Synthesis of pH-Sensitive Macromolecular Micelles from Amphiphilic Star Copolymers for Drug Delivery

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Abstract. Stimuli-responsive copolymers are a significant class of smart materials extensively studied for drug delivery systems. Two novel amphiphilic four-arm star copolymers poly(ε-caprolactone)-b-poly(hydroxyethyl methacrylate) (4sPCL-b-PHEMA) and poly(ε-caprolactone)-b-poly(diethylaminoethyl methacrylate)-b-poly(hydroxyethyl methacrylate) (4sPCL-b-PDEAM-b-PHEMA) with pH-responsive were designed and synthesized by a combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). The structure of the copolymers and the particle size of macromolecular micelles were investigated by FT-IR and DLS, and the controlled release kinetics of two copolymer micelles was detected using adriamycin (DOX), a hydrophobic antitumor drug. The results showed that, the both micelles have appropriate particle size and can be used for DOX carrier, the DLC of 4sPCL-b-PHEMA and 4sPCL-b-PDEAM-b-PHEMA micelles were 21.30% and 27.25%, respectively. The release process showed that, comparing to the diblock star copolymer, triblock star copolymer had more significant pH-sensitivity. The cumulative release percentage of DOX in triblock micelles gradually increased with the decrease of pH. The cumulative release percentage of 14 h at pH 2.2 was about 80%, which had significant pH-sensitivity. A pH-sensitive four-arm star copolymer was synthesized, which can be used as drug delivery system for further research.

1. Introduction
With the rapid development of materials science and nanotechnology, the application of nanomaterials provides a new way for cancer treatment[1]. Nanoscale drug carriers showed a wide application prospects in drug research and clinical application, with the advantages of reducing the side-effects of antitumor drugs, improving the stability in vivo, increasing blood circulation and drug solubility[2,3]. Drug carriers with a certain nanometer size can be accumulated in tumor tissues through EPR-mediated (enhanced permeability and retention), and effectively inhibit tumor growth by controlling specific drug release[4].

At present, nanocarrier materials mainly include mesoporous materials[5], graphene[6], semiconductor quantum dots[7], and polymers[8]. As a kind of polymer material, polymer has been widely used in the construction of antitumor drug carriers due to its advantages such as biocompatibility and modification[9]. However, the traditional polymer drug delivery system were often unable to release rapidly at the lesion location, resulting in multi-drug resistance of tumor cells and reducing the efficacy[10]. In order to solve these problems, a series of smart polymer micelles with stimuli-responsive were designed and synthesized by using the differences of tumor microenvironment, such as pH, reduced glutathione (GSH), and temperature, etc.[11-14]. These copolymer micelles were stable in normal tissues, but can release drugs rapidly by breaking chemical...
bond and depolymerizing structures at tumor sites [15,16]. Therefore, in the slow-release and controlled-release studies of antitumor drugs, stimuli-responsive polymers showed great application prospects.

Accordingly, we designed and synthesized two novel amphiphilic four-arm star copolymers with pH-responsive by a combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP) process, named poly(ε-caprolactone)-b-poly (hydroxyethyl methacrylate) (4sPCL-b-PHMEA) and poly (ε-caprolactone)-b-poly (diethylaminoethyl methacrylate)-b-poly(hydroxyethyl methacrylate) (4sPCL-b-PDEAM-b-PHEMA), which using diethylaminoethyl methacrylate (DEAM) and hydroxyethyl methacrylate (HEMA) as functional monomers, pentamethyldivinyltriamine (PMDETA) and CuBr as catalysts, 2-bromoisobutyrate poly(ε-caprolactone) (4sPCL-Br) as macroinitiator (Figure 1). The star copolymers were self-assembled into macromolecular micelles in aqueous medium, and the drug-loading properties and drug controlled release kinetics under different pH buffer solutions were also investigated.

2. Materials and Methods

2.1. Materials
ε-Caprolactone (ε-CL, 99%), 2-bromoisobutryl bromide (98%), pentamethyldivinyltriamine (PMDETA, 99%), doxorubicin hydrochloride (DOX-HCl, 98%), hydroxyethyl methacrylate (HEMA, 96%), diethylaminoethyl methacrylate (DEAM, 99%), stannous octoate (Sn (Oct)2, 95%) and CuBr were purchased from Aladdin (China). Pentaerythritol (99%) was purchased from Macklin (China). Pentaerythritol and ε-CL were dried under reduced pressure prior to use. The specifications of conventional reagents such as dichloromethane (DCM), tetrahydrofuran (THF), methanol (MeOH), N,N'-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were obtained from Tianjin Tianli Chemical Reagent Co. (China). Tetrahydrofuran (THF) and triethylamine (TEA) were further purified and distilled before used.

2.2. Preparation of Four-Arm Diblock Star Copolymer 4sPCL-b-PHEMA

2.2.1 Synthesis of hydroxy terminated poly(ε-caprolactone)(4sPCL-OH)
4sPCL-OH was synthesized by ROP process (Figure 1). The initiator pentaerythritol (0.068 g, 0.5 mmol), ε-CL (4.57 g, 40 mmol) and Sn(Oct)2 (0.1 wt% of ε-CL) were added to a 50 mL flask and dried under reduced pressure for 2 h. The reaction mixture was stirred at 120 °C for 24 h under the protection of nitrogen. Then, the concentrated liquid was dissolved with DCM, precipitated by cold MeOH, and washed three times. The white product (4sPCL-OH) was obtained by collecting precipitation and dried under vacuum at 35 °C for 24 h (yield: 3.7 g, 80%).

![Figure 1. Synthetic scheme of 4sPCL-b-PHEMA (a) and 4sPCL-b-PDEAM-b-PHEMA(b)](image-url)
2.2.2 Synthesis of the macroinitiator 2-bromoisobutyrate poly(ε-caprolactone) (4sPCL-Br)

4sPCL-OH (3.0 g) was dissolved in DCM (40 mL) in a 100 mL flask. After adding TEA (1 mL), the solution was cooled to 0 °C under the protection of nitrogen. 2-bromoisobutyric acid (1.2 g) was dissolved in DCM (15 mL) and slowly added into the 4sPCL-OH solution with a syringe. Then, the reaction mixture was stirred at room temperature for 48 h. After that, the reaction solution was washed with 5% NaHCO₃ solution and saturated NaCl solution for three times (3 × 30 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated by vacuum distillation. The concentrated liquid was precipitated by cold ether and washed three times. Finally, the yellow product (4sPCL-Br) was obtained by collecting precipitation and dried under vacuum at 35 °C for 24 h (yield: 2.3 g, 55%).

2.2.3 Synthesis of star copolymer 4sPCL-b-PHEMA

4sPCL-b-PHEMA was synthesized by ATRP process using 4sPCL-Br as initiator, CuBr as catalyst, PMDETA as ligand and HEMA as monomer (Figure 1). First, CuBr (29 mg), PMDETA (42 µL), 4sPCL-Br (0.2 g) and HEMA (0.3 g) were added to a schlenk flask and dissolved in 4 mL of 1,4-dioxane. The mixture was circulated through frozen, vacuumized, nitrogen filled and thawed for three times, and stirred at 60 °C for 24 h. Then, the reaction was terminated by exposing the mixture to air, and the remaining catalyst was removed through a neutral alumina column by using DCM as the eluent. The star copolymer was precipitated with cold petroleum ether and washed three times. The final product was dried under vacuum for 24 h (yield: 0.20 g).

2.3. Preparation of Four-Arm Triblock Star Copolymer 4sPCL-b-PDEAM-b-PHEMA

4sPCL-b-PDEAM was synthesized by ATRP process using 4sPCL-Br as initiator, CuBr as catalyst, PMDETA as ligand, and DEAM as monomer (Figure 1). 4sPCL-b-PDEAM-b-PHEMA was synthesized using 4sPCL-b-PDEAM as initiator, CuBr as catalyst, PMDETA as ligand and HEMA as monomer. The synthesis methods were same as 2.2.3. Yellow product (yield: 0.18 g) was finally obtained by two ATRP reactions.

2.4. Preparation of Macromolecular Micelles from Star Copolymers

Two star copolymers (1 mg 4sPCL-b-PHEMA or 4sPCL-b-PDEAM-b-PHEMA) were dissolved and dispersed in 1 mL THF and stirred at room temperature for 0.5 h. Then, 4 mL of water was slowly added to the above solution and stirred for 0.5 h to form the micelles. Finally, the THF and most of water were concentrated under reduced pressure using a rotary evaporator until the volume reached 2 mL.

2.5. Drug-Loading Content of Macromolecular Micelles

DOX-loaded micelles were synthesized as follows: 10 mg of star copolymers were dissolved in 1 mL THF/DMF mixed solvent (volume ratio = 3:1) to obtain solution (1), 3 mg of DOX·HCl and one drop of TEA were dissolved in 250 µL DMF to obtain solution (2), and the solution (2) was added to solution (1) and stirred for 0.5 h to obtain the solution (3). The mixture solution was dropped slowly in 5 mL water and stirred for 0.5 h to form the polymeric micelles. THF was removed with a rotary evaporator under reduced pressure. The resulting solution was transferred into a cellulose membrane bag (MWCO 3000 Da) and dialyzed against distilled water for 12 h. The distilled water was replaced every 1 h. After 12 h, the dialyzed solution was adjusted to 10 mL.

The drug-loading content (DLC%) of macromolecular micelles was measured as follows: 0.2 mL of DOX-loaded micelles was dissolved equally in 2 mL DMF/DMSO mixed solvent (volume ratio = 1:1). The DLC in macromolecular micelles was investigated by fluorescence spectrophotometer. Excitation wavelength was set to 470 nm, and fluorescence intensity at emission spectra from 500-700 nm were recorded. The concentration of DOX was calculated according to a standard curve of DMF/DMSO solution. From equation (1), the DLC of macromolecular micelles was calculated.

\[
\text{DLC(\%)} = \frac{\text{Weight of loaded drug}}{\text{Weight of copolymer}} \times 100\% \quad (1)
\]
2.6. In vitro Release of DOX from Macromolecular Micelles

The release kinetics of drug-loaded micelles in three different buffer solutions (pH = 2.2, 5.0 and 7.4) were studied. 2 mL of DOX-loaded micelles were transferred into dialysis bags (MWCO 8000-14000 Da). The dialysis bags were immersed in 20 mL PBS buffer solutions with different pH, and placed into a thermostatic water bath at 37 ºC. At different times, 2 mL of buffer solution outside the dialysis bag was taken out for analysis and it was replaced by an equal volume of fresh medium. The cumulative drug release percentage was determined by fluorescence spectrophotometer.

2.7. Characterization Methods

The structure of star copolymers were characterized by FT-IR spectroscopy (Bruker TENSOR 27). The particle size and polydispersity index (PDI) of macromolecular micelles were determined using dynamic laser light scattering (DLS; Malvern, ZEN3600). The drug-loading content and cumulative release percentage of micelles were carried out using fluorescence spectrophotometer (Perkin-Elmer, LS55).

3. Results and Discussion

3.1. Characterization of Star Copolymers

Figure 2 shows the FT-IR spectra of star copolymers. The wide absorption band at 3440 cm\(^{-1}\) was the characteristic absorption peak of –OH. The absorption band at 2855-2950 cm\(^{-1}\) were attributing to antisymmetric stretching of C-H from -CH\(_2\), -CH\(_3\), while the peaks at 1360-1450 cm\(^{-1}\) were belonged to symmetrical vibration and variable angle vibration peaks of C-H from -CH\(_2\), -CH\(_3\). The strong absorption band at 1729 cm\(^{-1}\) was stretching vibration peak of carbonyl (C=O). The absorption band at 1150-1290 cm\(^{-1}\) was twisted vibration of CH\(_2\). The absorption band at 724 cm\(^{-1}\) was in-plane rocking vibration of long-chain CH\(_2\), which was the characteristic peak of more than four CH\(_2\). In the spectrum of 4sPCL-\(b\)-PHEMA, wider and stronger –OH characteristic absorption peak can be observed, proving the structure of HEMA. In the spectrum of 4sPCL-\(b\)-PDEAM-\(b\)-PHEMA, the corresponding characteristic peaks can also be found, such as carbonyl, CH\(_2\), -OH, etc.

3.2. Characterization of Macromolecular Micelles

As shown in Table 1, The particle size and PDI of blank micelles were measured. These micellar sizes allow the micelles extravasate and accumulate in tumors via EPR effect, therefore these micelles should be suitable for anticancer drug delivery applications. The triblock copolymer micelles size was
much larger than diblock copolymer micelles, this is probably because the addition of DEAM can lead to an increase of micelles size.

| Table 1. The particle size and PDI of blank micelles |
|-----------------------------------------------|
| Blank micelles | Z-average(nm) | PDI   |
| 4sPCL-b-PHEMA  | 123.6         | 0.143 |
| 4sPCL-b-PDEAM-b-PHEMA | 201.1   | 0.239 |

The standard curve of DOX in mixed solvent ($V_{DMF}:V_{DMSO} = 1:1$) was $I = 8.8139 \rho + 7.9594$ ($r = 0.9984$), and the linear range was 0-20 $\mu$g/mL. The drug-loading contents were calculated using standard curve in mixed solvent and equation (1). The DLC of 4sPCL-b-PHEMA and 4sPCL-b-PDEAM-b-PHEMA micelles were 21.30% and 27.25%, respectively. The results showed that the DLC of triblock polymer was higher than diblock polymer. The high drug loading capacity maybe because that DEAM was a hydrophobic group which can increase the length of the hydrophobic chain of copolymer.

3.3. In vitro Release of DOX from Macromolecular Micelles

![Figure 3](image1.png)

**Figure 3.** Release of DOX from macromolecular micelles of 4sPCL-b-PHEMA (A) and 4sPCL-b-PDEAM-b-PHEMA (B) at different pH.

The standard curve of DOX in aqueous solution was $I = 60.5039 \rho + 7.4487$ ($r = 0.9978$), and the linear range was 0-10 $\mu$g/mL. Figure 3 shows the release kinetics of DOX from two star copolymer micelles in PBS buffer solutions with different pH. The results showed that pH had significant effect on the drug release of triblock copolymer micelles compared with diblock polymer micelles. When the pH decreased from 7.4 to 2.2, the DOX release of triblock micelles increased gradually. The cumulative release percentage of 14 h at pH 2.2 was about 80%, which had significant pH-sensitivity. At a lower pH, the DEAM molecule was protonated, which resulted in the dissociation of star copolymer, and DOX was released rapidly from micelles.

4. Conclusion

In summary, two amphiphilic four-arm star copolymers 4sPCL-b-PHEMA and 4sPCL-b-PDEAM-b-PHEMA were designed and synthesized by the combination of ROP and ATRP methods. The star copolymers can self-assembled into macromolecular micelles in aqueous medium. Both macromolecular micelles have appropriate particle sizes and can be used for DOX-loading. The release process of macromolecular micelles in different pH were studied. The results show that, comparing to the diblock star copolymer, triblock star copolymer had significant pH-sensitivity. The cumulative release percentage of DOX in triblock micelles gradually increased with the decrease of pH. The cumulative release percentage of 14 h at pH 2.2 was about 80%. A pH-sensitive four-arm star
Copolymer was synthesized, which can be used as drug delivery system for further research.

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