Amphiphilic block copolymeric micelles as chlorin e6 carriers☆

Yanling Guo[1,2,*], Jun Zhao[3], Ju Zuo[1], Wei Liu[2]

[1]*College of Chemistry*, *Nankai University*, *Tianjin* 300071, *China*; [2]*College of Science*, *Tianjin University of Science & Technology*, *Tianjin*, 300457, *China*; [3]*College of Material Science & Chemical Engineering*, *Tianjin University of Science & Technology*, *Tianjin*, 300457, *China*

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Abstract

Methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) (MePEG-PCL) block copolymeric micelles containing chlorin e6 (Ce6) were prepared by a dialysis method. The size of micelle formed was less than 100 nm, and the size distribution of the micelle showed a narrow and monodisperse unimodal pattern. The release rate of Ce6 from nanospheres was slow. [Life Science Journal. 2008; 5(1): 46 – 50] (ISSN: 1097 – 8135).

Keywords: MePEG-PCL; micelle; chlorin e6; controlled release

1 Introduction

Micelles based on amphiphilic block copolymers (ABCs) as drug delivery systems (DDS) have the functional properties of solubilization, stabilization and controlled release for drug therapy. For this reason, increased attention has been paid in recent years to the use of ABCs for drug carrying and controlled releasing[1].

ABCs in drug delivery often consist of poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO), non-toxic water-soluble polymer for hydrophilic block and poly (propylene oxide) (PPO), poly(ester)[2], e.g., poly(lactic acid) (PLA), poly(ε-caprolactone) for hydrophobic block. Especially, poly(ε-caprolactone) is a well-known biodegradable and biocompatible polymer for hydrophobic block[3].

ABC micelle intimately ties drug for its spherical supramolecular core-shell structure which forms above the ABCs critical micelle concentration (CMC).

Chlorin e6 (Ce6) (Figure 1) is a chlorophyll derivative as a photosensitizer used for photodynamic therapy (PDT). PDT has been approved in many countries for the treatment of lung, esophagus, bladder, skin and head and neck cancers[4]. Ce6 has improved efficacy[5] and has decreased side effects compared to first generation photosensitizers from hematoporphyrin derivatives. In order to enhance solubility and activity of Ce6, several water-soluble its derivatives[6] and polymer conjugation[7,8] have been designed and been synthesized. Conjugation of Ce6 to microspheres was found to be of higher specificity for the MGH-U1 human bladder carcinoma cells than Ce6 alone[9].

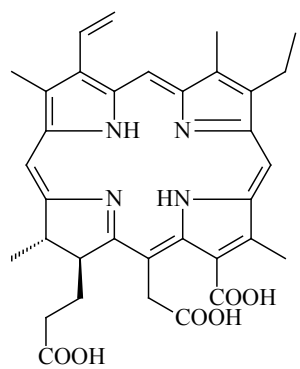

Figure 1. Structural formula of Chlorin e6 (Ce6).

In this report, we synthesized methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) (MePEG-PCL) amphiphilic block copolymers, and prepared its micelles. Then

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*Corresponding author. Email: nkylguo@yahoo.com.cn

Ce6 was introduced into the micelles, and its release behavior from micelles *in vitro* was studied.

2 Materials and Methods

2.1 Chemicals

Methoxy PEG (MePEG) with a molecular weight of 5,000, ε-caprolactone, stannous 2-ethyl hexanoate (stannous octoate, SnOct), pyrene were purchased from Aldrich. MePEG was first purified by precipitation into hexane from THF, and then the vacuum-dried precipitates were further dried by azeotropic distillation with dry toluene. ε-caprolactone was purified by vacuum distillation over CaH_2. Ce6 was obtained from Sigma. All other chemicals and solvents were analytical grades.

2.2 Synthesis of MePEG-PCL diblock copolymers

MePEG-PCL diblock copolymers with different PCL block lengths were synthesized by a ring opening polymerization of ε-caprolactone using MePEG as an initiator and SnOct as a catalyst. A weighed amount of MePEG was dissolved in toluene/xylene as cosolvent (12.5 ml toluene/g MePEG, 5 ml xylene/g MePEG) in a flask, and then was heated in an oil bath at 120 °C[10]. After the moisture was removed by azeotropic with evaporation of a small part of the toluene, the flask was cooled to 60 °C. One drop of SnOct (0.1 ml) and a predetermined amount of ε-caprolactone were added. The target molecular weights of PCL blocks were 5,000 and 8,000, respectively. Then the polymerization reaction was under dry nitrogen and 140 °C in oil bath for 24 h. After the reaction finished, the crude product was precipitated from excess amount of methanol, the precipitate was collected by centrifugate and washed several times with methanol. The final product was dried in a vacuum oven at 40 °C for 48 h.

The copolymers were characterized with ^{1}H NMR, GPC.

2.3 Preparation of MePEG-PCL diblock copolymer micelles

20 mg MePEG-PCL diblock copolymer was dissolved in 5 ml DMF. Then the solution was added drop by drop to 5 ml distilled pure water, and dialyzed against 400 ml pure water, which was renewed every 3 h during 24 h.

2.4 Measurement of micelles size distribution

The average size and the size distribution of micelles were obtained by Brookhaven 90Plus Particle Size Analyzer at 25 °C. Samples were filtered with 4.5 μm microfilter before measurements.

2.5 Measurement of critical micellar concentration (CMC) of copolymers

Acetone solvent of pyrene was added to 10 ml volumetric flasks, the acetone was then removed and polymeric aqueous solutions with various concentrations were added. The final concentration of pyrene in aqueous solution was 6×10^{-7} M. Pyrene fluorescence spectra were obtained by using Simadzu RF-5301 spectrofluorophotometer. The intensity ratio of peaks at 339 nm to those at 334 nm from pyrene excitation spectra versus the logarithm of the copolymer concentration was used to measure CMC[11].

2.6 Preparation of Ce6-loaded micelles

20 mg MePEG-PCL diblock copolymers and 5 mg Ce6 were dissolved in 5 ml DMF, the solution was added drop by drop to 5 ml distilled pure water, and dialyzed against 400 ml pure water, which was renewed every 3 h during 24 h, to removed DMF and formation of micelles. The micelles solutions were centrifugated to eliminate the uncomplicated Ce6 and aggregated particles.

2.7 *In vitro* drug release studies

10 ml Ce6-loaded micelle solution was introduced into dialysis membrane bag and the bag was placed in 100 ml phosphate buffer solution (PBS, pH 7.4) release media, and the media were stirred at 37 °C. At predetermined time, 10 ml aliquots of the aqueous solution were withdrawn from the release media, and 10 ml PBS was renewed into the release media[12]. The concentration of Ce6 in samples was monitored using a UV spectrophotometer at 405 nm.

3 Results

3.1 Copolymer synthesis

MePEG-PCL diblock copolymers with different hydrophobic PCL block lengths were synthesized by ring opening polymerization mechanism of ε-caprolactone (ε-CL) in the presence of MePEG, containing hydroxyl functional group at one end of the chain, with SnOct as catalyst (Figure 2).

The molecular weights of the copolymers were determined by ^{1}H NMR spectroscopy and GPC measurement. In ^{1}H NMR spectrum, typical signals at 3.65 ppm and 2.13 ppm were assigned to methylene proton of PEG chain and α-methylene proton in PCL respectively. The number average molecular weights detected by GPC were 11,734 and 14,100 for MePEG-PCL 5/5 and MePEG-PCL 5/8, the results exhibited the molecular weight of products were similar to the copolymers we predesigned.

3.2 Micelle preparation and size distribution

The diblock copolymer micelles were prepared by a dialysis method. DMF was selected as the organic solvent because it dissolves both copolymer and Ce6, and is miscible with water. Most of the micelles possess sizes < 100 nm in mean diameter. As the molecular weight of copolymer increased, the size of micelle formed increased. Particularly, they are 57.5 nm and 93.5 nm for MePEG-PCL 5/5 and MePEG-PCL 5/8 , respectively. The size of micelles were distributed with narrow peak (Figure 3).

Critical micelle phenomena in block copolymer systems occur at very much lower concentrations than in

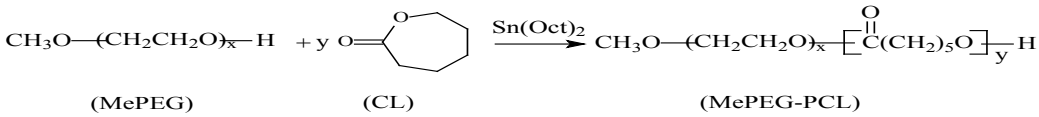

Figure 2. Synthetic route of MePEG-PCL diblock copolymers.

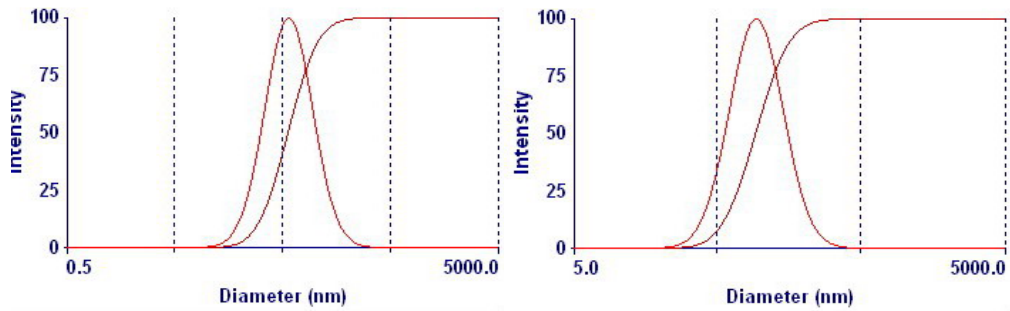

Figure 3. Size distributions of MePEG-PCL micelle in water (a: MePEG-PCL 5/5 micelle; b: MePEG-PCL 5/8 micelle).

low molecular amphiphiles. At concentration below the CMC, the collapsed blocks begin to associate to form loose aggregates. The CMC values were calculated to be 1.741×10^{-3} g/L and 1.413×10^{-3} g/L for MePEG-PCL 5/5 and MePEG-PCL 5/8. As the hydrophobic components in a copolymer increased, CMC values deceased.

3.3 Drug loading efficiency and release behaviours

We calibrate with Ce6 standard solutions (0 – 0.035 mg/ml) in water-DMF (1 : 9, v/v) at 405 nm UV absorbance. The polynomial calibration equation for Ce6 was Abs = 107.09849 × Conc. + 0.5491.

The amount of Ce6 introduced into the micelle was 2.04 mg and 1.83 mg for MePEG-PCL 5/5 and MePEG-PCL 5/8. It indicates that drug efficiency decreased with the increased molecular weights and hydrophobic chain lengths of a block copolymer.

Figure 4 shows a release profile of Ce6 from MePEG-PCL micelles prepared by copolymers of different molecular weights.

The release rate of Ce6 from diblock copolymeric micelles seems to decrease with an increase in molecular weight of MePEG-PCL. In the release patterns of all the samples, we could not observe an initial burst release effect, the drug release rate was constant (release profile shows nearly linear).

4 Discussions

MePEG-PCL diblock copolymers were successfully synthesized by solution polymerization. Results from ^{1}H NMR shows the molecular weight ratio of copolymers were identical to those we predetermined. In GPC analysis, it exhibited a narrow and symmetrical peak of the copolymers' molecular weight distribution.

The amphiphilic MePEG-PCL copolymers with hydrophilic MePEG blocks and hydrophobic PCL blocks can self-associate to form core-shell micelles in aqueous environment. The micelles in aqueous environment can easily be verified by particle size analyzer, which provides the mean diameters and size distribution of mi-

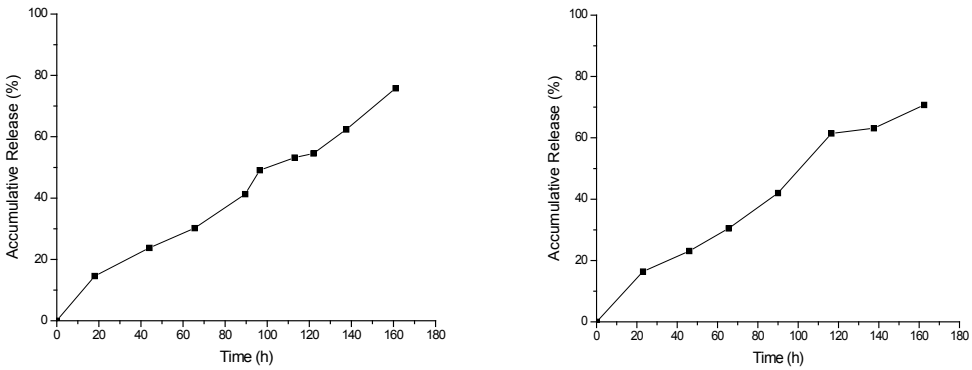

Figure 4. *In vitro* accumulated release amount of Ce6 from drug-loading micelles (a: MePEG-PCL 5/5; b: MePEG-PCL 5/8).

celles. The mean diameters of micelles prepared by the dialysis method were under 100 nm. The particle size increased as the PCL block length increased. It originated from the increase of hydrophobic property by the longer hydrophobic PCL chain in the aqueous milieu. All micelles showed a narrow size distribution.

To investigate the self-aggregation behavior of MePEG-PCL diblock copolymers in aqueous environment, pyrene was used as a fluorescence probe. The excitation spectra of pyrene (Figure 5) has small changes in the polymeric solutions which copolymer concentration lower than CMC, but has a remarkable increase of intensity and a red shift when the concentration increased over CMC. So we could use pyrene probe to calculate the CMC of the copolymers by crossover point of the intensity ratio of $I_{339/I334}$ from pyrene excitation spectra *vs*. log C for various copolymeric solutions (Figure 6).

The Ce6 released from drug-loading micelles were low, it could release for more than 180 hours, shows sustained release characteristics. The release rate of Ce6 from drug-loading micelles was determined by the length of copolymer hydrophobic chains. Ce6 owning its lipophilic character, is physically entrapped in hydrophobic core of micelles, the interaction between PCL and Ce6 as hydrophobic part affects its release behaviors *in vitro*. The results suggest MePEG-PCL was a considerable potential for sustained release drug delivery system to enhance the solublility of hydrophobic drug and minimize drug toxicity and maximize drug effectiveness.

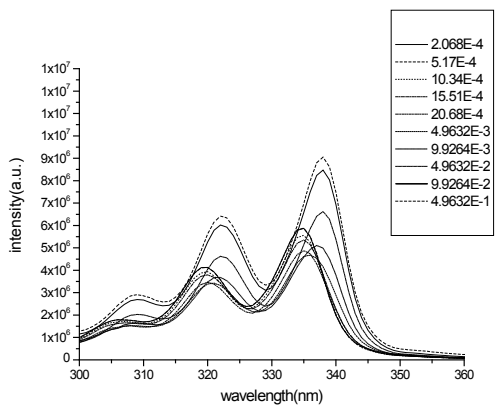

Figure 5. Excitation spectra of pyrene in various MePEG-PCL aqueous solutions.

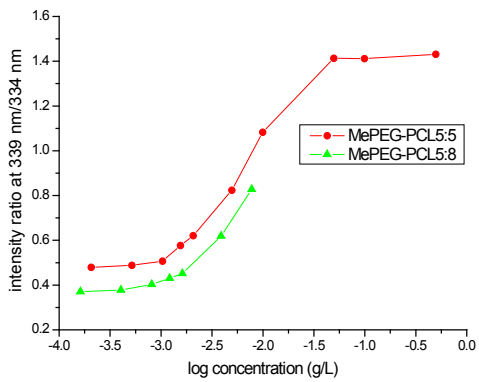

Figure 6. Intensity ratio ($I_{339/I334}$) from excitation spectra *vs*. log concentration of MePEG-PCL in aqueous solutions.

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