

Original Research Article

Study on synthesis of star poly(ϵ -caprolactone)-g-polylysine copolymer, characterization and its micelles'properties

ABSTRACT

Four-armed star poly(ϵ -caprolactone) (PCL) terminated with carboxy group was prepared by two-step reaction. The carboxy group of star PCL was activated and chemically conjugated to the amine group of PLL (polylysine) to produce sPCL-g-PLL copolymer. The structure of final product and the intermediates were characterized by IR and ^1H NMR. The sPCL-g-PLL copolymer can self-assemble into micelle in aqueous solution. The CMC (critical micelle concentration) of micelle ranged from 7.603×10^{-4} to $2.638 \times 10^{-4} \text{ mg} \cdot \text{mL}^{-1}$ for different sPCL-g-PLL copolymer. In addition, Curcumin as a hydrophobic drug can be entrapped in the sPCL-g-PLL micelle. The results indicated that the micelle exhibited 9.431% loading capacity for curcumin, and it can be used as a potential delivery carrier for the hydrophobic drug.

Keywords *Star amphiphilic block polymer; poly(ϵ -caprolactone)-g-polylysine; Biodegradable material; Polymer micelles; Curcumin*

ABBREVIATIONS

sPCL-b-PLL: star poly(ϵ -caprolactone)-b-polylysine copolymer ,

CMC: critical micelle concentration,

PLL: polylysine,

sPCL-COOH: star poly(ϵ -caprolactone) terminated with carboxyl group,

sPCL-OH: star poly(ϵ -caprolactone) terminated with hydroxyl Group,

DPH: 1,6 - diphenyl-1,3,5-hexatriene.

1. INTRODUCTION

Amphiphilic polymers can self-assemble into micelles in aqueous medium, which has a nanoscale hydrodynamic radius with core-shell structure. The micelles have the properties of long-circulation in blood, stealth and stability in vivo. Due to enhanced permeability and retention effect of the micellar particle, they can concentrate on tumor location to realize the passive

targetibility to tumor tissue. In addition, the micellar particle can enhance anti-tumor efficacy by increasing tumor drug deposition, and reduce drug toxicity by avoiding critical normal tissues^[1].

Currently, the considerable research has been focused on the synthesis of linear block copolymer^[2,3]. Compared with linear polymers, star copolymers can form micelles in aqueous medium more easily, which has the better stability and benefit the drug loading and delivery^[4,5].

Considering biosecurity of the material in vivo, amphiphilic polymers for drug delivery system should be biodegradable and biocompatible. The micelles have broad application in the field of medicine and biological technology^[6,7], which compose from PEG and polyester such as PCL, PLGA, polyaminoacids.

In this paper, a new four-armed star poly(ϵ -caprolactone)-g-polylysine copolymer (sPCL-g-PLL) was synthesized, the self-assembling behaviour of micelle was further examined. Curcumin was used as the hydrophobic model drug, and the drug loading capacity was investigated. It was hoped that sPCL-g-PLL micelle was explored as the potential drug carrier.

2 METHODOLOGY

2.1 Reagents and instruments

Reagents

The polylysine (PLL, $M_n=3000$) was purchased from Zhejiang Yinxiang Bioengineering Co. Ltd.; ϵ -caprolactone and stannous octoate were purchased from sigma company; Pentaerythritol and polyethylene glycol were purchased from Beijing Bailingwei Chemical Technology Co., Ltd.; Curcumin and succinic acid anhydride were purchased from the Guangfu Fine Chemical Research Institute of Tianjin city; N-Hydroxysuccinimide and N,N'-Dicyclohexylcarbodiimide were purchased from Shanghai Gil Chemical Co., Ltd..

Instruments

Bruker 500 plus NMR; Japanese JEM100CXII transmission electron microscope; Vcx-400 sonic&material Ultrasonic instrument; Malvern size and potential analyzer; SHA-C bath oscillator; Shimadzu UV-2401 ultraviolet spectrophotometer; FD-1C-50 freeze-dryer; Brook ALPHA Fourier infrared spectrometer.

2.2 Experimental

2.2.1 Synthesis and characterization of sPCL-g-PLL copolymer

A certain proportion of caprolactone (20 mL), pentaerythritol (0.1225g, 0.0009 mol) and $\text{Sn}(\text{Oct})_2$ (0.058 mL) was added into dichloromethane solution in a sealed tube for melting polymerization. The tube was repeatedly pumped vacuum and ventilated with nitrogen. The reaction carried out at 60 °C for 24 h. The mixture in the sealed tube was dissolved in dichloromethane after the reaction finished, and precipitated and purified twice with anhydrous methanol. Finally, the product sPCL-OH was dried under vacuum, whose molecular weight can be controlled by the ratio of initiator/monomer (1:50). The detailed synthetic procedure of sPCL-COOH was as follows. Two times amount of succinic anhydride reacted with sPCL-OH for 24 h at room temperature under continuous stirring. The solvent was removed after the reaction finished. Then the concentrated solution was dissolved in hot water and extracted three times with dichloromethane. sPCL-COOH was obtained by methanol recrystallizing and vacuum dried. The next step was to yield the activated ester. The mixture of sPCL-COOH, DCC (0.2 mmol, 0.0413 g), NHS (0.2 mmol, 0.0232 g) and triethylamine (0.2 mmol, 0.1 mL) was added to 30 mL CH_2Cl_2 solution (containing 1g PCL-COOH). The above reaction conducted for 12 h under N_2 atmosphere at room temperature. The reactant solution was filtered. Subsequently, a certain proportion of polylysine was added to above solution, in which the different feeding molar ratio $n(\text{sPCL-COOH}) : n(\text{PLL})$ were 1:1, 1:3 and 1:5 respectively. After 24 h reaction, the reaction solution was transferred to dialysis bags (MW, 7000 D) and dialyzed for three days to remove unreacted PLL and other small molecules. Finally, sPCL-g-PLL was obtained by freeze-drying, whose structure was further characterized by IR and ^1H NMR.

2.2.2 Amphiphilic behaviour investigation of sPCL-g-PLL micelle

Choosing 1,6-diphenyl-1,3,5-hexatriene (DPH) as a molecular probe, CMC of amphiphilic sPCL-g-PLL polymers were determined by the hydrophobic dye solubilization method^[9], sPCL-g-PLL copolymer dissolved into different concentration solution of (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mg/mL). 4 mL of the DPH/methanol solution (1 mM) was added to different glass tube, wherein removing methanol by flushed with nitrogen, then 4 mL of the sPCL-g-PLL solution with different concentrations was transferred to each DPH glass tube. All of the solution in glass tube were sonicated for 30 min in the dark to dissolve DPH completely. Finally, the absorbance of sample solution was determined by UV spectroscopy at 200-500 nm.

2.2.3 Curcumin loading sPCL-g-PLL micelles

Drug-loaded micelles were prepared by dialysis. A more detailed procedure is described

below.100 mg sPCL-g-PLL and 10 mg Curcumin dissolved in 10 ml DMSO, and the solution were added dropwise into 15 mL water under continuous stirring. After stirring for 3h at room temperature, the dialysis bag filled with the solution was placed in deionized water dialyzing for 48 h. Then the obtained drug-loaded micelles solution centrifuged at 10,000 rpm and filtered through 0.45 μm filtration membrane to remove free drug. Finally, the supernatant was freeze-dried to obtain the drug-loaded micelles. The diameter of drug-loaded micelles was measured by a laser particle size analyzer, and its morphology was observed by transmission electron microscopy. Taking drug loading amount and encapsulation efficiency as the index, the formulation of drug-loaded sPCL-g-PLL micelles was optimized.

2.2.4 drug loading capacity of drug-loaded micelles investigation

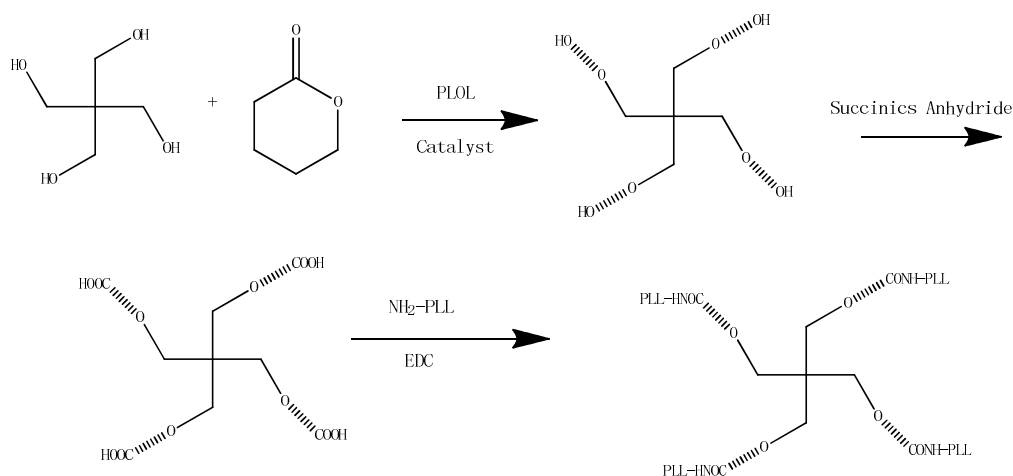
According to references[5] method, a certain amount of Curcumin-loaded micelles dissolved in DMSO to release the drug contained in micelles completely. The Curcumin content of drug-loaded sPCL-g-PLL micelles was determined by UV absorption spectroscopy at 428 nm. Encapsulation efficiency (EE%) and drug loading content (DL%) is calculated as follows:

$$\text{EE\%} = (\text{the amount of drug in the micelles} / \text{the amount of drug added initially}) / 100\%$$

$$\text{DL\%} = (\text{the amount of drug in the micelles} / \text{the amount of polymer carrier employed initially}) / 100\%$$

3 Results and discussion

3.1 Chemical Structure Characterization of sPCL-g-PLL



Scheme 1 The synthesis route of sPCL-g-PLL

Polylysine linked four-arm star-shaped PCL was realised by sPCL-COOH and the amino of PLL (varied feeded amount) by the activation of EDC and NHS. Synthetic route was shown as Scheme 1, wherein sPCL-OH was synthesized under the condition that the ratio of monomer and initiator was 1:50. The average molecular weight (M_n) of sPCL-OH was 9520.

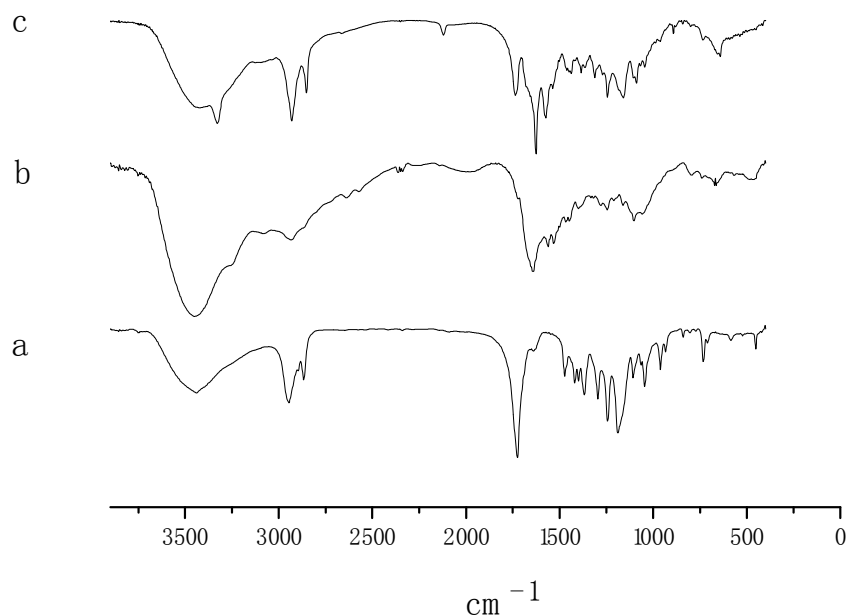


Fig.1 IR spectrum of sPCL-OH (a), PLL (b) and sPCL-g-PLL (c)

Figure 1 was IR spectra of sPCL-OH and sPCL-g-PLL. In figure 1(a), 3350 cm^{-1} was the absorption peak of sPCL-OH terminal hydroxyl groups, 2996 cm^{-1} was the methylene peak in PCL chain, 1640 cm^{-1} was the absorption peak of ester carbonyl in PCL. Figure 1(b) was the characteristic peak of polylysine in IR spectra. Compared with figure 1(a) and 1(b), figure 1(c) had the characteristic absorption peaks of both PCL and PLL. These results indicated that the sPCL-g-PLL copolymer was successfully synthesized.

The chemical structure of sPCL-OH, sPCL-COOH and sPCL-g-PLL was further confirmed by ^1H NMR. Figure 2 (A) was the ^1H NMR spectra of sPCL-OH, chemical shifts of 1.4-1.6, 2.4 and 4.1 respectively corresponded independent methylene protons, methylene protons of adjacent carbonyl and methylene protons of adjacent acyloxy oxygen atoms in PCL chain unit, these shifts proved the chemical structure of sPCL-OH polymer. After the reaction of sPCL-OH and succinic

anhydride finished, $\delta=2.7$ appeared as the new CH_2 chemical shifts of sPCL-COOH in figure 2 (B), it was corresponding to CH_2 chemical shift of succinic anhydride ($\delta=2.62$), which indicated that sPCL-COOH was produced by the reaction of sPCL-OH and succinic anhydride. Compared with the figure (B) and (C), figure(D) showed not only the PCL characteristic peak (1.4-1.6, 2.4 and 4.1), but also PLL characteristic peaks (3.82, 3.12, 1.27, 1.46 and 1.72), whereby the chemical structure of sPCL-g-PLL was further confirmed.. In addition, $\delta=2.5$ and $\delta=3.5$ were responding the peak of DMSO and water in the solvent D-DMSO.

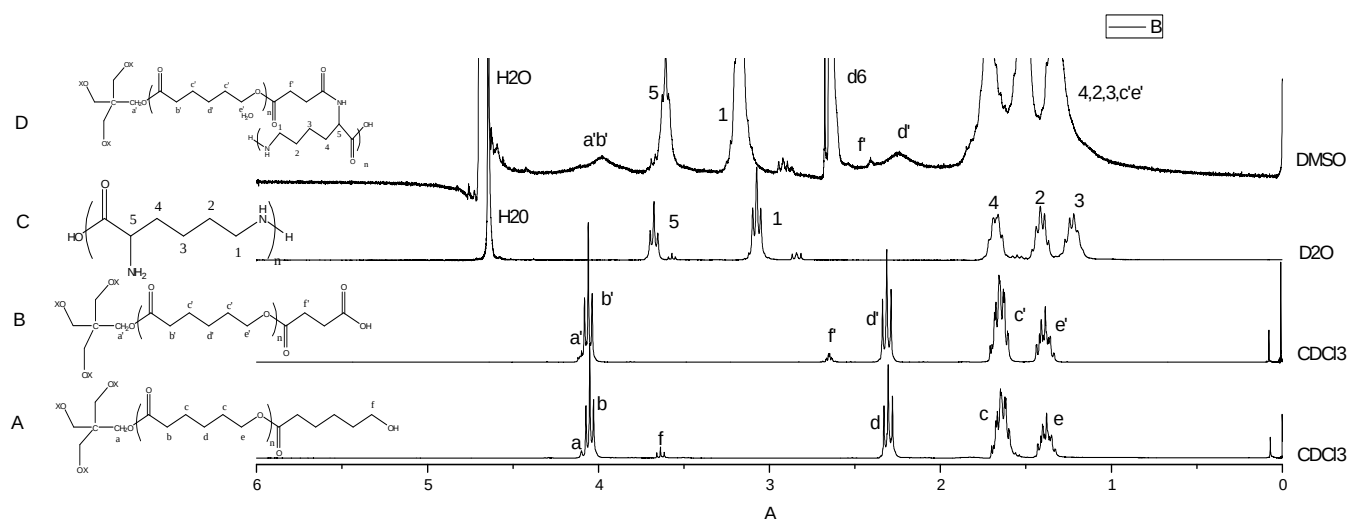


Fig.2 ^1H NMR spectrum of of sPCL-OH (A), sPCL-COOH (B) , PLL (C) and sPCL-g-PLL (D)

3.2 CMC detection of amphiphilic sPCL-g-PLL copolymer micelles

According the literature^[9], the CMC of star amphiphilic copolymer was measured by a hydrophobic dye solubilization method by taking DPH as molecular probes. Star amphiphilic polymer consisting of hydrophobic PCL and hydrophilic PLL can self-assemble into micelles by hydrophobic interaction in water. As a hydrophobic substance, DPH was very sensitive to polarity changes from environment. The absorbance greatly increased when the concentrations of copolymer solution was above the CMC (shown in figure 3). This phenomenon revealed that the hydrophobic DPH entered into hydrophobic regions of the aggregates, and its solubility increased in micelles. The results showed that from the table 1 the CMC of sPCL-g-PLL decreased as the PLL content of the sPCL-g-PLL copolymer increased .

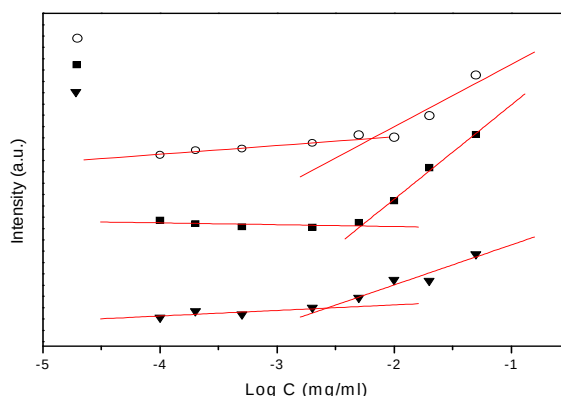


Fig. 3 Relationship of the absorption intensity at 356 nm for DPH as a function of the sPCL-g-PLL copolymer with different grafting ratio at the room temperature

3.3 drug-loaded content determination from the drug-loaded sPCL-g-PLL micelles

Three kinds of sPCL-g-PLL copolymer were obtained respectively by varied feeding ratio of PLL/sPCL-COOH, which were used to load Curcumin by the ratio of drug/carrier was 1:10. The drug-loaded content for different sPCL-g-PLL micelles was shown in table 1. It was shown that the encapsulation efficiency and drug loading content of sPCL-g-PLL₂ micelles were higher than the other carriers from the table 1. This fact indicated that it was beneficial to the drug loading when the ratio of the hydrophilic segment and the hydrophobic segment from amphiphilic polymer reached a certain proportion. TEM photograph of drug-loaded micelle was shown in figure 4, the drug-loaded micelle was nearly spherical and has small particle size (less than 100 nm).

Tab.1 Properties of the micelles formed by different sPCL-g-PLL copolymer

Copolymer	CMC(10^{-4} ,mg.mL ⁻¹)	Drug entrapment efficiency	Drug loading amount	Size(nm)
sPCL-g-PLL ₁ *	7.603	24.20%	2.42%	32.54
sPCL-g-PLL ₂ *	5.275	94.31%	9.43%	56.51
sPCL-g-PLL ₃ *	2.638	41.09%	4.26%	47.76

* sPCL-g-PLL_n corresponding to the different initial feeding molar ratio [n(sPCL-COOH):n(PLL)] were 1:1 (n=1), 1:3 (n=2) and 1:5 (n=3).

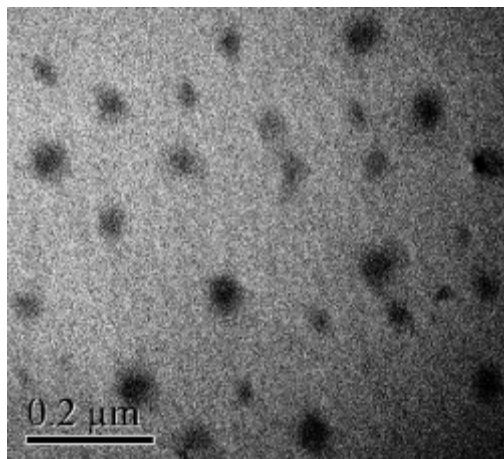


Fig.4 TEM photographs of drug-loaded sPCL-g-PLL₂ micelle

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167 **4. CONCLUSION**

168 Summary, amphiphilic sPCL-g-PLL copolymer was synthesized, whose structures were
 169 characterized by FT-IR and ¹H NMR. The copolymer can self-assemble into micelles, which has
 170 lower critical aggregation concentration than other ordinary micelles. It was also revealed that
 171 sPCL-g-PLL micelles had extraordinary loading ability of Curcumin, and sPCL-g-PLL micelles can be
 172 used as a new carrier of hydrophobic drugs.

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