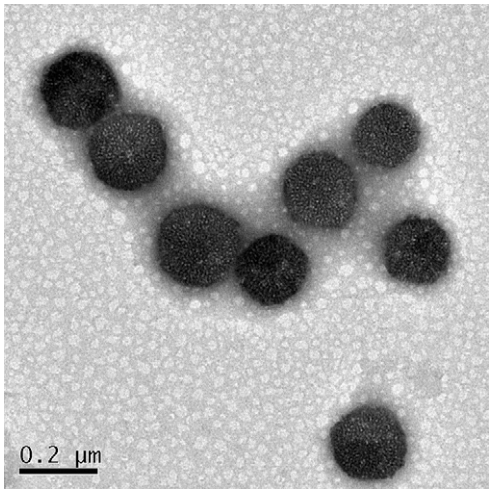


Fig. 3. TEM image of the nanoparticles formed by amphiphilic copolymer 3b.

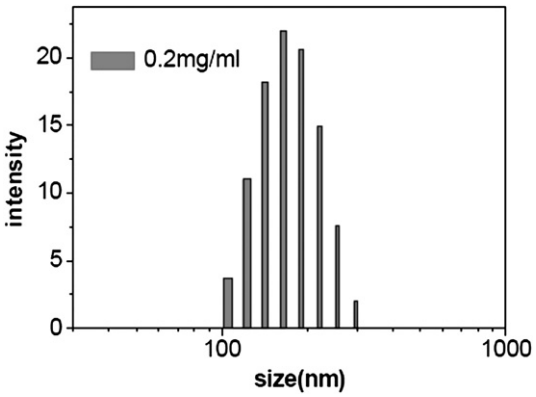


Fig. 4. Size distribution profile for nanoparticles of copolymer 3b measured by DLS.

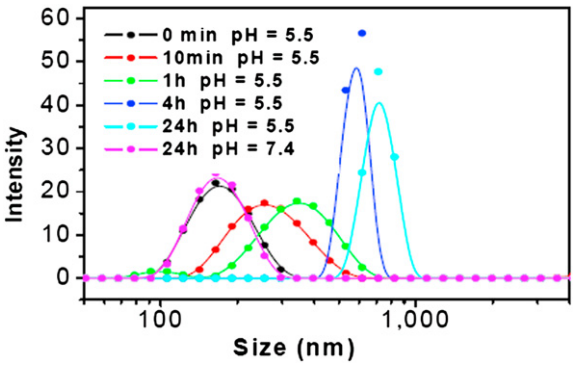


Fig. 6. Time dependence of nanoparticle size for copolymer 3b in pH = 5.5 and 7.4 measured by DLS.

and cultured at 37 °C in a humidified incubator (5% CO_2 in air, v/v). After grown for 24 h, the medium was discarded and 100 μL of 10% trichloroacetic acid in Hank's balanced salt solution was added and held at 4 °C for 1 h. Then, the stationary liquid was poured away; the cells were washed with deionized water for several times and stained with 100 μL of 0.4% SRB solution for 30 min. Following the remove of SRB, the cells were washed three times with 0.1% acetic acid solution. The SRB dye was extracted with 150 μL of 10 mmol/L tris (hydroxymethyl)-aminomethane solution (pH = 10.5). The absorbance at 531 nm was measured with a spectrophotometer.

3. Results and discussion

3.1. Monomer synthesis

According to the synthetic route of Scheme 1, we synthesized the monomer (PDM) containing an acetal, which was a pH-responsive moiety. The momomer (PDM) has two configurational isomers, herein we obtained a single crystal, and the single crystal structure of PDM was characterized by X-ray diffraction. Finally we found the configuration of the compound PDM synthesized in our laboratory was cis-PDM (Fig. 1).

3.2. Design and synthesis of amphiphilic copolymer

Four copolymers were synthesized with different feed ratios 3a–d (2:8, 4:6, 6:4 and 8:2). According to the ^{1}H NMR spectrum of polymer 3b, the hydrophilic and hydrophobic content could be calculated, which was coincided with the feed ratio of the two monomers (Table 1). The signals assignable to the methenyl in the acetals were at 5.5 and 4.5 ppm; the chemical shift of 3.4–4.4 ppm was ascribed to the methylene of acetals and HEA (Fig. 2). So the hydrophilic and hydrophobic content of the copolymer could be conveniently tuned through the adjustment of the monomers feed ratio.

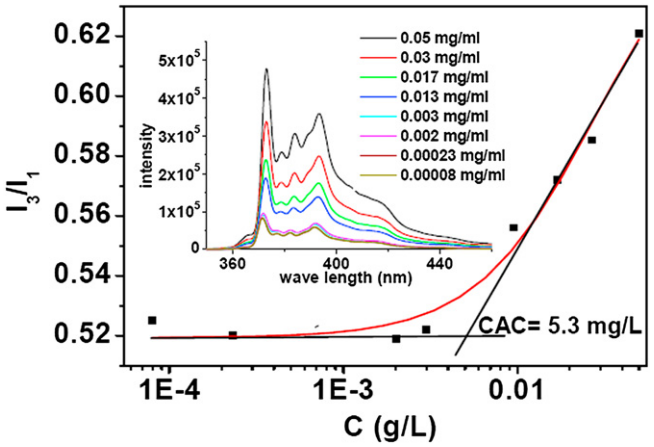


Fig. 5. Intensity ratios (I_3/I_1) obtained from the fluorescence emission spectra of pyrene (inset) plotted versus the concentrations of copolymer 3b at pH = 7.4 and 25 °C.

3.3. Nanoparticle formation and behavior of the amphiphilic copolymer 3b

The micellar characteristics of copolymer 3b in aqueous solution were investigated using a fluorescence technique, TEM, and DLS [40]. Fig. 3 shows a TEM image of the copolymer 3b nanoparticles, and samples were stained with 0.1 wt% phosphotungstic acid before measurements. It can be seen that the nanoparticles are spherical in shape with an average diameter of about 170 nm. The particle size and size distribution of the nanoparticles were investigated further by DLS (Fig. 4). DLS results show that the nanoparticles have an average diameter of 163 nm, with a narrow size distribution (PDI = 0.03), which are consistent with the TEM results. Interestingly, although the copolymer 3b was random rather than block amphiphilic copolymer, it could be self-assembled to nanoparticles well.

The CAC of copolymer 3b was determined by a fluorescence technique using pyrene as a fluorescence probe [41]. Fig. 5 shows the intensity ratios (I_3/I_1) obtained from the fluorescence emission spectra of pyrene (inset) plotted versus the concentrations of copolymer 3b (M_n = 30 500, M_w/M_n = 2.1) at pH = 7.4 and 25 °C. I_3/I_1 was fluorescent emission intensity ratio of third band (383 nm, I_3) and first band (372 nm, I_1) of pyrene in aqueous media containing amphiphilic polymer 3b. At a certain concentration, the intensity

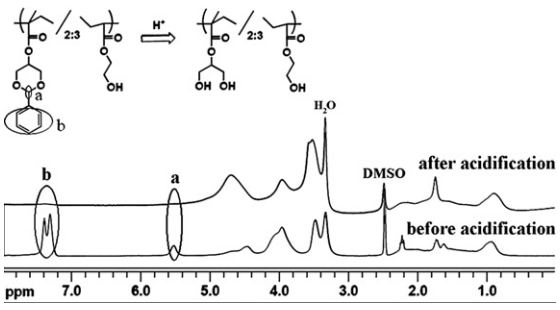


Fig. 7. ^{1}H NMR of copolymer 3b before acidification and after acidification.

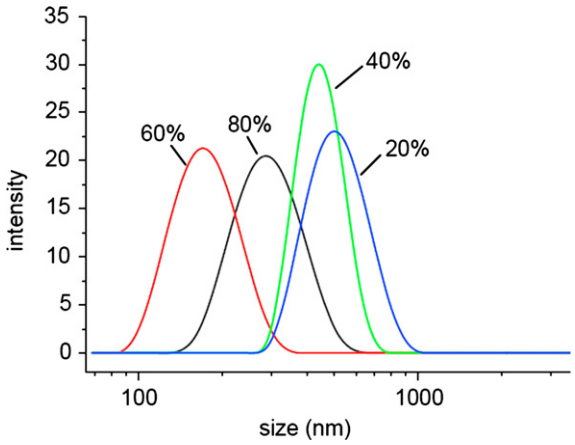


Fig. 8. Size distribution of nanoparticles in different composition (the molar ratio of HEA in polymer chain as 20%, 40%, 60% and 80%).

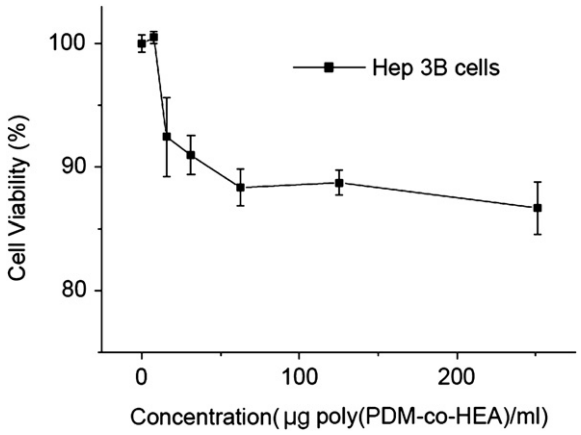


Fig. 10. In vitro cell viabilities, measured by the SRB assay, after culture of Hep3B cells with amphiphilic poly(PDM-*co*-HEA) as functions of different concentrations (0, 7.844, 15.69, 31.38, 62.75, 125.5, 251 μg/mL).

ratio started to increase dramatically. This increase reflects the incorporation of pyrene into the hydrophobic cores of the nanoparticles. The CAC was determined from the crossover point in the low concentration range. The CAC of the copolymer 3b was 5.3 mg/L, which is not only strong evidence to the formation of nanoparticles but also an important parameter in evaluating the stability of the nanoparticles in the blood post administration, owing to the low CAC value. The CAC/CMC of polymeric nanoparticles/micelles is typically of the order of 10^{-6}–10^{-7} M, while that of low molecular weight surfactant micelles is of the order of 10^{-3}–10^{-4} M [42]. Herein, the CAC of nanoparticle 3b was calculated to be 1.7×10^{-7} M (CAC = 5.3 mg/L), which is less prone to dissociation at low concentrations.

Amphiphilic polymer with a high CAC value seems to be unsuitable for drug delivery because the nanoparticles may be dissociated after being administered into the body due to the dilution effect. Here we have obtained the amphiphilic copolymer with very low CAC value (CAC = 5.3 mg/L) and this nanoparticle could be used for potential drug carrier.

3.4. *pH-responsive nanoparticle of amphiphilic copolymer 3b*

To determinate whether the nanoparticles of copolymer 3b were sensitive to acid, one drop of dilute HCl was added to the nanoparticles, the pH was adjusted to about 5.5, which was consistent to the mildly acidic environment of endosomes and lysosomes (pH = 5.5–6.5) [32]. Rapid and remarkable swelling of nanoparticles instead of nanoparticle disruption was observed according to DLS result (Fig. 6). The nanoparticle size increased from 167 nm to 255 nm within 10 min, and then reached about 800 nm within 24 h. In contrast, no change of nanoparticle size was observed over 1 day at pH = 7.4. The reason for this phenomenon could be ascribed to the cleavage of hydrophobic cyclic acetals moieties of the polymer. Namely, the acid-sensitive cyclic acetal functionality could be cleaved under mildly acidic conditions and transferred into hydrophilic dihydroxypropyl methacrylate unit, which was highly hydrophilic but not soluble in water, together with releasing of benzaldehyde. Environmentally responsive polymer capsules ranging in the size from nanometers to microns have been interesting for the material scientists due to their potential and practical utilization in controlled release [43].

To further explain the phenomenon of the nanoparticles swelling, and to make sure that the acetal has been hydrolyzed, copolymer 3b, which was set in mildly acidic solution for 1 h, was characterized via ^{1}H NMR. Compared to ^{1}H NMR of copolymer 3b before acidification (Fig. 7), the specific chemical shifts attributing to the protons of methenyl group (a) and phenyl group (b) in the

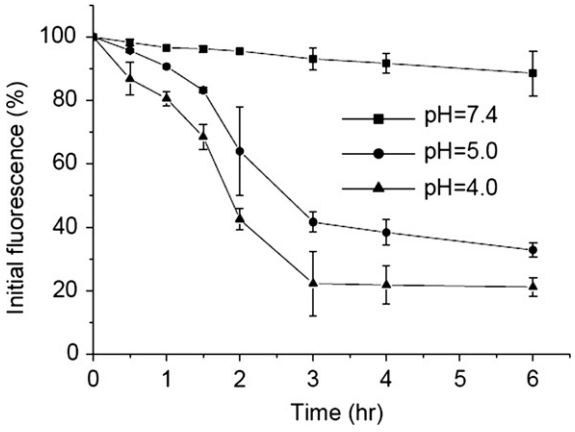


Fig. 9. Nile Red fluorescence in micelles 3b as a function of time at different pH at 37 °C.

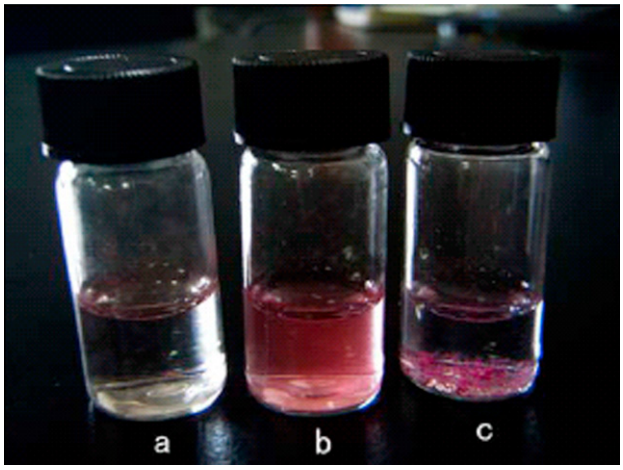


Fig. 11. The pictures of Nile Red in different conditions (a). Nile Red in aqueous solution; (b). Nile Red in nanoparticles; (c). 15 min after drops of sodium acetate buffer at pH = 4 in nanoparticles.

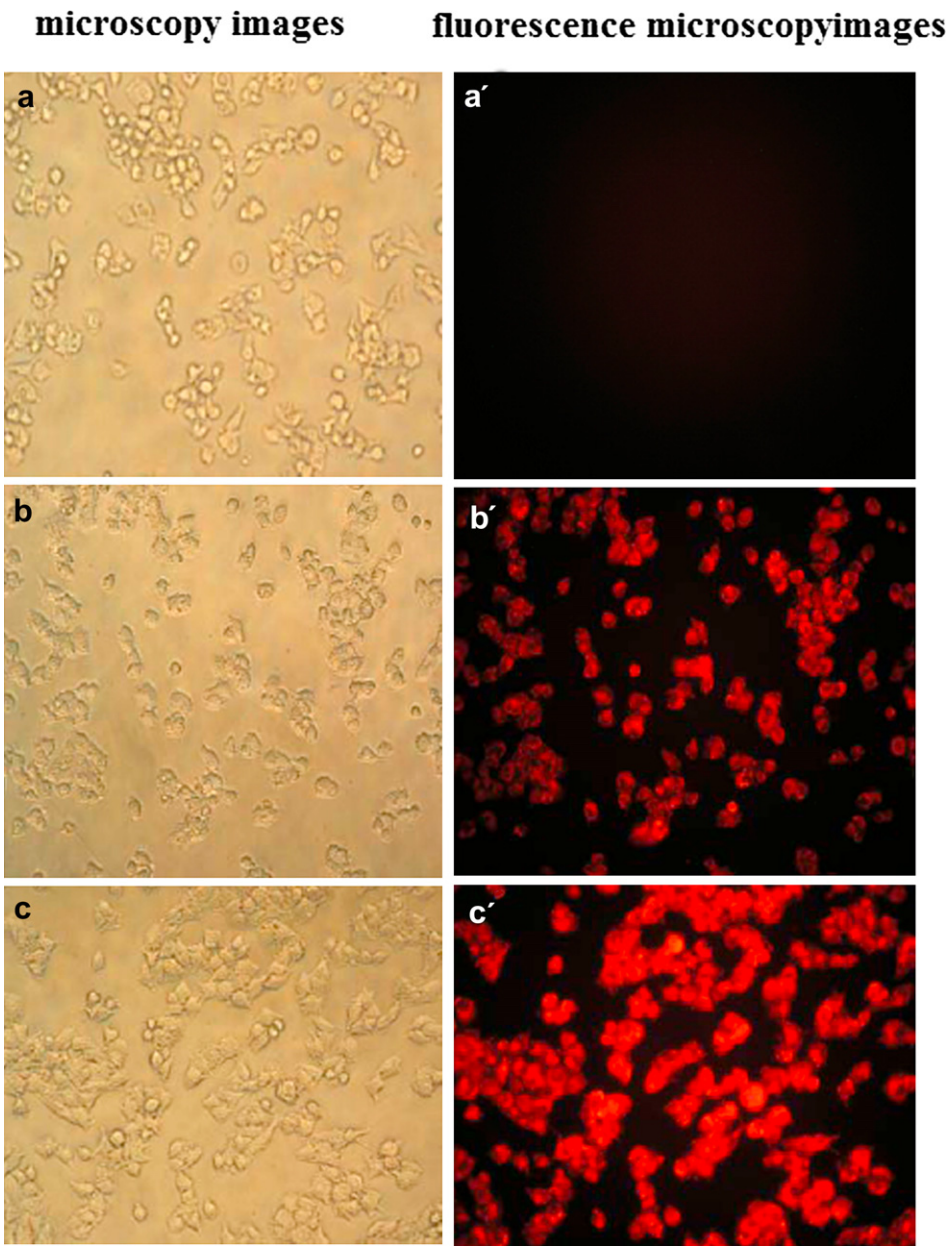


Fig. 12. Fluorescence microscopy images of Hep3B cells being treated with free Nile Red and Nile Red-loaded 3b nanoparticle with different incubation time at pH = 7.4 (a). a′-free Nile Red, 0.5 h; (b). b′-Nile Red-loaded 3b nanoparticle, 0.5 h; (c). c′-Nile Red-loaded 3b nanoparticle, 2.0 h.

polymer chain were disappeared after acidification. So it could conclude that remarkable swelling of nanoparticles under mildly acidic conditions was due to the hydrolysis of the acetals.

3.5. Size distribution of the nanoparticles in different chemical composition

We have prepared the micelles from the amphiphilic copolymers with different chemical composition of PDM and HEA (2:8, 4:6, 6:4 and 8:2). As shown in Fig. 8, the result of DLS shows that the size of self-aggregates decreased from 510 to 270 nm as the hydrophilic ratio increased from 20 to 80%, indicating formation of more dense hydrophobic cores, which is consistent with the papers reported by Ick Chan Kwon etc before [44,45]. However when the hydrophilic ratio is 60%, the copolymer can self-assemble to nanoparticles with the size of 167 nm. As for this phenomenon, the copolymer with hydrophilic ratio of 60% may be the right chemical composition to self-assemble to nanoparticles used for drug carrier. So we choose copolymer 3b as potential biomaterials.

3.6. Encapsulation and release of Nile Red in vitro

The encapsulation and release of Nile Red from micelles 3b were investigated at 37 °C at different pHs (i.e., pH 4.0, 5.0, and 7.4). The release of Nile Red was followed by measurement of fluorescence and the results showed clearly a pH-dependent release behavior from micelles 3b, in which the fluorescence of Nile Red decreased almost 70 and 80% at pH 5.0 and 4.0, respectively, within 6 h, while little change was observed for the sample at pH 7.4 (Fig. 9).

3.7. In vitro cytotoxicity of the PDM-co-HEA nanoparticles

Hep3B cells were used to assess the cytotoxicity of poly(PDM-*co*-HEA). We expected little cytotoxicity because the copolymer was synthesized from biocompatible and nontoxic monomers. In fact, the cells cultured after 24 h in the presence of copolymer with different concentration retained high cell viability (above 85%) in SRB assays even at high concentrations (350 μg/mL) (Fig. 10). This result suggests that amphiphilic poly(PDM-*co*-HEA) may be safe drug carriers in vivo.

3.8. Encapsulation of Nile Red by the nanoparticles and in vitro cellular uptake by Hep3B cells

To assess whether the nanoparticles of amphiphilic copolymer 3b could carry and controllably release drugs, Nile Red, as guest molecules, was encapsulated by the nanoparticles solution of 3b (0.2 mg/mL, above the CAC). According to Fig. 11, the hydrophobic Nile Red, insoluble in water (Fig. 11-a), could be solubilized by amphiphilic copolymers in aqueous media into a homogeneous state (Fig. 11-b). When drops of sodium acetate buffer solution were added into the solution (pH = 4), encapsulated Nile Red was deposited from the solution within 15 min (Fig. 11-c). So polymer 3b was a promising candidate for nanoparticle carrier of hydrophobic drugs to passively target cancer tissue by EPR effect [46,47] and controllably release drugs triggered by changing of pH values.

To mimic the in vitro drug controllable delivery and release behavior of polymer 3b nanoparticles, Nile Red was also used as guest molecules and Hep3B cell as target cell. By using fluorescence microscopy, the process of drug (Nile Red) delivery and release with assistance of nanoparticles could be observed. The result was shown in Fig. 12-a′ showed that the free Nile Red in water with very low solubility, so there was little Nile Red in solution interacting with the cells, resulting in fewer uptakes and almost no fluorescent image. In contrast, the Nile Red could be solubilized in the nanoparticles at the concentration of 20 μg/mL, strong Nile Red fluorescence was observed in the cells after 0.5 h incubation with Nile Red loaded 3b micelles (Fig. 12-b′). It has been reported that polymeric micelles can be rapidly uptaken by the cells via endocytosis [48]. After a longer incubation time of 2 h, Nile Red fluorescence intensity became stronger, indicating Nile Red escaped from the micelles (Fig. 12-c′). It has been shown that acetals moiety could be rapidly cleaved in the intracellular compartment caused in comparatively acidic condition, such as endosomes and lysosomes of cells and based on this intriguing phenomenon, intracellular delivery systems for drugs and DNA have successfully been developed [21,49].

4. Conclusion

We have successfully developed pH-responsive nanoparticles based on amphiphilic copolymers comprising of a novel acid-labile cyclic acetal as hydrophobic moiety. The copolymer could undergo self-association in aqueous media to form its nanoparticles, with a narrow PDI. The sizes of nanoparticles increased with the prolonging of the time in acidic aqueous solution due to the hydrolysis of the acid-labile acetals. CAC of the copolymer was 5.3 mg/L (1.7×10^{-7} M), which was rather low, and the nanoparticles can solubilize hydrophobic guest molecules in dilute solution. Hydrophobic Nile Red could be solubilized, controllably delivered and released into liver cancer cell Hep3B. So the present pH-sensitive nanoparticles can be used as a potential smart drug carrier for cancer therapy.

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