Journal of Molecular Liquids 266 (2018) 405–412

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

pH responsive self-assembly and drug release behavior of aliphatic liquid crystal block polycarbonate with pendant cholesteryl groups

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ARTICLE INFO

Article history:
Received 2 April 2018
Received in revised form 13 June 2018
Accepted 21 June 2018
Available online 22 June 2018

Keywords:
Aliphatic polycarbonate
Liquid crystal
pH response
Self-assembly
Drug release

ABSTRACT

An amphiphilic liquid crystal (LC) block copolymer based on aliphatic polycarbonate with pendant cholesteryl groups was synthesized by the coupling reaction. The structure and LC properties of the obtained block copolymer were characterized using FT-IR, ^{1}H NMR, DSC, POM and XRD. The results showed the copolymer exhibited broken focal conic texture and fan-shaped texture of a smectic A phase during the heating and cooling process. Moreover, this copolymer showed mesophase state below body temperature, and it would be promising candidate as self-assembly material for applications in drug delivery. In addition, the self-assembly, drug loading and release behavior of the LC copolymer were investigated with UV–vis, DLS and SEM. It was found that the initial copolymer concentration and the pH value had a critical influence on the self-assembly behavior and the particle size of the micelles. What's more, the drug release also presented a pH response and the drug-loaded copolymer micelles were stable.

1. Introduction

Biodegradable polymeric materials have extensive applications in biomedical uses, such as drug delivery, tissue engineering and surgical suture, and so on [1]. In recent years, in order to meet the requirements of biomedicine and other fields, the preparation of nanosystems with high biosafety, biocompatibility, biodegradability and environmental response has become a new development trend. At present, aliphatic polycarbonates have become the research focus due to its non-acidic degradation products and adjustable structure [2]. What's more, the degradation mechanism of aliphatic polycarbonates is surface erosion, different from the bulk degradation of polyesters. And there is no acid created in the degradation process of polycarbonates, which may occur during that of polyesters, and the acid product may be hazardous to the cells and tissues of human body [3,4].

Poly(trimethylene carbonate) (PTMC), as a representative aliphatic polycarbonate, has been investigated in drug delivery vehicles and degradation by some researches [5–9]. However, it is found that PTMC cannot satisfy many special application requirements with some flaws such as high hydrophobicity, improper degradation profile and low degradation rate, lack of functional primitives to link bioactive molecules like siRNA, plasmid DNA, peptide, proteins and drugs. In order to improve the mechanical performance of PTMC, several copolymers were developed with other ester monomers such as ε-caprolactone (CL) [10–12], L-lactide and D,L-lactide [13–15], but there is a major drawback of the copolymer micelles that the stability in body is relatively poor, which would lead to the rapid decomposition of the drug-loaded system and burst release of drug [16]. To solve the problem, one of the innovative approaches is to introduce functional groups such as hydroxyl, carboxyl and amino groups in the side. So the poly(5-hydroxy-trimethylene carbonate) (PHTMC) gradually becomes the focus of research. PHTMC is a kind of hydroxyl-enriched polycarbonates with high hydrophilicity and biocompatibility, and the degradation rate is much faster than that of PTMC, and can be further constructed as drug delivery nanocarriers [17,18].

Polycarbonates bearing several other functional groups such as poly (ethylene glycol) (mPEG) and some bioactive or biocompatible molecules including cholesterol, diosgenin, lipid, vitamin, hormone and peptide have also been developed and investigated to adjust the properties for biomedical applications [3,19]. mPEG is used extensively in the delivery of various bioactive molecules due to its good water solubility, biocompatibility and non-toxicity, and it could not only enhance biocompatibility but also favorably affect the performance of drug loading and degradation [20,21]. As known, cholesterol is an important structural component in mammalian membranes with highly hydrophobic skeleton, good biocompatibility with cells and definite biodegradability, and amphiphilic polymers with cholesteryl functionalized have become a major part of self-assembly research [22–26]. On the other hand,

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https://doi.org/10.1016/j.molliq.2018.06.086
0167-7322/

cholesteryl derivatives can exhibit liquid-crystal (LC) phases, and LC can form an orientational order, which may provide driving force for molecule self-assembly. Zhuo and coworkers [1,27] reported the end-capped polycarbonates using cholesterol as an initiator to initiate the ring-opening of CL and TMC, and they had low cytotoxicity and excellent performance of drug release with the introduction of cholesteryl group. Lee et al. [28,29] reported a novel polymer with pendant cholesteryl groups, and the polymer micelles had a relatively high drug loading efficiency. So it is very significant to study the self-assembly and drug loading behavior of new PTMC with LC cholesteryl groups.

In the previous work, we reported the synthesis and self-assembled morphology of new aliphatic polycarbonate copolymers with diosgenin as side groups [30]. In this study, an aliphatic LC block polycarbonate functionalized with pendant cholesterol was synthesized, named as $mPEG_{43}$-*b*-P[$(TMC\text{-}C)_{28}$-$HTMC_3$]. In the designed copolymer structure, the aliphatic polycarbonate PHTMC was used as the basic skeleton, and mPEG was introduced and part of the hydroxyl group was retained to improve hydrophilicity. And LC cholesteryl groups were introduced to improve biocompatibility and driving force for molecule self-assembly. The structure of the LC block copolymer was characterized by Fourier transform infrared spectrum (FT-IR), proton nuclear magnetic resonance spectrum (^{1}H NMR), and the LC properties were investigated using polarizing optical microscopy (POM), differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Furthermore, the pH responsive self-assembly, drug loading and release behavior were studied with UV–vis, dynamic light scattering (DLS) and scanning electron microscope (SEM).

2. Experimental method

2.1. Materials

All chemicals were obtained from the indicated sources. Cholesterol was purchased from Xiayi Beier Biological Products Co., Ltd. (Xiayi, China) and purified by recrystallization with ethyl alcohol. Glycerol, benzaldehyde, benzyl chloride and triethylamine were purchased from Tianjin Bodi Chemical co., Ltd. (Tianjin, China). Ethyl chloroformate was obtained from Xinyi Huili Fine Chemical Co., Ltd. (Xinyi, China). Palladium on activated carbon (10% Pd), palladium hydroxide on Carbon (wetted with ca. 50% Water), adipic acid, hexadecyl trimethyl ammonium bromide, dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shenyang, China). Stannous octoate [Sn$(Oct)_2$] and methoxypolyethylene glycols ($mPEG_{43}$, M_n = 1900) were purchased from Sigma-Aldrich (Shanghai, China). Doxorubicin hydrochloride (DOX·HCl) was purchased from Ark Pharm, Inc. (Arlington, IL, USA). Toluene was dried and distilled over Na before use. All other solvents and reagents used were purified by standard methods.

2.2. Measurements

2.2.1. FT-IR

The samples were pressed into KBr pellets to be measured on a PerkinElmer spectrum One (B) spectrometer (PerkinElmer, Foster City, USA).

2.2.2. ^{1}H NMR spectra

^{1}H NMR spectra were performed on a Bruker ARX 600 (Bruker, Karlsruhe, Germany) high resolution NMR spectrometer, and tetramethylsilane (TMS) was used as an internal standard to report chemical shifts in ppm.

2.2.3. Differential scanning calorimetry (DSC)

The thermal behavior was determined with a Netzsch 204 DSC (Netzsch, Hanau, Germany) with a heating and cooling rate of 10 °C/min in a nitrogen atmosphere.

2.2.4. Polarizing optical microscope (POM)

The LC optical textures were observed and recorded with a Leica DMRX POM (Leica, Wetzlar, Germany) equipped with a Linkam THMSE-600 (Linkam, London, UK) cool and hot stage.

2.2.5. X-ray diffraction (XRD)

XRD measurements were performed with a nickel-filtered Cu-Kα radiation (λ = 1.54 Å) and a Bruker D8 Advance (Bruker, Karlsruhe, Germany) powder diffractometer.

2.2.6. UV–vis

The absorption spectra during the process of self-assembly and drug-loading were recorded using a TU-1901 double beam UV–visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China).

2.2.7. Dynamic light scattering (DLS)

The particle size and distribution of the polymer micelles were measured on a Zetasizer Nano S instrument (Malvern Instruments Ltd., Worcestershire, UK) equipped with a He-Ne laser (633 nm) set at 173° for scattering angle at 25 °C.

2.2.8. Scanning electron microscope (SEM)

The morphology of the samples was determined using a Hitachi ×650 (Hitachi, Tokyo, Japan). The samples must be pretreated with a thin gold film before measured.

2.3. Synthesis of $mPEG_{43}$-*b*-P[$(TMC\text{-}C)_{28}$-$HTMC_3$]

The synthetic route of the block copolymer $mPEG_{43}$-*b*-P[$(TMC\text{-}C)_{28}$-$HTMC_3$] is outlined in Scheme 1. The cyclic monomer BTMC, the LC monomer 6-cholesteroxy-6-oxocaproic acid (C), the copolymers $mPEG_{43}$-*b*-$PBTMC_{31}$ and $mPEG_{43}$-*b*-$PHTMC_{31}$ were synthesized according to our previous works [31–33].

The LC monomer C (4.63 g, 9 mmol) and dichloromethane (50 mL) were placed in a 250 mL of three-necked flask and stirred until fully dissolved. DCC (2.06 g, 10 mmol) and DMAP (0.21 g, 4 mmol) were dissolved in 10 mL of dichloromethane and added dropwise to the above solution and then stirred for 0.5 h. $mPEG_{43}$-*b*-$PHTMC_{31}$ (1.79 g, 10 mmol for —OH groups) was dissloved in 50 mL of dichloromethane and added dropwise. After the mixture was reacted for 48 h at room temperature, 20 mL of deionized water was added and stirred for 1.0 h. The mixture was filtered to remove *N*,*N*′-dicyclohexyl urea, and the filtrate was evaporated to dryness. The crude polymer was purified in dichloromethane and precipitated in methanol, and then dried in vacuum at room temperature until constant weight was obtained. IR (KBr, cm^{-1}): 2951, 2868(—CH_2—, —CH_3); 1745 (C═O); 1674 (C═C); 1256, 1171 (C—O—C); ^{1}H NMR (δ, $CDCl_3$, 600 MHz): δ = 5.37 (—C***H***═C in cholesteryl), δ = 5.27 (—OCH_2—C***H***—CH_2O— in PHTMC), δ = 4.61 (—COOC***H***< in cholesteryl), δ = 4.38–4.26 (—OC***H***$_2$—CH—C***H***$_2$O), δ = 3.64 (—OC***H***$_2$— in mPEG), δ = 2.39–0.68 (the rest of the protons from PTMC, cholesteryl and —COO(C***H***$_2$)$_4$COO—).

2.4. Preparation of the LC copolymer aggregates

The LC block copolymer $mPEG_{43}$-*b*-P[$(TMC\text{-}C)_{28}$-$HTMC_3$] with different mass (1.7 mg, 2.5 mg, 5.0 mg) was dissolved in 10 mL of THF and the solution was stirred overnight. Then the distilled water with different pH of 5–9 was added dropwise with gentle agitation, and the pH was adjusted by the addition of hydrochloric acid or ammonia. The solution was allowed to stand for 5 min to balance and the transmittance (T%) of the mixed solution was monitored with UV–vis. The final solution was transferred to dialysis membrane with a molecular weight cut-off (MWCO) of 3500 Da and dialyzed for 48 h in distilled water with corresponding pH value and the water was changed every 6 h. The