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Novel self-assembled micelles of amphiphilic poly(2-ethyl-2-oxazoline)-poly(L-lactide) diblock copolymers for sustained drug delivery

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Abstract

A series of poly(2-ethyl-2-oxazoline)-poly(L-lactide) (PEOz-PLA) diblock copolymers were synthesized by ring-opening polymerization of L-lactide using a monohydroxy terminated PEOz-OH macro-initiator in the presence of stannous octoate as catalyst. The resulting diblock copolymers were characterized by ¹H nuclear magnetic resonance and gel permeation chromatography. Self-assembled micelles were prepared using the co-solvent evaporation method in water and in phosphate buffered saline (PBS) at pH 7.4, 6.5 and 5.0. The resulting micelles exhibit different morphologies, such as spherical micelles and worm-like micelles depending on the hydrophilic/hydrophobic balance. Spherical micelles were exclusively observed for PEOz-PLA copolymers with short PLA blocks, whereas co-existence of worm-like and spherical micelles was observed for copolymers with long PLA blocks. The micelle size increases with decreasing pH due to the electrostatic repulsion between PEOz chains resulting from ionization of the tertiary amide groups along PEOz chains. A hydrophobic anti-tumor drug, paclitaxel, was entrapped in PEOz-PLA micelles. High loading efficiency up to 86.7% was obtained for copolymers with long PLA blocks. Drug release was performed in PBS at different pH values. During the 30-day release period, faster release was obtained for copolymers with shorter PLA blocks than for copolymers with longer PLA blocks, at acidic pH than at pH 7.4. It is thus concluded that pH-responsive PEOz-PLA copolymer micellss could be promising as carrier of anti-tumor drugs.

Keywords: poly(2-ethyl-2-oxazoline); poly(L-lactide); block copolymer; self-assembly; micelles; paclitaxel; drug delivery

1. Introduction

Paclitaxel (PTX) is a natural anti-cancer drug extracted from the bark of the pacific yew tree *Taxus brevifolia*. Since its approval by the U.S. Food and Drug Administration (FDA) for advanced ovarian cancer treatment in December 1992, the clinical applications of PTX have been continuously growing [1,2]. Nowadays, PTX is considered as one of the most efficient anti-cancer drugs for the treatment of a variety of tumors, especially ovarian cancer, acellular lung cancer and metastatic breast cancer [3]. Its main mechanism of action is to promote the formation of microtubules by polymerization of tubulin dimer and to prevent depolymerization to stabilize microtubules, which results in the inability of cells to form spindle and spindle filaments during mitosis. PTX also exhibits an antitumor effect through inhibiting cell division and proliferation. Nevertheless, despite its great potential as anti-tumor drug, the clinical application of PTX is considerably restrained by its poor aqueous solubility [4]. The commonly used drug formulations of PTX are Taxol[®] and Abraxane[®]. The former is a co-solvent formulation composed of 50:50 Cremophor EL and dehydrated alcohol which allows to improve the bioavailability of PTX [5]. The annual market of Taxol[®] attained a maximum of \$1.6 billion in 2000 before decreasing. In fact, serious side effects have been reported with the use of Cremophor EL, such as hypersensitivity reaction, nephrotoxicity, neurotoxicity and cardiotoxicity [6]. Abraxane[®] is an injectable formulation of albumin-bound paclitaxel nanoparticles which gained more and more importance in the past years, largely exceeding Taxol[®] in clinical uses [7]. However, neurotoxicity of Abraxane[®] has also been reported [8]. Therefore, recent research has focused on the development of novel delivery systems of PTX, including prodrugs, emulsions, microspheres, micelles, liposomes and nanoparticles [9,10]. Among them, micelles prepared by self-assembly of amphiphilic block copolymers appear most promising as drug carrier because of their outstanding properties, such as biocompatibility, nano-size, sustained drug release, high stability in vivo and in vitro, and easy binding with tumor-specific targeting ligands. Self-assembled micelles exhibit a core-shell structure: an inner core constituted by aggregation of hydrophobic segments, and an outer shell formed by hydrogen bonding between hydrophilic segments and surrounding water molecules. The outer shell ensures stability and long circulation of the micelle system, whereas the inner core allows to encapsulate drug molecules which will be transported to the targeted site at much higher concentration than the drug's water solubility [11,12].

Poly(ethylene glycol) (PEG) is a FDA-approved biocompatible polyether with outstanding physico-chemical and biological properties [13]. It's most commonly used as a hydrophilic block in amphiphilic copolymers for pharmaceutical uses. On the other hand, polylactide (PLA) is a bioresorbable and biocompatible polyester widely studied for biomedical applications [14]. PLA/PEG block copolymers are able to self-assemble to yield various aggregates, including polymersomes, worm-like or filomicelles, rod-like micelles, and spherical micelles. The morphology of aggregates mainly depends on the hydrophobic to hydrophilic balance. Filomicelles are of great interest as drug carrier due to their higher drug load and longer circulation time in the bloodstream compared to spherical micelles [15].

Although the biomedical safety of PEG has been well admitted, it has been reported that PEG may cause complement activation in certain conditions, resulting in rapid clearance after repeated injections of PEG-containing carriers. In addition, PEG as a polyether is prone to peroxidation, thus adversely affecting cells [16,17]. Therefore, great effort has been made in the past years to search for hydrophilic alternatives of PEG.

It is known that the extracellular pH value of tumor is between 6.5 and 7.2, and that of its lysosomal/endosomal vesicles is in the range of 4.5-5.0 and 5.5-6.5. The lower pH around solid tumor tissues inspired researchers to conceive micelles which are responsive to acid-specific stimulation [18]. Poly(2-ethyl-2-oxazoline) (PEOz) is a hydrophilic polymer which can be ionized at lysosomal/endosomal's pH values. Besides, PEOz presents outstanding biocompatibility, which makes it a promising substitute of PEG [19-21]. Wang and Hsiue synthesized poly(L-lactide)-poly(2-ethyl-2-oxazoline)-poly(L-lactide) (PLLA-PEOz-PLLA) triblock copolymers, and investigated the polymers' pH-sensitive properties as potential drug carrier [19]. Gulyuz et al. prepared PEOz-PLA and poly(2-ethyl-2-oxazoline)-poly(ϵ -caprolactone) (PEOz-PCL) diblock copolymers, and evaluated their cytotoxicity in vitro [22]. Zhao et al. studied the release of doxorubicin (DOX) from poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) (PEOz-PDLLA) micelles. Data showed that the release of DOX was faster at pH 5.0 than at pH 7.4 [18]. Wang et al. studied the in vitro release, cellular uptake and in vitro cytotoxicity of PEOz-PDLLA micelles co-encapsulating PTX and honokiol (HNK) [23]. The dual drug-loaded PEOz-PLA micelles exhibited small size and high drug encapsulation

efficiency, and favorable pH-dependent drug release characteristics. Qiu et al. attached folic acid to PEOz-PCL, and investigated the cellular internalization and anticancer activity of DOX-loaded micelles of FA-PEOz-PCL [24]. Wang et al. synthesized PEOz-PDLLA-PEOz triblock copolymers. The resulting hydrogels showed good cytocompatibility in vitro [25]. Gao et al. synthesized HOOC-PEOz-PDLLA to prepare YPSMA-1-modified micelles and cyclic RGDyK (cRGDyK)-conjugated micelles for targeted delivery of paclitaxel [26,27].

Recently, Wang et al. investigated the mechanisms of pH-sensitivity and cellular internalization of a series of PEOz-PDLLA micelles with various hydrophilic/hydrophobic ratios [28]. The authors suggested that electrostatic repulsion between PEOz chains resulting from ionization of the tertiary amide groups along PEOz chain at pH lower than its pKa was responsible for pH-sensitivity of PEOz-PDLLA micelles. Nevertheless, few studies focused on the self-assembly behavior of PEOz-PLLA copolymers under different pH conditions. In fact, it has been shown that the morphology of self-assembled micelles is dependent on the hydrophilic/hydrophobic ratio, block length, and chain structure regularity of copolymers [15]. Various micelle structures, including spherical, rod-like and worm-like micelles can be obtained from PEG-PLA, PEG-PCL, and PEG-P(CL/GA) copolymers, which strongly affects the drug release behavior and blood circulation time of micelles [15,29].

In this paper, a series of PEOz-PLLA copolymers were synthesized by ring-opening polymerization of L-lactide, using hydroxyl-terminated PEOz-OH as macro-initiator and stannous octoate as catalyst. Self-assembled micelles of the copolymers were prepared using co-solvent evaporation method and characterized. The drug loading and drug release properties of PTX-loaded PEOz-PLA micelles were evaluated, considering the influence of copolymer composition and pH value. The results are reported herein in comparison with literature.

2. Materials and methods

2.1. Materials

L-lactic acid was obtained from Sigma (St Louis, MO, USA). L-lactide was synthesized according to literature method, and purified by recrystallization three times from ethyl acetate [30]. 2-Ethyl-2-oxazoline supplied by Sigma–Aldrich

(Shanghai, China) and methyl *p*-toluenesulfonate (MeOTs, Aldrich) were dried over CaH₂ and distilled before use. So were acetonitrile and chlorobenzene purchased from Aladdin (Shanghai, China). Stannous octoate obtained from Sigma (St Louis, MO, USA), and paclitaxel and Tween 80 supplied by Aladdin (Shanghai, China) were used as received. All other organic reagents were of analytical grade.

2.2. Synthesis of PEOz-PLA copolymers

Amphiphilic PEOz-PLA diblock copolymers were synthesized as reported in literature [31]. Typically, 2-ethyl-2-oxazoline (20.0 g, 200 mmol) and methyl *p*-toluene sulfonate (1.24 g, 6.67 mmol) were added to dry acetonitrile (60 mL), and the temperature was gradually increased to 100 °C. The reaction then proceeded at reflux for 30 h under nitrogen atmosphere. After cooling down to room temperature, 0.1 M potassium hydroxide in methanol was added to the solution, and the reaction continued 4 more hours to introduce hydroxyl groups. The crude product was filtered through silica gel, and the filtrate was added to cold diethylether. The precipitated product PEOz-OH was dried under vacuum for 24 h.

Secondly, PEOz-OH (1.0 g), stannous octoate (5 mg), L-lactide (0.58 g, 4 mmol), and chlorobenzene (15 mL) were introduced in a Schlenk flask. The reaction proceeded at 120 °C for 30 h under N₂ atmosphere. The product was filtered through silica gel, precipitated by addition of diethylether and finally dried under vacuum up to constant weight. And the yield of the final product is about 90%.

2.3. Preparation of polymeric micelles and drug-loaded micelles

PEOz-PLA micelles were prepared by using co-solvent evaporation method [32]. Briefly, 20 mg copolymer was dissolved in 1 mL chloroform. The solution was slowly added to 20 mL phosphate buffered saline (PBS) at pH 7.4, and stirred for 4 h to allow self-assembly of micelles along with solvent evaporation. The resulting micelle solution was filtered through 0.8 μm filter and stored at 4 °C.

PTX-loaded micelles were prepared using a two-step procedure according to the literature [29]. Blank micelles were first prepared as mentioned above. 1 mg PTX dissolved in 1 mL methanol was then added to 10 mL blank micelle solution. The solution was vigorously stirred for 4 h, and gently stirred for 24 h to complete solvent evaporation. The solution was finally centrifuged at 3 000 rpm for 10 min to remove

unloaded drug. The supernatant was collected as drug-loaded micelle solution.

2.4. Characterization

¹H nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker AVANCE III 500 spectrometer (Billerica, MA, USA) operating at 500 MHz, using CDCl₃ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

Gel permeation chromatography (GPC) measurements were performed on a Shimadzu apparatus (Kyoto, Japan) equipped with a Waters 410 refractometer and a PLgel 5 μ m MIXED-C column. Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1.0 mL/min. The sample solution at a concentration of 1.0 mg/mL was injected for analysis. Polystyrene standards were used for calibration.

Dynamic light scattering (DLS) was realized on a Nano-ZS90 nanosizer (Malvern, Worcestershire, UK). Measurements were performed at 25 °C with a 90° scattering angle.

Transmission electron microscopy (TEM) was performed on JEM-1200EX microscope (JEOL, Japan). A small amount of micelle solution in PBS was dropped on a carbon coated copper grid. Then the excess fluid was removed with a filter paper, followed by staining using 1.0 wt.% phosphotungstic acid solution and air drying. Examination of micelles was realized at an acceleration voltage of 80 kV.

The critical micelle concentration (CMC) of PEOz-PLA copolymers was measured by fluorescence spectrophotometer (F-4600) using pyrene as fluorescent probe [33]. Briefly, 1 mL pyrene solution (2×10^{-5} M in benzene) was added to 10 mL volumetric flask, and the solvent was evaporated. Different volumes of micelle solutions were added to the flask. Then distilled water was added to a total volume of 10 mL. The resulting micellar concentrations ranged from 1.0×10^{-3} to 0.5 mg/mL, and the final concentration of pyrene was 2.0×10^{-6} M. After equilibrium at room temperature for 24 h, the fluorescence excitation spectra of the micellar solutions were recorded from 350 to 450 nm at an excitation wavelength of 334 nm. The intensity ratio at 375 nm and 395 nm was plotted *versus* copolymer concentration. The CMC value was obtained from the cross-over point of two regression lines.

High-performance liquid chromatography (HPLC) was performed using a Waters HPLC (Milford, MA, USA) equipped with a UV detector and a C18 column. The detection wavelength was set at 227 nm. The mobile phase was a mixture of water and acetonitrile (v/v 45/55). The column temperature was 30 °C. 10 µL solution was injected for each analysis at a flow rate of 1.0 mL/min.

2.5. In vitro release of PTX from PEOz-PLA micelles

PTX release from PEOz-PLA micelles was realized under in vitro conditions. Briefly, 3 mL drug-loaded micelle solution was introduced in a cellulose dialysis bag (molecular weight cutoff 3500) which was then placed in 30 mL PBS containing 0.1% Tween 80. The pH of the drug release media was 7.4, 6.5, or 5.0, respectively. Drug release was performed at 37 °C in a water bath shaker at 80 rpm. At pre-determined time points, 1 mL of the release medium was removed, and replaced with the same volume of medium. The content of PTX in the release medium was determined by using HPLC.

3. Results and discussion

3.1. Characterization of PEOz-PLA copolymers

Amphiphilic PEOz-PLA block copolymers were synthesized by ring-opening polymerization of L-lactide as illustrated in **Fig. 1**. Hydroxyl-terminated PEOz-OH was first synthesized by polymerization of 2-ethyl-2-oxazoline using methyl tosylate as initiator, followed by chain termination with methanolic KOH. The hydroxyl endgroup of PEOz-OH was used to initiate the ring-opening polymerization of L-lactide in the presence of stannous octoate as catalyst, yielding PEOz-PLA diblock copolymers with different PEOz and PLA block lengths.

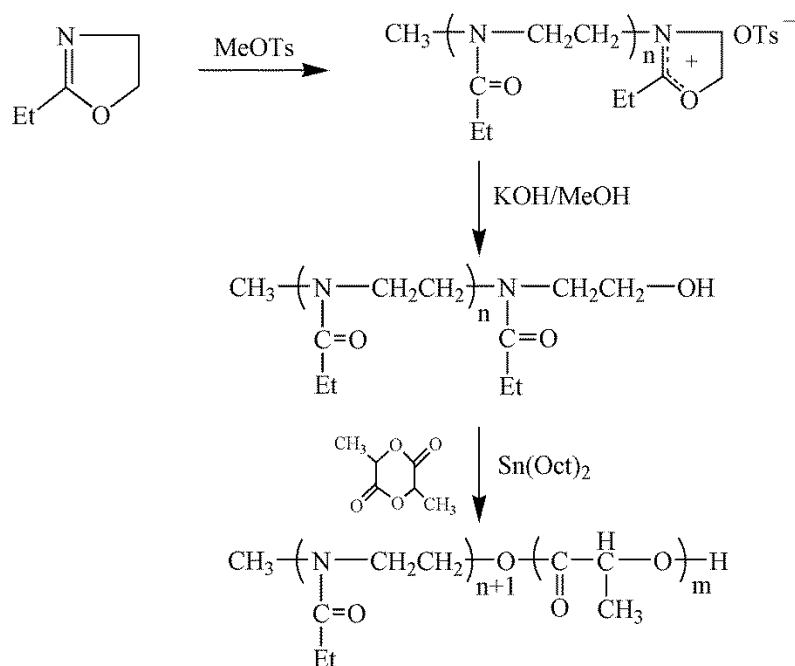


Fig. 1. Synthesis route of PEOz-PLA copolymers.

^1H NMR spectroscopy was used to determine the composition of the copolymers. **Fig. 2** shows the ^1H NMR spectra of PEOz-OH and PEOz-PLA. In **Fig. 2A**, the 3 main signals at 3.44 ppm (peak *c*), 2.29 and 2.38 ppm (peak *b*), and 1.11 ppm (peak *a*) are attributed to the CH_2 protons of PEOz main chain, the CH_2 of ethyl side chain, and the CH_3 of ethyl side chain, respectively. Two small signals at 3.75 ppm (peak *d*) and 3.0 ppm (peak *e*) are assigned to the CH_2 connected to the hydroxyl endgroup and the terminal CH_3 group. New signals of PLA are detected in **Fig. 2B**. The signals at 5.17 ppm (peak *f*) and 1.59 ppm (peak *g*) belong to the methine and methyl protons of PLA main chain, respectively. On the other hand, disappearance of the signal at 3.75 ppm is detected, in agreement with the attachment of PLA segment. A small signal appears at 4.40 ppm which is assigned to the methine proton of PLA connected to the hydroxyl endgroup.

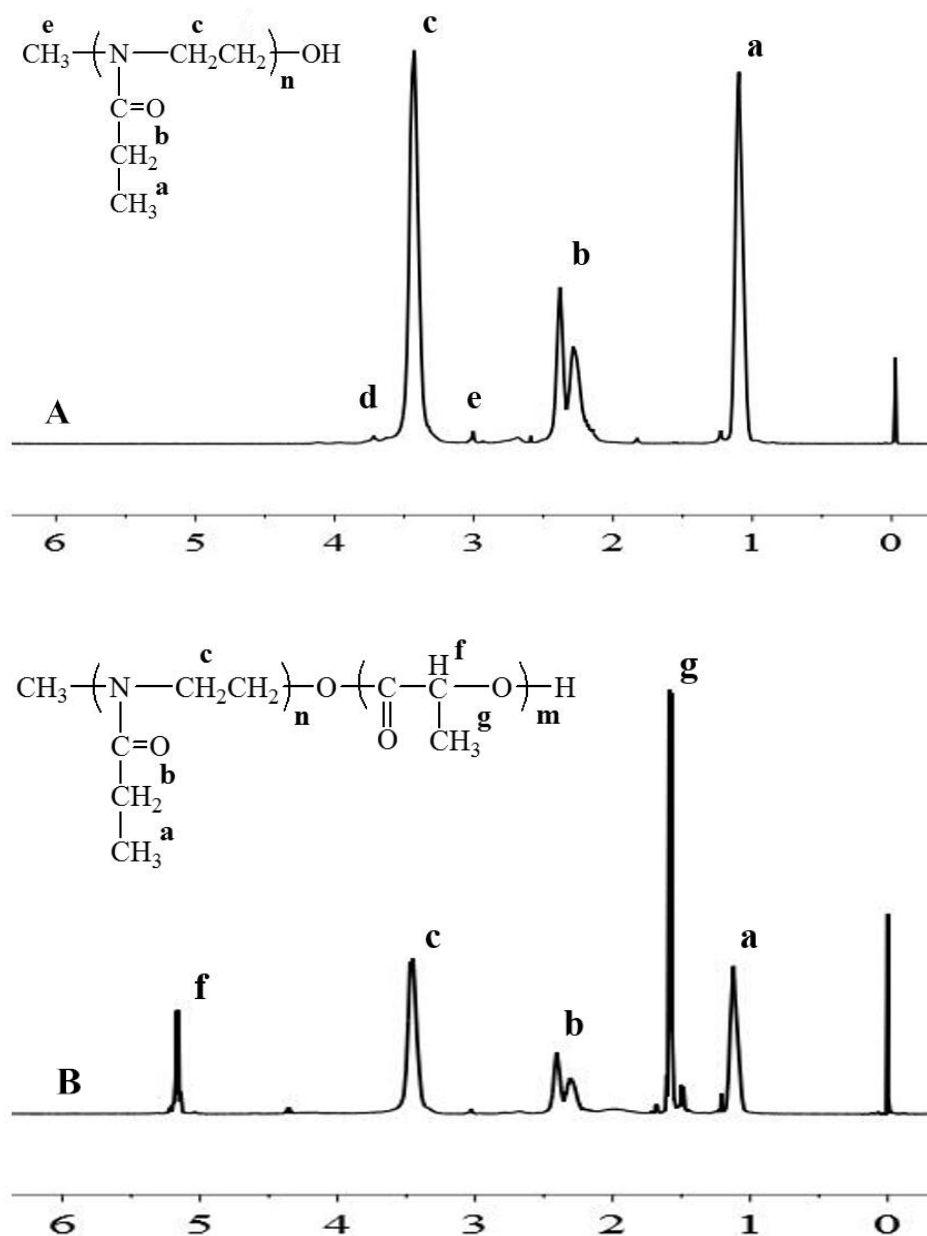


Fig. 2. $^1\text{H-NMR}$ spectra of PEOz-OH (A) and PEOz-PLA copolymer (B).

The degree of polymerization (DP) of PEOz-OH is calculated from the integration ratio of signals *a* and *e* corresponding to the methyl group of ethyl side chain and that of the chain end (2A), respectively. Two PEOz-OH macro-initiators with DP_{PEOz} of 28 and 18 were synthesized in this work. The [LA/EOz] molar ratio of the block copolymers is determined from the integrations of signals *f* and *a* corresponding to the methine group of PLA and the methyl group of ethyl side chain of PEOz (2B), respectively. The [LA/EOz] ratios are slightly lower than those in the feed as shown in Table 1, suggesting that the conversion of L-lactide is not complete. In fact, the yield

of the reactions was 80 to 90%. The DP_{PLA} and the number-average molar mass (M_n) of the copolymers are then obtained from the following relationships:

$$DP_{PLA} = DP_{PEOz} \times [LA/EOz] \quad (\text{eq. 1})$$

$$M_n = DP_{PEOz} \times 99 + DP_{PLA} \times 72 \quad (\text{eq. 2})$$

Where 99 and 72 are the molar mass of EOz and LA repeat units, respectively.

The compositions of the various copolymers are summarized in **Table 1**. The copolymers are named as EOz_xL_y for the sake of clarity. In this acronym, EOz and L represent PEOz and PLLA blocks, respectively, while x and y designate the number-average degree of polymerization of corresponding blocks. The DP_{PLA} varies from 42 to 6 for the $EOz_{28}L_y$ series, with M_n varying from 5820 to 3230 g/mol. And a copolymer with composition of $EOz_{18}L_5$ and M_n of 2160 was also synthesized.

The M_n and dispersity ($D = M_w/M_n$) of the copolymers are also determined from GPC measurements, as summarized in **Table 1**. The $M_{n(GPC)}$ values are close or slightly superior to $M_{n(NMR)}$ ones. In fact, the $M_{n(GPC)}$ is determined with polystyrene standards and is usually higher than those from NMR, as reported in literature [29]. On the other hand, the dispersity varies from 1.47 for $EOz_{28}L_{42}$ to 1.18 for $EOz_{18}L_5$, in agreement with narrow molar mass distributions.

The hydrophilic-lipophilic balance (HLB) is a major parameter of amphiphilic polymers which can be determined by Griffin's method. A low HLB value indicates strong hydrophobicity, and *vice versa*. Table 1 shows that the HLB varies from 9.6 for $EOz_{28}L_{42}$ to 17.3 for $EOz_{28}L_6$. Obviously samples with higher PEOz content have a higher HLB, in agreement with higher hydrophilicity.

Table 1

Structural characteristics of PEOz-PLLA diblock copolymers.

Copolymer	[LA/EOz] ^a	DP _{PEOz}	DP _{PLA}	M _n (NMR)	M _n (GPC)	D	CMC (mg/mL)	HLB ^b
PEOz 1800	--	18	--	1800	1700	1.13	--	--
PEOz 2800	--	28	--	2800	2900	1.09	--	--
EOz ₂₈ L ₄₂	1.50 (1.70)	28	42	5800	5900	1.47	0.0041	9.6
EOz ₂₈ L ₂₁	0.75 (0.80)	28	21	4300	4500	1.30	0.0046	13.0
EOz ₂₈ L ₁₀	0.35 (0.50)	28	10	3500	3800	1.26	0.0069	15.9
EOz ₂₈ L ₆	0.20 (0.30)	28	6	3200	3100	1.27	0.0067	17.3
EOz ₁₈ L ₅	0.25 (0.30)	18	5	2200	2400	1.18	0.0076	16.7

^a [LA/EOz] is calculated from NMR, and data in parentheses are the feed ratios.

^b $HLB_{\text{copolymer}} = (M_{n\text{PEOz}}/M_{n\text{copolymer}}) \times 20$.

3.2. Self-assembly of copolymers

3.2.1. Critical micelle concentration

The CMC is an essential parameter that determines the behavior of micelles in vivo, especially in terms of micelle stability after intravenous injection. The CMC of PEOz-PLA copolymers was obtained from fluorescence spectrophotometer using pyrene as the hydrophobic probe. The fluorescence emission spectra of pyrene were registered in the range of 350 to 450 nm with increasing copolymer concentration from 1.0×10^{-3} to 0.5 mg/mL (**Fig. S1**). The emission intensity of samples at low concentrations is almost the same, but a significant increase occurs when the concentration reaches a critical value. The intensity ratio I_{375}/I_{395} vs. $\log C$ changes of micelles were plotted, and the crossover point of the two regression lines is taken as the CMC (**Fig. S1**). The CMC data of the various copolymers are shown in **Table 1**. It appears that the CMC is closely related to the PLA block length. The longer the hydrophobic PLA block, the lower the CMC value. In fact, amphiphilic block copolymers with longer hydrophobic blocks are more inclined to self-assemble in aqueous medium, thus leading to lower CMC and higher structural stability [34]. It is noteworthy that the CMC values of all samples are very low (0.004-0.008 mg/mL) compared to low molar mass surfactants, which should ensure the stability of micelles after administration in the bloodstream [35].

3.2.2. Morphology of self-assembled structures

Micelle size and morphology strongly affect the drug loading and drug release properties. PEOz-PLA micelles were prepared by self-assembly in pH 7.4 PBS using co-solvent evaporation method. The morphology of PEOz-PLA blank and

PTX-loaded micelles was examined by TEM as shown in **Fig. 3**. Both spherical and worm-like micelles are observed for EOZ₂₈L₄₂ (**Fig. 3A**). The spherical micelles exhibit a diameter of 20-50 nm, and the worm-like micelles a length of about 200 nm and a diameter of about 30 nm. In contrast, uniformly dispersed spherical micelles are observed for EOZ₂₈L₂₁ (**Fig. 3B**). The TEM images of micelles after PTX loading are shown in **Fig. 3A'** and **Fig. 3B'**. Obviously drug loaded micelles exhibit the same structures as blank micelles. Similar results are obtained in the case of EOZ₂₈L₁₀, EOZ₂₈L₆ and EOZ₁₈L₅ micelles (**Fig. S3**). Thus copolymers with HLB value above 13.0 only yield spherical micelles.

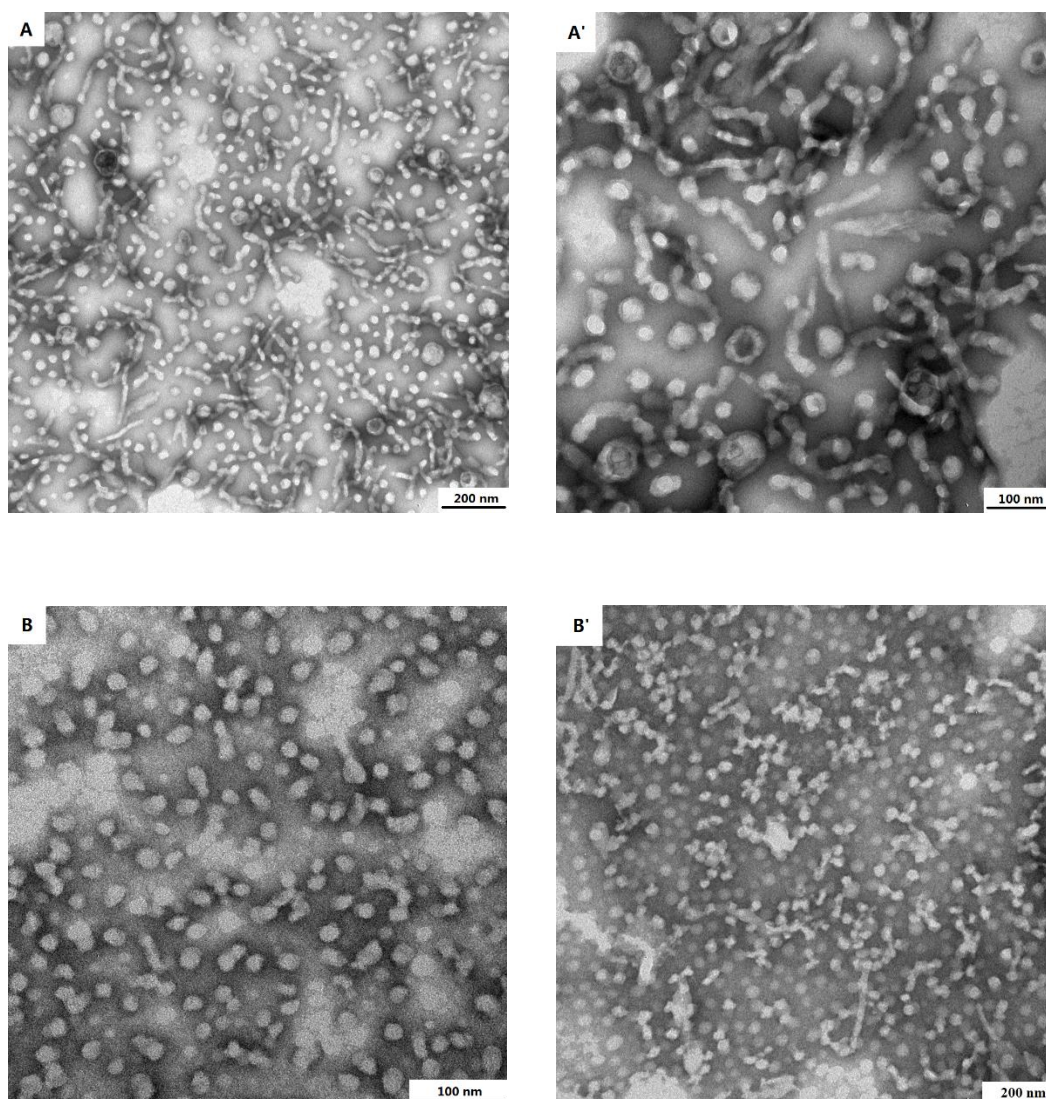


Fig. 3. TEM images of PEOz-PLLA blank micelles (left) and PTX-loaded micelles (right): EOZ₂₈L₄₂ (**A, A'**), EOZ₂₈L₂₁ (**B, B'**).

DLS was used to determine the size and size distribution of spherical micelles in

aqueous solution (**Fig. S2**). A uniform micelle size distribution is observed in all cases. The effect of pH on the micelle size was investigated as PEOz exhibits some pH-sensitivity. The various copolymers were allowed to self-assemble in water and in PBS at pH = 7.4, 6.5, 5.0 by using co-solvent evaporation method, and characterized by using DLS. All the micelle size data are summarized in **Table 2**. Data of EO_{Z28}L₄₂ are not available as it forms both spherical and worm-like micelles. For the two poorly water-soluble copolymers (EO_{Z28}L₂₁ and EO_{Z28}L₁₀), the diameter of micelles decreases with decreasing PLA block length. In fact, the size is 103.6 nm for EO_{Z28}L₂₁ and 74.6 nm for EO_{Z28}L₁₀, which is in agreement with literature [28]. This finding could be explained by the increased aggregation number of copolymer micelles with longer PLA blocks which attracted more copolymer chains in a micelle. It is also of interest to note that the diameter obtained from TEM is much lower than that from DLS, which can be attributed to the dehydration and shrinkage of micelles during TEM measurements.

On the other hand, it appears that the size of micelles self-assembled in water is smaller than that in PBS, the micelle size increases with decreasing pH. These findings could be related to the presence of tertiary amide bonds in PEOz structure. In a weakly acidic environment, the tertiary amide bond allows adsorption of protons, resulting in the formation of PEOz intrachain or interchain hydrogen bonds. Meanwhile, the electrostatic repulsion between PEOz chains resulting from ionization of the tertiary amide groups along PEOz chain also contributes to micelle size increase with decreasing pH. The situation is more complicated in the case of water-soluble copolymers (EO_{Z28}L₆ and EO_{Z18}L₅). In contrast to kinetically “frozen” micelles obtained from water insoluble copolymers, EO_{Z28}L₆ and EO_{Z18}L₅ copolymer micelles are dynamic systems with permanent exchanges between micelle-forming molecules and free molecules in solution, continuously breaking and reforming [36]. Surprisingly, little difference of micelle size was observed in water and in PBS at different pH values probably because of the dynamic nature of micelles which allows self-adjustment of micelle architecture and size [28,29]. It is also noted that the polydispersity index of micelles ranges from 0.21 to 0.35 in all cases.

Table 2

Diameter of blank micelles in different media and loading efficiency (LE) and loading content (LC) data of PTX-loaded micelles.

Copolymer	Water		PBS 7.4		PBS 6.5		PBS 5.0		LE ^b (%)	LC ^b (%)
	D _h /nm ^a	PDI ^a	D _h /nm	PDI	D _h /nm	PDI	D _h /nm	PDI		
EOZ ₂₈ L ₄₂	--	--	--	--	--	--	--	--	62 ± 3	5.8 ± 0.3
EOZ ₂₈ L ₂₁	104	0.26	115	0.22	116	0.24	126	0.24	87 ± 3	8.0 ± 0.2
EOZ ₂₈ L ₁₀	75	0.29	77	0.23	92	0.27	108	0.31	80 ± 5	7.4 ± 0.4
EOZ ₂₈ L ₆	82	0.26	89	0.21	84	0.22	90	0.27	44 ± 2	4.2 ± 0.2
EOZ ₁₈ L ₅	99	0.33	96	0.28	92	0.24	102	0.21	41 ± 3	3.9 ± 0.3

^a D_h and PDI of micelles determined by DLS.

^b LE and LC determined by HPLC. Data represent mean value ± S.D., n = 3.

3.3. In vitro drug release

PTX was used as a hydrophobic model drug to evaluate the drug loading and release properties of PEOz-PLA copolymers. Drug loading was realized by using a two-step procedure consisting in adding drug solution into a pre-formed micelle solution under stirring. The drug loading content (LC) was defined as the ratio of the amount of loaded drug to the total amount of drug loaded micelles, and the loading efficiency (LE) as the ratio of the amount of loaded drug to the amount of initially introduced drug (theoretical drug loading) according to the following equations [37,38]:

$$LE = \frac{\text{weight of loaded drug}}{\text{theoretical drug loading}} \times 100\% \quad (1)$$

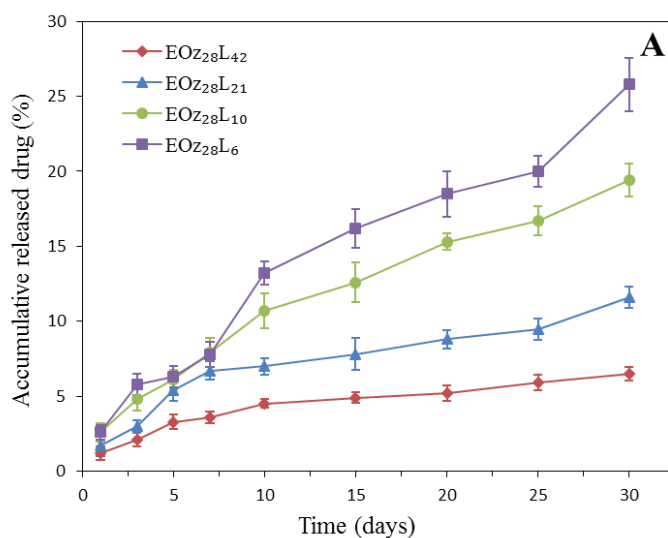
$$LC = \frac{\text{weight of loaded drug}}{\text{weight of drug-loaded micelles}} \times 100\% \quad (2)$$

Table 2 presents the LC and LE data of PTX-loaded micelles. EOZ₁₈L₅ exhibits the lowest LE of 40.6% and LC of 3.9%, whereas EOZ₂₈L₂₁ presents the highest LE of 86.7% and LC of 8.0%. Obviously the LC and LE values mainly depend on the length of the hydrophobic block of copolymers. The longer the hydrophobic block, the higher LE and LC values of copolymer micelles, and *vice versa*. However, lower LE and LC values are obtained for EOZ₂₈L₄₂ as compared to EOZ₂₈L₁₀ and EOZ₂₈L₂₁. In fact, EOZ₂₈L₄₂ has a high hydrophobic PLA content or low HLB value. Thus the micelles tend to aggregate and precipitate during the self-assembly process, leading to

lower drug loading efficiency in spite of the co-existence of spherical and worm-like micelles.

Fig. 4A presents the drug release profiles from 4 micelle samples (EO_{Z28}L₄₂, EO_{Z28}L₂₁, EO_{Z28}L₁₀ and EO_{Z28}L₆) with different PLA block lengths in pH 7.4 PBS at 37 °C. No burst release is detected. It appears that the PTX release ratio is improved for copolymers with short PLA blocks. EO_{Z28}L₆ exhibits the highest release ratio with 25.8% of released drug in 30 days, in contrast to EO_{Z28}L₄₂ which has only 6.5% drug release. EO_{Z28}L₁₀ and EO_{Z28}L₂₁ exhibit intermediate release ratios of 19.4% and 11.6%, respectively. EO_{Z28}L₄₂ with low drug content shows the lowest release rate. This finding could be assigned to the most compact core structure of EO_{Z28}L₄₂ micelles which disfavors drug diffusion to the medium.

It is also of interest to compare the released drug amounts from different micelle systems. For example, EO_{Z28}L₂₁ exhibits a release percentage of 11.6% in 30 days, but it has a high loading efficiency of 86.7%. In contrast, EO_{Z28}L₆ presents the highest release rate (25.8% in 30 days), but its loading efficiency is only 43.5%. In 30 days, the total released drug from EO_{Z28}L₂₁ is approximately 0.030 mg, and that from EO_{Z28}L₆ is about 0.034 mg.



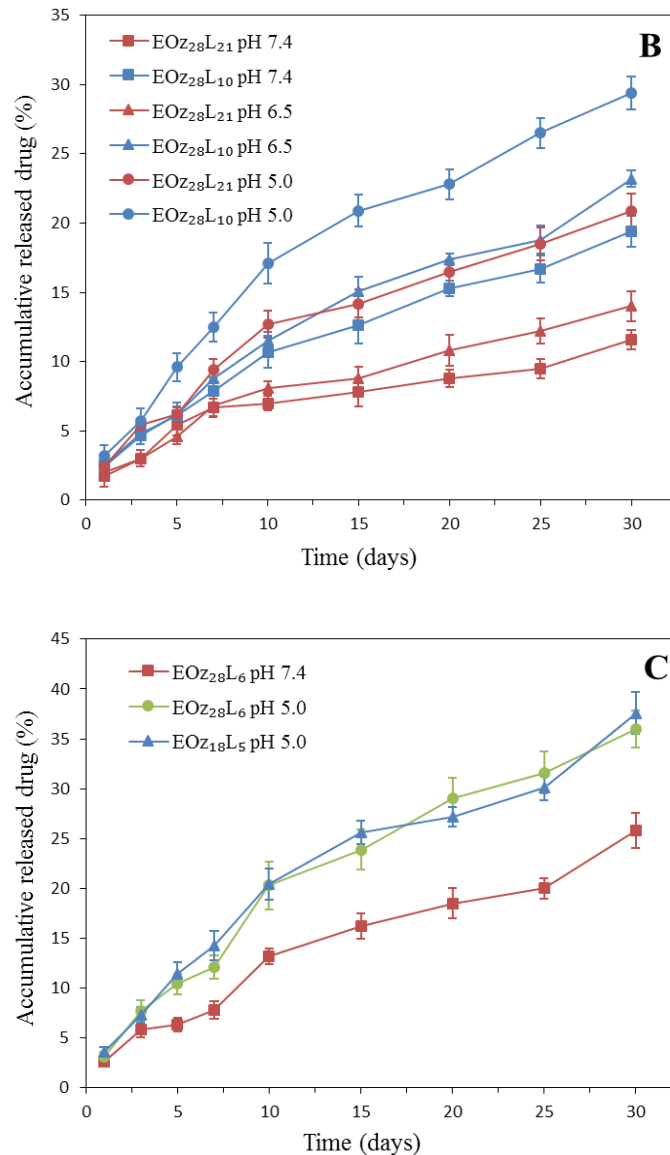


Fig. 4. (A) In vitro release profiles of paclitaxel from EOZ₂₈L₄₂, EOZ₂₈L₂₁, EOZ₂₈L₁₀ and EOZ₂₈L₆ copolymer micelles at pH = 7.4 PBS at 37 °C; (B) In vitro release profiles of paclitaxel from EOZ₂₈L₂₁ and EOZ₂₈L₁₀ copolymer micelles at pH = 7.4, 6.5, 5.0 PBS at 37 °C; (C) In vitro release profiles of paclitaxel from EOZ₂₈L₆ and EOZ₁₈L₅ copolymer micelles at pH = 7.4 and pH = 5.0 PBS at 37 °C. All data are represented as the mean ± SD (n = 3).

Considering the overall performance of the loading content, loading efficiency and release rate, EOZ₂₈L₂₁ and EOZ₂₈L₁₀ micelles were used to evaluate the effect of pH on drug release. Three pH values (7.4, 6.5, 5.0) were used to mimic the physiological pH, extracellular pH of tumor, and that of lysosome/endosome of tumor. **Fig. 4B**

shows the PTX release profiles from EOZ₂₈L₂₁ and EOZ₂₈L₁₀ copolymer micelles at different pH values. Drug release appears rather smooth without burst effect. The release ratio from EOZ₂₈L₂₁ is 11.6% at pH 7.4, 14% at pH 6.5, and 20.9% at pH 5.0 in 30 days. Similarly, the release ratio from EOZ₂₈L₁₀ is 19.4% at pH 7.4, 23.2% at pH 6.5, and 29.4% at pH 5.0. These findings indicate that the release rate is only slightly enhanced at pH 6.5, and significantly enhanced at pH 5.0 as compared to pH 7.4. It has been reported that the pKa of PEOz-PLA was 6.5 [28]. Thus the hydrophilic PEOz block could be ionized at pH 5.0 which is below its pKa, leading to swelling of micelles and increase of micelle size which should accelerate drug release.

In fact, protonation of amide groups is an intermediate step of the acid hydrolysis of poly(2-alkyl-2-oxazoline)s (PAOx) to obtain polyethylenimine (PEI) [39]. During the hydrolysis process, the amide group is activated by protonation of the carbonyl functional group, followed by formation of a tetrahedral intermediate via nucleophilic attack of water. Finally the carboxylic acid is detached from the polymer backbone to yield PEI. It should be noticed that amide groups are very stable and hydrolysis of PAOx only occurs under strong acidic or basic conditions [39-41]. In the present work, protonation of the amide group in PEOz chain well agrees with the variation in micelle size and release rate at various pH values.

It is also noteworthy that acidic environments could enhance the degradation of PLA blocks, which could also accelerate drug release. However, it has been reported that the cumulative release of PTX from PEG-PLLA micelles at pH 5.5 is only 2.5% higher than that of pH 7.4 in 30 days, and the difference of release rates between pH 7.4 and pH 3.0 was less than 3% [38]. Thus it can be assumed that the pH-responsiveness of PEOz-PLLA micelles is the main reason for the enhanced drug release at acidic pH.

It has been reported that PEOz with higher molar mass may have higher pH-responsive effect [19]. Thus the release profiles of PTX from EOZ₂₈L₆ and

EOZ₁₈L₅ micelles were comparatively studied, as shown in **Fig. 4C**. The release ratio from EOZ₂₈L₆ and EOZ₁₈L₅ is 36% and 37.6% at pH 5.0, respectively. This finding could be attributed to the small difference of molar masses between the two samples. It is also noted that 25.8% drug release is obtained for EOZ₂₈L₆ at pH 7.4, which is much lower than that at pH 5.0, in agreement with the enhanced drug release at acidic pH.

The release rate of PTX from of PEOz-PLLA micelles appears much slower as compared to the results reported in literature [28]. In fact, Wang et al. reported that PTX release from the micelles was pH dependent and markedly accelerated with decreasing pH value. 67% of PTX were released within 48 h from PEOz6-*b*-PLA4 micelles with Mn of PEOz of 6000 and Mn of PEOz of 4000, whereas PTX release was 75% and 90% at pH 6.5 and 5.0, respectively. The differences could be assigned to the chemical composition and release conditions. In the present work, 0.1% Tween 80 was added in the release media, in contrast to 0.2% Tween 80 in literature. Higher Tween content should enhance drug release as it improves drug solubility. On the other hand, PLLA block used in this study has lower degradability than PDLLA block used in literature. And faster degradation of the polyester block favors drug release [38]. Last but not least, as PLLA degrades very slowly in PBS at pH 7.4, 6.5 and 5.0 [38], drug molecules are more likely to be released through diffusion from the interior of PEOz-PLLA micelles.

It is also of interest to compare the self-assembly and drug release behaviors of PEOz-PLLA micelles and other micelle systems [15,29,36-38]. Various micelle structures, including spherical, rod-like and worm-like micelles have been observed from PEG-PLLA, PEG-PDLLA, PEG-PCL, and PEG-P(CL/GA) copolymers, depending on the hydrophilic/hydrophobic ratio, block length, and chain structure regularity of copolymers. In particular, spherical micelles are exclusively obtained for PEG-PDLLA copolymers, whereas both spherical and worm-like micelles are formed from PEG-PLLA copolymers [15]. Jelonek et al. studied PTX release from various PEG-PLA micelles. The LE of all samples ranges from 48% to 69%, and LC from 4.7%

to 6.5%, which is comparable to PEOz-PLLA micelles. Nevertheless, less than 25% of drug was released during 71 days [15]. On the other hand, little difference was found between the release rates of PEG-PLLA micelles at pH 7.4, 5.5 and 3.0 during the first 5 weeks [41]. Therefore, pH-sensitive PEOz-PLLA micelles could be a promising carrier of hydrophobic anti-tumor drugs.

4. Conclusions

In this work, amphiphilic PEOz-PLA copolymers were successfully synthesized by ring-opening polymerization and considered as a potential drug carrier for controlled delivery of anti-tumor drugs. Self-assembled micelles were prepared using the co-solvent evaporation method in water and in PBS at pH 7.4, 6.5 and 5.0. The resulting micelles exhibit different morphologies, such as spherical micelles and worm-like micelles depending on the hydrophilic/hydrophobic balance. The micelle size increases with decreasing pH due to the electrostatic repulsion between PEOz chains resulting from ionization of the tertiary amide groups along PEOz chains. The PTX-loading and release properties of micelles were also closely related to the length of PLA. Copolymers with longer PLA segments present higher LE and LC values. Faster release was obtained for copolymers with shorter PLA blocks than for copolymers with longer PLA blocks, at acidic pH than at pH 7.4. It is thus concluded that pH-responsive PEOz-PLA copolymers could be promising as carrier of anti-tumor drugs. Further studies are underway in terms of micelle morphology, drug release, *in vitro* cytotoxicity, and *in vivo* cellular uptake, in particular the differences between spherical and worm-like micelles.

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References

- [1] Y.J. Li, X. Zhang, W.X. Luo, D.X. Wang, L. Yang, J.G. Wang, L. Zhang, S.Y. Zhang, S.Y. Luo, Y. Wang, Dual-functionalized nanoparticles loaded microbubbles for enhancement of drug uptake, *Ultrasonics* 87 (2018) 82-90.
- [2] D. Mandal, T.K. Shaw, G. Dey, M.M. Pal, B. Mukherjee, A.K. Bandyopadhyay, M. Mandal, Preferential hepatic uptake of paclitaxel-loaded

- poly-(d-l-lactide-co-glycolide) nanoparticles - A possibility for hepatic drug targeting: Pharmacokinetics and biodistribution, *Int. J. Biol. Macromol.* 112 (2018) 818-830.
- [3] H. Meng, M.Y. Wang, H.Y. Liu, X.S. Liu, A. Situ, B. Wu, Z.X. Ji, C.H. Chang, A.E. Nel, Use of a lipid-coated mesoporous silica nanoparticle platform for synergistic gemcitabine and paclitaxel delivery to human pancreatic cancer in mice, *ACS Nano.* 9 (2015) 3540-3557.
- [4] F.W. Zhang, S.Y. Zhang, S.F. Pollack, R.C. Li, A.M. Gonzalez, J.W. Fan, J. Zou, S.E. Leininger, A. Pavia-Sanders, R. Johnson, L.D. Nelson, J.E. Raymond, M. Elsabahy, D.M. Hughes, M.W. Lenox, T.P. Gustafson, K.L. Wooley, Improving paclitaxel delivery: in vitro and in vivo characterization of PEGylated polyphosphoester-based nanocarriers, *J. Am. Chem. Soc.* 137 (2015) 2056-2066.
- [5] G. Bajaj, M.R. Kim, S.I. Mohammed, Y. Yeo, Hyaluronic acid-based hydrogel for regional delivery of paclitaxel to intraperitoneal tumors, *J. Control Release* 158 (2012) 386-392.
- [6] K. Patel, A. Patil, M. Mehta, V. Gota, P. Vavia, Medium chain triglyceride (MCT) rich, paclitaxel loaded self nanoemulsifying concentrate (PSNP): a safe and efficacious alternative to Taxol, *J. Biomed. Nanotechnol.* 9 (2013) 1996-2006.
- [7] Q. Chen, X. Wang, C. Wang, L.Z. Feng, Y.G. Li, Z. Liu, Drug-induced self-assembly of modified albumins as nano-theranostics for tumor-targeted combination therapy, *ACS Nano.* 9 (2015) 5223-5233.
- [8] V.A. de Weger, J.H. Beijnen, J.H.M. Schellens, Cellular and clinical pharmacology of the taxanes docetaxel and paclitaxel--a review, *Anticancer Drugs* 25 (2014) 488-494.
- [9] Y.N. Zhong, K. Goltsche, L. Cheng, F. Xie, F.H. Meng, C. Deng, Z.Y. Zhong, R. Haag, Hyaluronic acid-shelled acid-activatable paclitaxel prodrug micelles effectively target and treat CD44-overexpressing human breast tumor xenografts in vivo, *Biomaterials* 84 (2016) 250-261.
- [10] F. Ravar, E. Saadat, M. Gholami, P. Dehghankelishadi, M. Mahdavi, S. Azami, F.A. Dorkoosh, Hyaluronic acid-coated liposomes for targeted delivery of paclitaxel, in-vitro characterization and in-vivo evaluation, *J. Control Release* 229 (2016) 10-22.
- [11] M. Almeida, M. Magalhães, F. Veiga, A. Figueiras, Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer, *J. Polym. Res.* 25 (2018) 31.

- [12] D. Niu, Y.S. Li, J.L. Shi, Silica/organosilica cross-linked block copolymer micelles: a versatile theranostic platform, *Chem. Soc. Rev.* 46 (2017) 569-585.
- [13] A.A. D'souza, R. Shegokar, Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications, *Expert Opin. Drug Deliv.* 13 (2016) 1257-1275.
- [14] S.M. Li, "Synthetic biodegradable medical polyesters", in "Science and Principles of Biodegradable and Bioresorbable Medical Polymers: Materials and Properties", X. Zhang, ed., Elsevier Woodhead Publishing - S&T Book Production, Chennai, (2016) 37-78.
- [15] K. Jelonek, S.M. Li, X.H. Wu, J. Kasperczyk, A. Marcinkowski, Self-assembled filomicelles prepared from polylactide/poly(ethylene glycol) block copolymers for anticancer drug delivery, *Int. J. Pharm.* 485 (2015) 357-364.
- [16] T. Ishida, H. Kiwada, Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes, *Int. J. Pharm.* 354 (2008) 56-62.
- [17] M. Barz, R. Luxenhofer, R. Zentel, M.J. Vicent, Overcoming the PEG-addiction: well-defined alternatives to PEG, from structure-property relationships to better defined therapeutics, *Polym. Chem.* 2 (2011) 1900-1918.
- [18] Y. Zhao, Y.X. Zhou, D.S. Wang, Y.J. Gao, J.W. Li, S.J. Ma, L. Zhao, C. Zhang, Y. Liu, X.R. Li, pH-responsive polymeric micelles based on poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) for tumor-targeting and controlled delivery of doxorubicin and P-glycoprotein inhibitor, *Acta Biomater.* 17 (2015) 182-192.
- [19] C.H. Wang, G.H. Hsiue, New amphiphilic poly(2-ethyl-2-oxazoline)/poly(L-lactide) triblock copolymers, *Biomacromolecules* 4 (2003) 1487-1490.
- [20] C.H. Wang, C.H. Wang, G.H. Hsiue, Polymeric micelles with a pH-responsive structure as intracellular drug carriers, *J. Control Release* 108 (2005) 140-149.
- [21] E. Vlassi, A. Papagiannopoulos, S. Pispas, Amphiphilic poly(2-oxazoline) copolymers as self-assembled carriers for drug delivery applications, *Eur. Polym. J.* 88 (2017) 516-523.
- [22] S. Gulyuz, U.U. Ozkose, P. Kocak, D. Telci, O. Yilmaz, M.A. Tasdelen, In-vitro cytotoxic activities of poly(2-ethyl-2-oxazoline)-based amphiphilic block copolymers prepared by CuAAC click chemistry, *Express Polym. Lett.* 12 (2018) 146-158.
- [23] Z.Q. Wang, X.R. Li, D.S. Wang, Y. Zou, X.Y. Qu, C.Y. He, Y.Q. Deng, Y. Jin, Y.H. Zhou, Y.X. Zhou, Y. Liu, Concurrently suppressing multidrug resistance and

- metastasis of breast cancer by co-delivery of paclitaxel and honokiol with pH-sensitive polymeric micelles, *Acta Biomater.* 62 (2017) 144-156.
- [24] L.Y. Qiu, L. Yan, L. Zhang, Y.M. Jin, Q.H. Zhao, Folate-modified poly(2-ethyl-2-oxazoline) as hydrophilic corona in polymeric micelles for enhanced intracellular doxorubicin delivery, *Int. J. Pharm.* 456 (2013) 315-324.
- [25] X. Wang, X. Li, Y. Li, Y. Zhou, C. Fan, W. Li, S. Ma, Y. Fan, Y. Huang, N. Li, Y. Liu, Synthesis, characterization and biocompatibility of poly(2-ethyl-2-oxazoline)-poly(d,l-lactide)-poly(2-ethyl-2-oxazoline) hydrogels, *Acta Biomater.* 7 (2011) 4149-4159.
- [26] Y.J. Gao, Y.F. Li, Y.S. Li, L. Yuan, Y.X. Zhou, J.W. Li, L. Zhao, C. Zhang, X.R. Li, Y. Liu, PSMA-mediated endosome escape-accelerating polymeric micelles for targeted therapy of prostate cancer and the real time tracing of their intracellular trafficking, *Nanoscale* 7 (2015) 597-612.
- [27] Y.J. Gao, Y.X. Zhou, L. Zhao, C. Zhang, Y.S. Li, J.W. Li, X.R. Li, Y. Liu, Enhanced antitumor efficacy by cyclic RGDyK-conjugated and paclitaxel-loaded pH-responsive polymeric micelles, *Acta Biomater.* 23 (2015) 127-135.
- [28] D.S. Wang, Y.X. Zhou, X.R. Li, X.Y. Qu, Y.Q. Deng, Z.Q. Wang, C.Y. He, Y. Zou, Y.G. Jin, Y. Liu, Mechanisms of pH-sensitivity and cellular internalization of PEOz-b-PLA micelles with varied hydrophilic/hydrophobic ratios and intracellular trafficking routes and fate of the copolymer, *ACS Appl. Mater. Interfaces* 9 (2017) 6916-6930.
- [29] X.K. Sun, X. Liu, C.L. Li, Y.D. Wang, L. Liu, F. Su, S.M. Li, Self-assembled micelles prepared from poly(ϵ -caprolactone)-poly(ethylene glycol) and poly(ϵ -caprolactone/glycolide)-poly(ethylene glycol) block copolymers for sustained drug delivery, *J. Appl. Polym. Sci.* 135 (2018) 45732.
- [30] H.P. Zhang, J.M. Ruan, Z.C. Zhou, Y.J. Li, Preparation of monomer of degradable biomaterial poly(L-lactide), *J. Cent. South Univ. Technol.* 12 (2005) 246-250.
- [31] S.C. Lee, Y. Chang, J.S. Yoon, C. Kim, I.C. Kwon, Y.H. Kim, S.Y. Jeong, Synthesis and micellar characterization of amphiphilic diblock copolymers based on poly(2-ethyl-2-oxazoline) and aliphatic polyesters, *Macromolecules* 32 (1999) 1847-1852.
- [32] K. Rajagopal, A. Mahmud, D.A. Christian, J.D. Pajerowski, A.E.X. Brown, S.M. Loverde, D.E. Discher, Curvature-coupled hydration of semicrystalline polymer amphiphiles yields flexible worm micelles but favors rigid vesicles: polycaprolactone-based block copolymers, *Macromolecules* 43 (2010)

9736-9746.

- [33] A. Dominguez, A. Fernandez, N. Gonzalez, E. Iglesias, L. Montenegro, Determination of critical micelle concentration of some surfactants by three techniques, *J. Chem. Educ.* 74 (1997) 1227-1231.
- [34] R.T. Liggins, H.M. Burt, Polyether-polyester diblock copolymers for the preparation of paclitaxel loaded polymeric micelle formulations, *Adv. Drug Deliv. Rev.* 54 (2002) 191-202.
- [35] J.T. Zhu, R.C. Hayward, Spontaneous generation of amphiphilic block copolymer micelles with multiple morphologies through interfacial instabilities, *J. Am. Chem. Soc.* 130 (2008) 7496-7502.
- [36] L. Yang, Z.X. Zhao, J. Wei, A.E. Ghzaoui, S.M. Li, Micelles formed by self-assembling of polylactide/poly(ethylene glycol) block copolymers in aqueous solutions, *J. Colloid Interface Sci.* 314 (2007) 470-477.
- [37] L. Yang, X.H. Wu, F. Liu, Y.R. Duan, S.M. Li, Novel biodegradable polylactide/poly(ethylene glycol) micelles prepared by direct dissolution method for controlled delivery of anticancer drugs, *Pharm. Res.* 26 (2009) 2332-2342.
- [38] K. Jelonek, S.M. Li, J. Kasperczyk, X.H. Wu, A. Orchel, Effect of polymer degradation on prolonged release of paclitaxel from filomicelles of polylactide/poly(ethylene glycol) block copolymers, *Mater. Sci. Eng. C Mater. Biol. Appl.* 75 (2017) 918-925.
- [39] M.A. Mees, R. Hoogenboom, Full and partial hydrolysis of poly(2-oxazoline)s and subsequent post-polymerization modification of the resulting polyethylenimine (co)polymers, *Polym. Chem.* (2018) 10.1039.C8PY00978C-.
- [40] K.M. Kem, Kinetics of the hydrolysis of linear poly[(acylimino)-ethylenes], *J. Polym. Sci. Pol. Chem.* 17 (1979) 1977-1990.
- [41] V.R.D.L. Rosa, E. Bauwens, B.D. Monnery, B.G.D. Geest, R. Hoogenboom, Fast and accurate partial hydrolysis of poly(2-ethyl-2-oxazoline) into tailored linear polyethylenimine copolymers, *Polym. Chem.* 5 (2014) 4957-4964.