Journal of Controlled Release 142 (2010) 40–46

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

pH-Sensitive degradable polymersomes for triggered release of anticancer drugs: A comparative study with micelles

Wei Chen, Fenghua Meng *, Ru Cheng, Zhiyuan Zhong *

Biomedical Polymers Laboratory and Jiangsu Key Laboratory of Organic Chemistry, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China

ARTICLE INFO

Article history:
Received 22 June 2009
Accepted 24 September 2009
Available online 3 October 2009

Keywords:
Degradation
Polymersome
Micelle
pH-sensitive
Drug delivery
Anticancer drugs

ABSTRACT

pH-Sensitive degradable polymersomes and micelles were prepared based on diblock copolymer of poly (ethylene glycol) (PEG) and an acid-labile polycarbonate, poly(2,4,6-trimethoxybenzylidenepentaerythritol carbonate) (PTMBPEC). Polymersomes of PEG(1.9k)–PTMBPEC(6k) revealed average sizes of 100–200 nm. The acetals of polymersomes, similar to those of PEG(5k)–PTMBPEC(5.8k) micelles, though stable at pH 7.4 were prone to fast hydrolysis at mildly acidic pH of 4.0 and 5.0, with half lives of 0.5 and 3 d, respectively. The acetal hydrolysis resulted in significant size increase of polymersomes, to over 1000 nm in 24 h at pH 4.0. Drug encapsulation studies revealed that polymersomes were able to simultaneously load paclitaxel (PTX, hydrophobic) and doxorubicin hydrochloride (DOX·HCl, hydrophilic), whereas micelles loaded PTX only. Notably, polymersomes showed lower drug loading efficiencies for PTX than micelles (30.0–37.7% versus 61.4–65.2%). The *in vitro* release studies demonstrated that release of PTX and DOX·HCl from polymersomes was highly pH-dependent, *i.e.* significantly faster drug release at mildly acidic pH of 4.0 and 5.0 compared to physiological pH. Furthermore, much higher release rates were observed for PTX release from the polymersomes compared to that from the micelles under otherwise the same conditions. These pH-sensitive nano-sized degradable polymersomes hold great promise for combination therapy for cancers.

1. Introduction

In the past decade, polymersomes [1,2] have attracted rapidly growing interest due to their intriguing aggregation phenomena, cell and virus-mimicking dimensions and functions, as well as tremendous potential applications in medicine, pharmacy, and biotechnology [3–9]. As for liposomes, polymersomes have aqueous cores that are separated from the outside medium by the hydrophobic membranes, and can be used to deliver both hydrophilic and hydrophobic species such as anti-cancer drugs, therapeutic proteins and diagnostic probes. Unlike liposomes self-assembled from low molecular weight lipids, polymersomes are formed from macromolecular amphiphiles of various architectures including amphiphilic diblock [1,10,11], triblock [12,13], graft [14,15] and dendritic [16] copolymers. As a result, polymersomes usually display much higher stability as compared to liposomes.

Recently, stimuli-sensitive polymersomes have emerged as novel programmable delivery systems in which the release of drugs can be readily modulated by exerting an appropriate stimulus (e.g. temperature, pH, glucose, glutathione, etc.) [7,8,17]. The stimuli-responsive release may result in significantly enhanced therapeutic efficacy and minimal side effects. Among all applied stimuli, acidic pH as an internal stimulus is particularly appealing due to the mildly acidic pH encountered in tumor and inflammatory tissues as well as in the intracellular compartments such as endosomes and lysosomes of cells [18]. The existing tumoral pH variation has been considered as an ideal trigger for the selective release of anticancer drugs in tumor tissues and/or within tumor cells, accomplishing tumor-targeted drug delivery. For example, Armes and coworkers reported pH-sensitive polymersomes based on poly(2-(methacryloyloxy) ethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) for controlled release of DOX [19] and for DNA delivery [20]. Hubbell and coworkers reported that polymersomes based on PEG–PVP dissolve quickly when pH drops from 7.4 to 5.5 [21]. Deming and coworkers developed pH sensitive polymersomes based on polypeptides [22]. These polymersomes are, nevertheless, not readily degradable.

For drug delivery applications, usually degradable polymersomes are desired. Feijen and coworkers prepared biodegradable polymersomes from block copolymers based on poly(ethylene glycol) (PEG) and biodegradable polyesters or polycarbonates [11]. These biodegradable polymersomes were investigated as a basis for artificial cells for drug encapsulation and release [3]. Lee and coworkers reported biodegradable polymersomes of poly(2-hydroxyethyl aspartamide) grafted with lactic acid oligomers [15]. Discher and coworkers found that biodegradable polymersomes of PEG–PLA and PEG–PCL are self-porating, leading to controlled release of DOX [23]. Biodegradable polymersomes loaded with both PTX and DOX were reported to

* Corresponding authors. Tel./fax: +86 512 65880098.
E-mail addresses: fhmeng@suda.edu.cn (F. Meng), zyzhong@suda.edu.cn (Z. Zhong).

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doi:10.1016/j.jconrel.2009.09.023

permeate and shrink tumor [24]. Recently, degradable polymersomes were applied as non-viral carriers for siRNA and antisense oligonucleotides [25], and mouse-anti-rat monoclonal antibody OX26 conjugated PEG–PCL polymersomes was investigated for peptide brain delivery [26]. It is worthy to note that none of these degradable polymersomes possess stimuli-sensitivity.

In this paper, we report on novel pH-sensitive nano-sized degradable polymersomes for triggered release of both hydrophilic and hydrophobic anticancer drugs (Scheme 1). These polymersomes were based on diblock copolymer of PEG and an acid-labile polycarbonate containing trimethoxybenzylidene acetals at the sides (PTMBPEC). Acid-labile acetals have been exploited by Frechet and coworkers for the development of rapidly pH-sensitive micelles, nanoparticles and hydrogels [27–33], and by Bulmus and coworkers for acid-labile polymeric core–shell particles and core-crosslinked micelles [34]. Trimethoxybenzylidene acetals are of particular interest because they show high sensitivity to mildly acidic pH and micelles containing trimethoxybenzylidene acetals exhibit low cytotoxicity [27–33]. Recently, we reported pH-responsive degradable micelles based on PEG–PTMBPEC for triggered release of hydrophobic anticancer drugs [35]. Interestingly, pH-sensitive degradable polymersomes developed in this study were able to release PTX and DOX·HCl in a controlled and pH-dependent manner, in which significantly faster drug release was observed at mildly acidic pH of 4.0 and 5.0 compared to physiological pH. Furthermore, release of PTX from polymersomes revealed a higher sensitivity towards mildly acidic pH compared to micelles. These pH-sensitive nano-sized degradable polymersomes hold great promise for combination therapy for cancers.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol) (PEG, $M_n = 1900$ or 5000, Fluka) was dried by azeotropic distillation from anhydrous toluene. Dichloromethane (DCM) were dried under an argon atmosphere by refluxing over CaH_2 and distilled prior to use. Zinc bis[bis[trimethylsilyl]amide] (97%, Aldrich) was used as received. Doxorubicin hydrochloride (DOX·HCl) and paclitaxel (PTX) were obtained from Beijing Zhongshuo Pharmaceutical Technology Development Co. Ltd., and used as received. Mono-2,4,6-trimethoxybenzylidene-pentaerythritol carbonate (TMBPEC) was synthesized according to a previously reported procedure [35].

2.2. Synthesis of PEG–PTMBPEC diblock copolymers

PEG–PTMBPEC block copolymers were prepared by ring-opening polymerization of TMBPEC in DCM at 50 °C using PEG ($M_n = 1900$ or 5000) as an initiator and zinc bis[bis[trimethylsilyl]amide] as a catalyst, as reported previously. Typically, in a glove-box under a nitrogen atmosphere, to a solution of PEG ($M_n = 1900$, 0.2 g, 0.1 mmol) and TMBPEC (1.0 g, 2.94 mmol) in DCM (10 mL) was quickly added zinc bis[bis[trimethylsilyl]amide] (18 mg, 0.05 mmol). The reaction vessel was sealed and the polymerization was allowed to proceed at 50 °C under stirring for 8 d. The resulting PEG–PTMBPEC copolymer was isolated by twice precipitation from cold diethylether and dried in vacuo at r.t. Monomer conversion: 56%. ^{1}H NMR showed an M_n of 1900–6000. Similarly, using PEG 5000 as an initiator, PEG–PTMBPEC diblock copolymer with an M_n of 5000–5800 was prepared.

2.3. Preparation of polymersomes and micelles

The polymersomes and micelles were prepared by dropwise adding 4.0 mL of phosphate buffer (10 mM, pH 7.4) into 0.2 mL of dioxane solution containing 0.5 wt.% PEG–PTMBPEC block copolymer followed by dialysis overnight against phosphate buffer (10 mM, pH 7.4) with a molecular weight cut-off (MWCO) of 3500 at r.t. The final concentration of polymersomes and micelles was ca. 0.2 mg/mL.

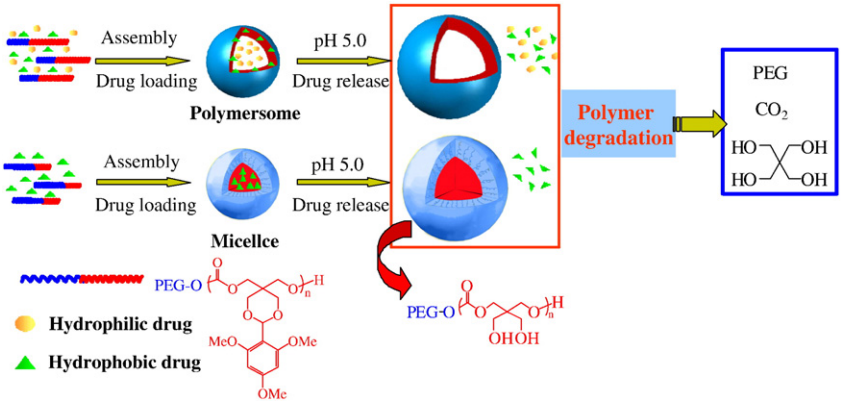
The critical aggregation concentration (CAC) was determined by using pyrene as a fluorescence probe. The concentration of block copolymer was varied from 2.0×10^{-5} to 0.2 mg/mL and the concentration of pyrene was fixed at 1.0 μM. The fluorescence spectra were recorded using FLS920 fluorescence spectrometer with the excitation wavelength of 330 nm. The emission fluorescence at 372 and 383 nm were monitored. The CMC was estimated as the cross-point when extrapolating the intensity ratio I_{372}/I_{383} at low and high concentration regions.

2.4. Determination of the pH-dependent hydrolysis rate of acetals in the polymersomes and micelles

The acetal hydrolysis was followed by UV/vis spectroscopy by measuring the absorbance at 290 nm, according to the previous reports by Frechet and coworkers. The polymersomes and micelles solutions (0.1 wt.%) were prepared and divided into three aliquots (2 mL). Their pH was adjusted to 4.0, 5.0 and 7.4, respectively, by addition of 50 μL of 4.0 M pH 4.0 and 5.0 acetate buffer or pH 7.4 phosphate buffer. The solutions were shaken at 37 °C. At desired time intervals, 80 μL aliquot was taken out and diluted with 3.5 mL phosphate buffer (0.1 M, pH 7.4). The absorbance at 290 nm was monitored.

2.5. Encapsulation and release of PTX

PTX was loaded into polymersomes and micelles by dropwise adding 8 mL of phosphate buffer (10 mM, pH 7.4) to 1 mL of dioxane



Scheme 1. Illustration of pH-sensitive degradable polymersomes based on PEG–PTMBPEC diblock copolymer for triggered release of both hydrophilic and hydrophobic anticancer drugs. In comparison, pH-sensitive degradable micelles are typically applied for release of hydrophobic drugs only.

solution of block copolymer (5 mg) and 100 or 200 μL of PTX solution in DMSO (0.5 wt.%), sonicating for 1 h, and dialysis against phosphate buffer (10 mM, pH 7.4) with a MWCO of 3500 at r.t. The final concentration of polymersomes and micelles was ca. 0.5 mg/mL. 0.2 mL of polymersome or micelle solution was freeze-dried, the residue was dissolved in acetonitrile, and the amount of PTX was determined by HPLC (Waters 1525) with UV detection at 227 nm using a 1/1 (v/v) mixture of acetonitrile and water as a mobile phase.

Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

$$\text{DLC(wt.\%)} = (\text{weight of loaded drug} / \text{weight of polymer}) \times 100\%$$

$$\text{DLE(\%)} = (\text{weight of loaded drug} / \text{weight of drug in feed}) \times 100\%.$$

The release profiles of PTX from polymersomes and micelles were studied at 37 °C in three different media, i.e. (a) acetate buffer, pH 4.0; (b) acetate buffer, pH 5.0; and (c) phosphate buffer, pH 7.4. The concentrations of the release media were 0.1 M. The above prepared PTX-loaded polymersomes were divided into three aliquots (each 1 mL). Their pH was adjusted to 4.0 or 5.0 using acetate buffer and to pH 7.4 using phosphate buffer, and immediately transferred to a dialysis tube with a MWCO of 12,000–14,000. The dialysis tube was immersed into 20 mL corresponding buffer (0.1 M) and the media was stirred at 37 °C. At desired time intervals, 5 mL of release media was taken out for HPLC measurement and replenished with an equal volume of fresh media. The amount of PTX was determined by HPLC (Waters 1525) with UV detection at 227 nm using a 1/1 (v/v) mixture of acetonitrile and water as a mobile phase. The release experiments were conducted in triplicate. The results presented are the average data.

2.6. Encapsulation and release of DOX·HCl

DOX·HCl was loaded into polymersomes by dropwise adding 3 mL of ammonium sulfate solution (10 mM, pH 4.5) to 375 μL of 0.8 wt.% copolymer solution dissolved in dioxane, adjusting its pH to 8.0 with sodium hydroxide solution (0.1 M). The solution was divided into three samples with the same volume, adding 20, 40 or 60 μL of DOX·HCl solution in water (0.5 wt.%, to result in theoretical DLC of 10, 20 and 30% respectively), sonicating at 50 °C for 5 h in dark, and dialysis against phosphate buffer (10 mM, pH 7.4) with a MWCO of 3500 to remove the organic solvent and free DOX·HCl if present. The final polymersome concentration was ca. 0.5 mg/mL.

The release studies were carried out in a similar way as for PTX except that the whole procedure was performed in the dark and the amount of DOX·HCl was determined using fluorescence (FLS920) measurement (excitation at 600 nm). For determination of DLC, DOX·HCl-loaded polymersomes were dissolved in DMSO and analyzed with fluorescence spectroscopy, wherein calibration curve was obtained with DOX·HCl/DMSO solutions with different DOX·HCl concentrations. To determine the amount of DOX·HCl released, calibration curves were run with DOX·HCl in corresponding buffer solutions at pH 4.0, 5.0 and 7.4, respectively. The emission spectra were recorded from 500 to 680 nm.

2.7. Simultaneous encapsulation and release of PTX and DOX·HCl from polymersomes

PTX and DOX·HCl were loaded into polymersomes by dropwise adding 6 mL of citrate buffer (0.1 mM, pH 5.2) to a mixture of 1 mL of copolymer solution in dioxane (0.5 wt.%) and 0.2 mL of PTX solution in DMSO (0.5 wt.%), adjusting its pH to 7.4 with phosphate buffer (4 M, pH 7.4), adding 200 μL of DOX·HCl solution in water (0.5 wt.%), sonicating at 40 °C for 5 h in the dark, and dialysis against phosphate buffer (10 mM, pH 7.4) with a MWCO of 3500 to remove the organic solvent and free DOX·HCl and PTX if present. The final polymersomes concentration was approximately 0.5 mg/mL. The release studies were carried out in a similar way as for DOX·HCl. The release samples obtained at different time intervals were divided into two aliquots, one was taken for HPLC measurements to determine the amount of PTX and the other was taken for fluorescence analysis to determine the amount of DOX·HCl.

3. Results and discussion

3.1. Design and synthesis of PEG–PTMBPEC diblock copolymers

Whether an amphiphilic block copolymer can self-assemble into polymersomes is determined mainly by the weight fraction of its hydrophilic block (f_{phil}), its molecular weight, and the effective interaction parameter of its hydrophobic block with H_2O (χ) [7]. For block copolymers with a high χ, vesicles are favored when f_{PEG} = 20–40%, whereas micelles are predominantly formed at f_{PEG} = 45–70% [36,37]. In this study, in order to obtain polymersomes and micelles with similar block length of pH-sensitive polycarbonate hydrophobe, two PEGs with different M_n of 1900 and 5000 were used. PEG–PTMBPEC diblock copolymers were readily prepared by ring-opening polymerization as previously reported [35]. ^{1}H NMR spectrum as well as signal assignments of PEG(1.9k)–PTMBPEC block copolymer is shown in Fig. S1 (Appendix A). The M_n of PTMBPEC block was calculated to be 6000 by comparing signals of PEG and PTMBPEC blocks (Table 1). This copolymer is denoted accordingly as PEG(1.9k)–PTMBPEC(6k). GPC measurement showed a unimodal distribution with a low polydispersity index (PDI) of 1.17 (Table 1), corroborating successful synthesis of PEG(1.9k)–PTMBPEC(6k) block copolymer. In a similar way, PEG(5k)–PTMBPEC(5.8k) with a PDI of 1.21 was prepared (Table 1). The weight fractions of PEG (f_{PEG}) in PEG(1.9k)–PTMBPEC(6k) and PEG(5k)–PTMBPEC(5.8k) copolymers are 24.1 and 46.3%, which correspond to compositions to yield polymersomes and micelles, respectively. These two copolymers have comparable PTMBPEC block lengths, facilitating comparisons between polymersomes and micelles with regard to degradation in response to acidic pH, drug loading efficiency, and pH-triggered drug release profiles.

Table 1
Synthesis of PEG–PTMBPEC diblock copolymers.[a]

Block copolymer	PEG/TMBPEC weight ratio in feed	Conv[b] %	M_n theory	M_n[c] ^{1}H NMR	PDI[d] GP	f_{PEG}[e] %	CAC[f] mg/L
PEG(1.9k)–PTMBPEC(6k)	1/5	56	1900–5600	1900–6000	1.17	24.1	0.40
PEG(5k)–PTMBPEC(5.8k)	1/2	52	5000–5200	5000–5800	1.21	46.3	0.25

[a] Polymerizations were carried out in CH_2Cl_2 at 50 °C for 8 d using methoxy PEG (M_n = 1900 or 5000) as an initiator and zinc bis[bis[trimethylsilyl]amide] as a catalyst. The monomer concentration is 0.3 M.
[b] Determined from ^{1}H NMR spectrum of the polymerization mixture.
[c] Calculated by ^{1}H NMR.
[d] Determined by GPC measurement.
[e] The weight fraction of PEG block.
[f] Critical aggregation concentration determined by fluorescence microscopy using pyrene as a probe.

3.2. Formation of polymersomes and cytotoxicity assessments

Polymersomes were conveniently prepared by dropwise adding phosphate buffer (10 mM, pH 7.4) into 0.2 mL of dioxane solution containing PEG(1.9k)–PTMBPEC(6k) block copolymer followed by dialysis overnight against phosphate buffer (MWCO 3500) at r.t. DLS analysis revealed that these polymersomes had average sizes of about 120 nm, which correlated well with that observed by TEM measurement (Fig. 1). Moreover, TEM micrographs showed clearly existence of hollow interiors, indicating formation of polymeric vesicles. To further confirm their vesicular structures, polymersomes were loaded with both hydrophilic (DOX·HCl) and hydrophobic (nile red) fluorescent molecules and then observed with CLSM. As shown in Fig. S2 (Appendix A), fluorescence of DOX·HCl and nile red co-localized and furthermore fluorescence area of DOX·HCl was slightly smaller than that of nile red, in line with vesicular structure containing a watery interior to encapsulate DOX·HCl and a hydrophobic membrane to encapsulate nile red. We found that polymersomes with average sizes ranging from 100 to 200 nm were formed at different polymer concentrations from 0.5 wt.% to 0.1 wt.%. In the following studies, polymer concentration was fixed at 0.5 wt.%. In comparison, micelles were prepared similarly from PEG(5k)–PTMBPEC(5.8k) block copolymer. DLS showed sizes of about 35 nm. As expected, CLSM revealed that these micelles were not able to load DOX·HCl. The critical aggregation concentration (CAC) was determined by fluorescence study using pyrene as a probe. The results showed that PEG(1.9k)–PTMBPEC(6k) and PEG(5k)–PTMBPEC(5.8k) block copolymers had CAC values of 0.40 and 0.25 mg/L, respectively (Table 1). The cytotoxicity of PEG(1.9k)–PTMBPEC(6k) polymersomes was studied by MTT assay using L929 fibroblasts and HeLa cells. The results revealed that these polymersomes had low cytotoxicity, as over 80% cells were viable at polymersome concentrations ranging from 20 μg/mL to 500 μg/mL (Fig. 2A). In a similar way, trimethoxybenzaldehyde (TMBA), which is a degradation product from acetal hydrolysis, was also shown to have low cytotoxicity up to a tested concentration of 200 μg/mL (Fig. 2B).

3.3. pH-Triggered hydrolysis of acetals in the polymersomes and micelles

The hydrolysis of polycarbonate acetals within polymersome membranes and micelle cores was investigated at three different pHs of 4.0, 5.0 and 7.4 at 37 °C. The extent of acetal hydrolysis was conveniently determined using UV/vis spectroscopy by monitoring the absorbance at 290 nm, which is the characteristic absorbance of

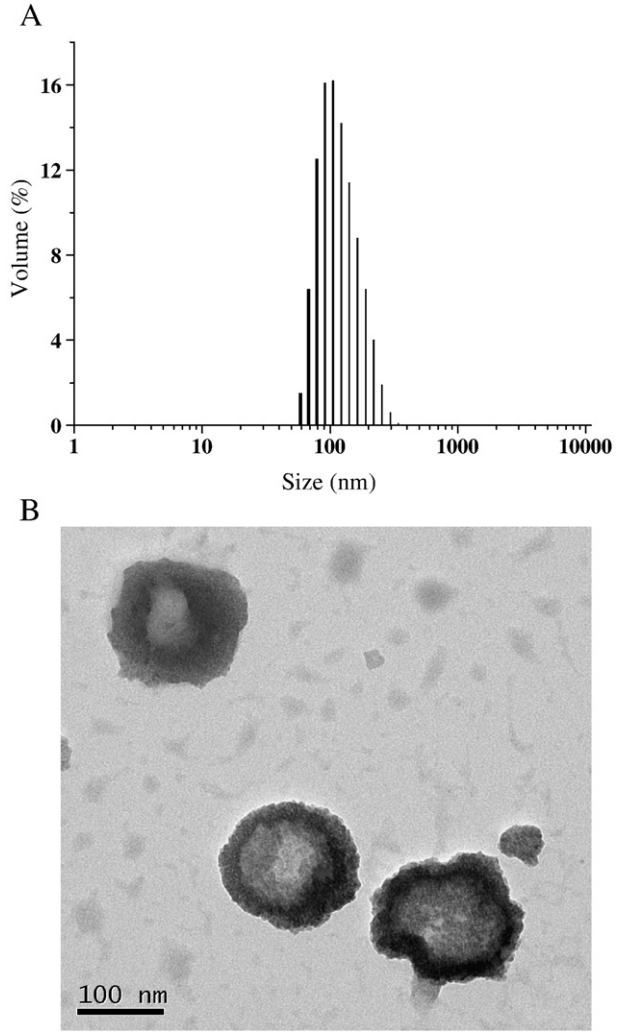


Fig. 1. Size distribution profiles for pH-sensitive degradable polymersomes (1 wt.% staining agent) determined by DLS (A) and TEM (B).

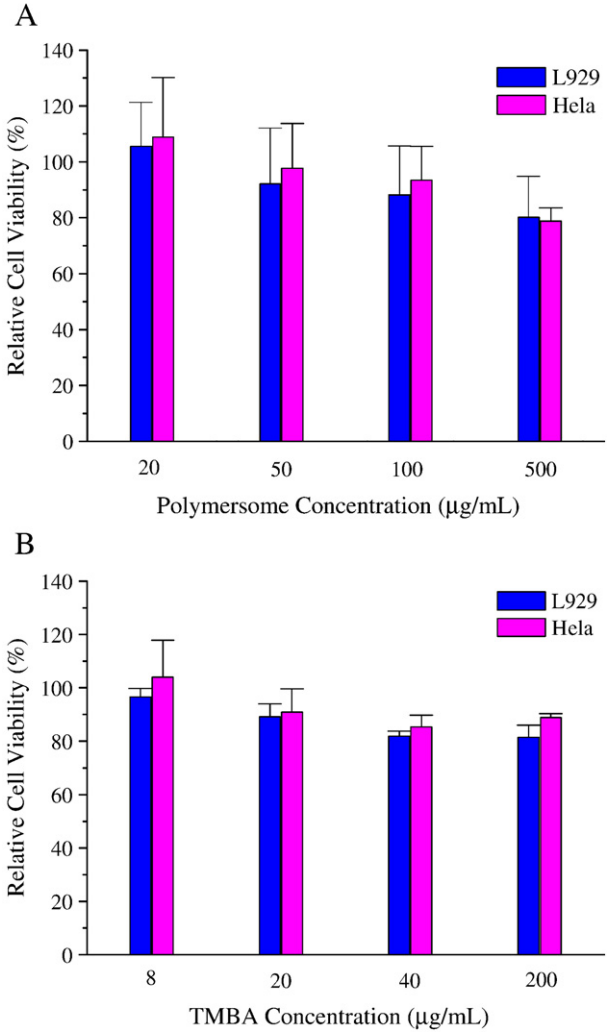


Fig. 2. Cytotoxicity of PEG(1.9k)–PTMBPEC(6k) polymersomes (A) and trimethoxybenzaldehyde (TMBA) (B). L929 fibroblasts and HeLa cells were incubated with either polymersomes or TMBA for 48 h. The cell viability was determined by MTT assay ($n=4$).

the hydrolysis products, 2,4,6-trimethoxybenzaldehyde [27,29]. The results showed that the hydrolysis rate of the acetals of polymersomes from PEG(1.9k)–PTMBPEC(6k) is highly pH dependent (Fig. 3A). While negligible hydrolysis was observed after 5 d at pH 7.4, rapid hydrolysis took place at pH 4.0 and 5.0, with half lives of approximately 12 h and 3 d, respectively. Interestingly, nearly identical rates of hydrolysis were observed for PEG(5k)–PTMBPEC (5.8k) micelles (Fig. 3B). It should be noted, however, that the rates of acetal hydrolysis are much lower compared to PEG(5k)–PTMBPEC micelles with shorter PTMBPEC block (3.7 kDa), in which half lives of about 1 and 6 h were reported at pH 4.0 and 5.0, respectively [35]. It appeared, therefore, that rates of acetal hydrolysis are critically dependent on the molecular weight of PTMBPEC block rather than on aggregation forms. The relatively slow hydrolysis of PEG(5k)–PTMBPEC(5.8k) micelles might be due to their better packing of PTMBPEC chains in the micellar core as a result of enhanced hydrophobic interactions.

The size change of polymersomes and micelles in response to acetal hydrolysis at acidic pH was followed by DLS measurements. Interestingly, placement of polymersomes into pH 4.0 acetate buffer (120 mM) resulted in rapid and remarkable swelling of polymersomes (Fig. S3A, Appendix A). The polymersome size increased from 120 nm to ca. 700 nm in 8 h, reaching over 1000 nm after 1 d. In contrast, no change of polymersome size was observed over 2 d at pH 7.4 at the same buffer concentration. Similar swelling phenomena were observed for PEG(5k)–PTMBPEC(5.8k) micelles (Fig. S3B, Appendix A). It has been reported previously that hydroxy polycarbonate derived from pentaerythritol is highly hydrophilic but not soluble in water [38], which could explain the maintenance of vesicular or micellar structures even after complete acetal hydrolysis.

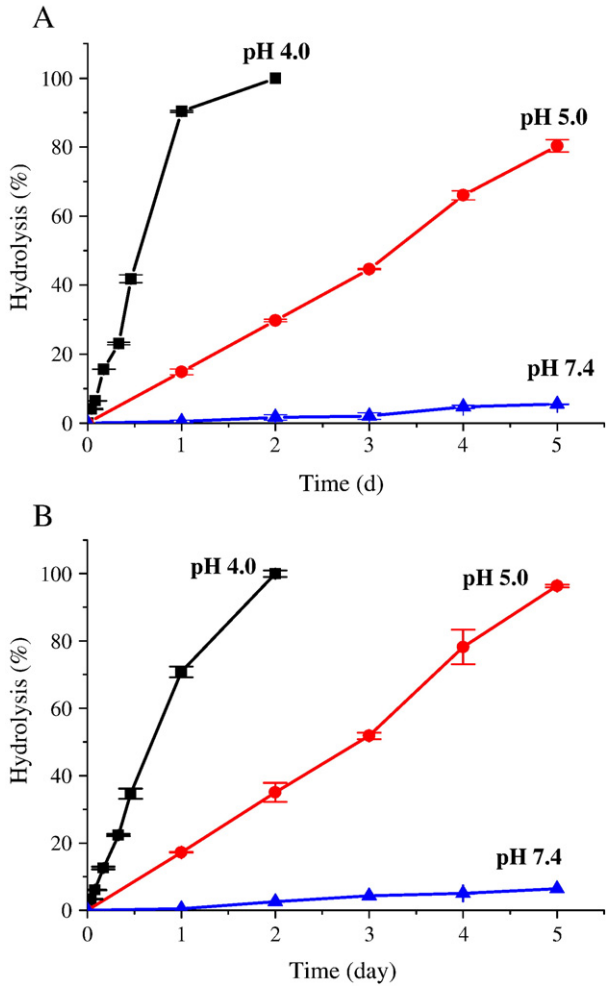


Fig. 3. pH-Dependent hydrolysis of acetals in the polymersomes (A) and micelles (B).

3.4. Encapsulation and pH-triggered release of PTX and DOX·HCl

Loading and release of both hydrophilic and hydrophobic anticancer drugs, DOX·HCl and PTX, into polymersomes was investigated. The theoretical drug loading contents were set at 10, 20 or 30 wt.%. The results showed that these polymersomes had drug loading efficiencies of 30.0–37.7% for PTX and 19.5–26.2% for DOX·HCl, respectively (Table 2). In consistence with CLSM experiments, PEG (5k)–PTMBPEC(5.8k) micelles were not able to load DOX·HCl. However, for hydrophobic drug PTX, micelles showed considerably higher drug loading efficiencies (61.4–65.2%) as compared to polymersomes (Table 2).

The release studies were carried out at 37 °C at three different pHs, i.e. (a) acetate buffer, pH 4.0; (b) acetate buffer, pH 5.0; and (c) phosphate buffer, pH 7.4. The results revealed a significantly faster release of PTX from polymersomes at mildly acidic pHs compared to physiological pH (Fig. 4A), in agreement with the acetal hydrolysis results. For example, within 1 d, approximately 80%, 45%, and 25% PTX were released from polymersomes at pH 4.0, 5.0 and 7.4, respectively. Release of PTX from PEG(5k)–PTMBPEC(5.8k) micelles appeared also pH-dependent (Fig. 4B). The rates of drug release were, nevertheless, much lower than those of polymersomes at otherwise the same conditions. For instance, within 1 d, only about 44%, 32% and 19% PTX were released from micelles at pH 4.0, 5.0 and 7.4, respectively. To achieve 80% cumulative release at pH 4.0, 4 d was required for micelles, whereas only 1 d for polymersomes. It has been shown that rates of acetal hydrolysis are similar for micelles and polymersomes (Fig. 3). The higher PTX release rates witnessed for the polymersomes compared to the micelles are most likely due to their higher diffusion rates resulting from comparatively shorter diffusion pathway than micelles. Release of DOX·HCl from polymersomes appeared also highly pH-dependent, in which approximately 65%, 45% and 32% DOX·HCl were released in 1 d at pH 4.0, 5.0 and 7.4, respectively (Fig. 4C). Notably, for both hydrophilic and hydrophobic drugs, no initial burst release from polymersomes was observed.

Finally, simultaneous loading and release of PTX and DOX·HCl from pH-sensitive degradable polymersomes was studied. Interestingly, both PTX and DOX·HCl were released in a pH-dependent manner (Fig. 5). The release rates of PTX were close to those for PTX-loaded polymersomes (Fig. 5A *versus* Fig. 4A). However, for DOX·HCl, significantly enhanced release was observed at pH 5.0 as compared to DOX·HCl-loaded polymersomes (Fig. 5B *versus* Fig. 4C). About 66%, 60% and 28% DOX·HCl were released in 12 h at pH 4.0, 5.0 and 7.4, respectively. In comparison, for DOX·HCl-loaded polymersomes after 12 h only 52% and 38% drug were released at pH 4.0 and 5.0, respectively (Fig. 4C). The much faster release of DOX·HCl from PTX/DOX·HCl-loaded polymersomes than from DOX·HCl-loaded polymersomes

Table 2
Drug loading content and drug loading efficiency for PTX and DOX·HCl using pH-sensitive degradable polymersomes and micelles.

Type of carrier	Anticancer drug	Theoretical drug loading content (wt.%)	Drug loading content[a] (wt.%)	Drug loading efficiency (%)
Polymersomes	PTX	10	3.0	30.0
		20	7.5	37.7
	DOX·HCl	10	2.14	21.4
		20	3.89	19.5
		30	7.85	26.2
Micelles	PTX	10	6.5	65.2
		20	12.3	61.4

[a] Drug loading content for PTX and DOX·HCl was determined by HPLC analysis and fluorescence measurement, respectively.

at pH 5.0 might be due to formation of channels or cavities in the vesicular membrane as a result of release of PTX, which facilitates penetration of DOX·HCl through the membrane.

These pH-sensitive degradable polymersomes capable of simultaneous release of both hydrophilic and hydrophobic drugs in response to mildly acidic pHs are particularly appealing in combination therapy for cancers. It has been reported that a combination of two anticancer drugs or a combination of chemotherapeutics with anti-angiogenic agents, proteins, or genes can act synergistically against cancer cells *in vitro* and tumors *in vivo* [24,39–45].

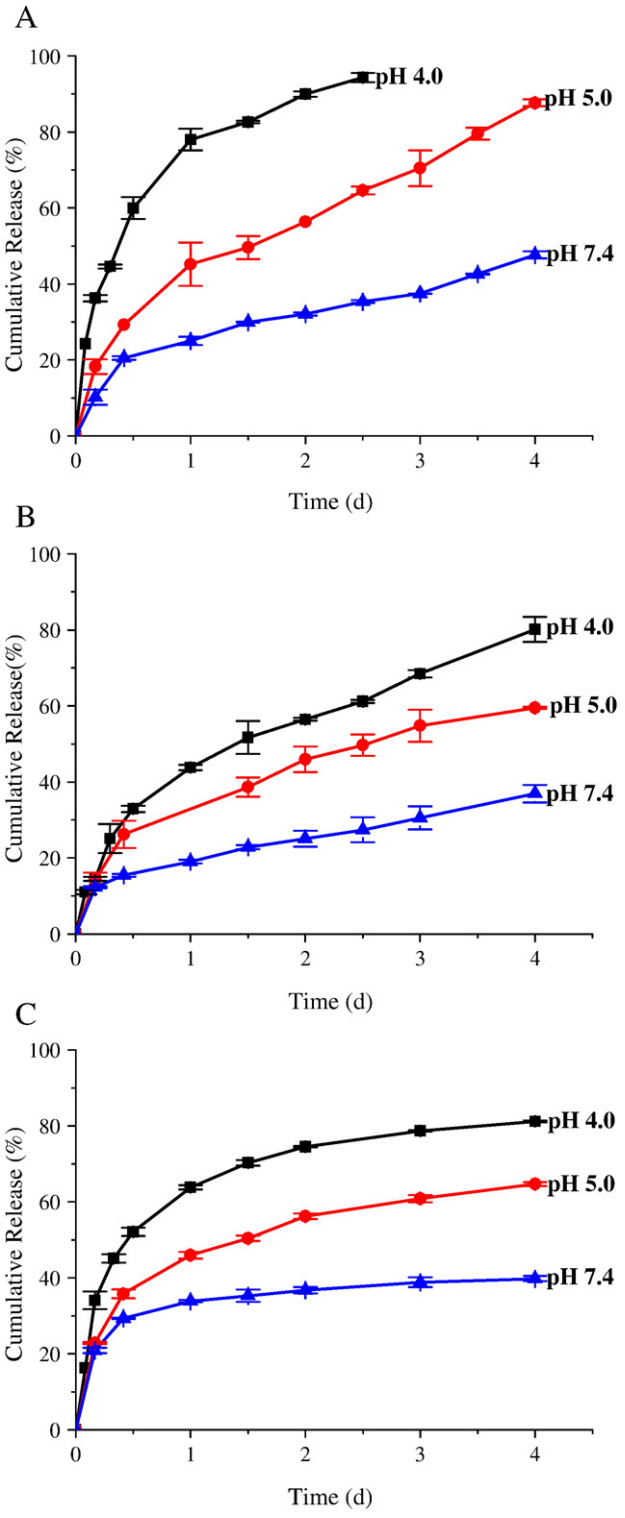


Fig. 4. pH-triggered drug release at 37 °C. (A) PTX from polymersomes; (B) PTX from micelles; and (C) DOX·HCl from polymersomes.

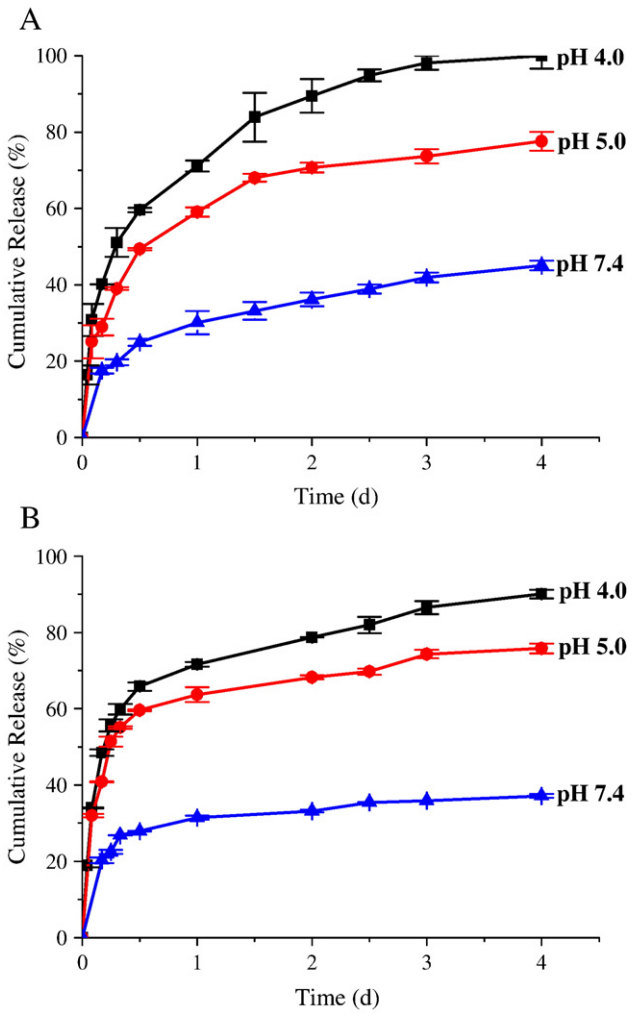


Fig. 5. pH-Triggered simultaneous release of PTX (A) and DOX·HCl (B) from pH-sensitive degradable polymersomes at 37 °C.

4. Conclusions

We have developed pH-sensitive nano-sized degradable polymersomes based on PEG–PTMBPEC diblock copolymer. These polymersomes are able to load both hydrophilic and hydrophobic anticancer drugs, and more importantly both drugs are released in a controlled and pH-dependent manner wherein significantly faster drug release is achieved at mildly acidic pH as compared to physiological pH. To the best of our knowledge, this represents a first report on stimuli-sensitive degradable polymersomes. Furthermore, we have compared pH-sensitive polymersomes with micelles in terms of degradation, drug encapsulation, and pH-triggered drug release behaviors. It turns out that polymersomes release drugs more rapidly in response to mildly acidic pH as compared to micelles. These pH-sensitive nano-sized degradable polymersomes are particularly appealing for targeted intracellular release of a combination of hydrophilic and hydrophobic anticancer drugs or a combination of anticancer drug with therapeutic proteins (combination therapy for cancers).

Acknowledgements

This work was supported by National Natural Science Foundation of China (NSFC 50703028, 50973078, 20874070 and 20974073), the

Natural Science Foundation of the Jiangsu Higher Education Institutions of China (08KJB150016), and Program of Innovative Research Team of Soochow University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jconrel.2009.09.023.

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