Poly(caprolactone)–Pluronic– poly(caprolactone) amphiphilic copolymer nanoparticles for controlled 5-Fluorouracil release

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ABSTRACT

In this work amphiphilic triblock copolymers poly(ζ-caprolactone)/Pluronic/ poly(ζ-caprolactone) were synthesized by Lipase B catalyzed ring-opening polymerization of ε- caprolactone monomer initiated by Pluronic. Nanoparticles were prepared using prepared copolymers by solvent evaporation method. 5- Fluorouracil as a model drug was loaded in these nanoparticles to investigate the drug release behavior. The properties of the prepared nanoparticles were extensively studied by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The particle sizes obtained by dynamic light scattering of these nanoparticles were in the range of 100–115 nm, and increased as the hydrophobic property of the nanoparticles increased. TEM showed that the nanoparticles were in a well-defined spherical shape with a uniform size distribution. We also investigated the entrapment and in vitro release behavior in presence of ultrasound for controlled release, which indicated that the release speed of 5-FU could be well-controlled.

Keywords: poly(ζ-caprolactone) (PCL), 5-fluorouracil, nanoparticles, controlled release

1 INTRODUCTION

Over the past decades, polymeric nanoparticles have emerged as a versatile carrier system for targeted drug delivery due to its augmentation of the local drug concentration and the desired controlled and sustained release [1-3]. poly(ζ-caprolactone) (PCL) is widely used in the fabrication of nanoparticles for drug delivery due to its high permeability to drugs, excellent biocompatibility, outstanding non-immunogenicity[4-6] and less acidic degradation products as compared to polylactide and polyglycolide[7]. However, because of its great hydrophobicity and slow biodegradation rate, further applications of PCL homopolymer in biomedicine have been quite limited [8]. In a series of studies, hydrophilic PEG was incorporated into PCL main chain, and the resultant amphiphilic copolymer PCL/PEG was developed and widely used as controlled drug delivery system [9]. This work aimed to synthesized biodegradable and biocompatible poly(ζ-caprolactone)/Pluronic/ poly(ζ-caprolactone) (PLC-Plu-PLC) triblock copolymer series of different molecular weight, followed by nanoparticles prepartion from PCL-Plu-PCL prepared series to encapsulate 5-fluorouracil as an anticancer drug.

2 METHODS

Enzymatic Polymerization of poly(ζ-caprolactone)

Amphiphilic triblock copolymers PCL/Pluronic/PCL (CUC series) was synthesized by Lipase B catalyzed ring-opening polymerization of ε-CL monomer initiated by Pluronic. Pluronic F68,consisted of 80% hydrophile made of poly(ethylene oxide) and its molecular weight was 8400, was used in this study. For all of the reactions under study, the ratio of the enzyme catalyst to ε-CL monomer was 1/10 (w/w). The molecular weights of the copolymers were determined by gel permeation chromatography (GPC). The structure of block tripolymer composed of pluronic and poly(ε-caprolactone) was confirmed by Fourier transform infrared (FT-IR) spectroscopy [10].

Processing of prepared polymer into nanoparticles

30 mg of copolymer were dissolved in 15 ml of DCM and the solution was stirred at room temperature until complete dissolution. The solution was added to 10 ml distilled water. The emulsion was stirred for 24 h. A milky-like suspension was obtained and the DCM was removed at reduced pressure. The solution was then filtered through a 5 μm filter to remove any eventual polymer aggregate before being analyzed for determination of particle size [11]. The loading of 5-fluorouracil onto the prepared triblock copolymer nanoparticles was achieved by the incubation procedures that involved single drug surface adsorption onto the preformed polymeric NPs. The drug loading efficiency and entrapment efficiency were presented by Eqs. (1)-(2), respectively [12, 13].

$$DLE = \frac{\text{weight of drug in nanopaticles}}{\text{weight of nanopaticles}} \times 100\% \quad (1)$$

$$EE = \frac{\text{weight of drug in nanopaticles}}{\text{weight of drug added}} \times 100\% \quad (2)$$

5- FU free and encapsulated nanoparticles were characterized in terms of size and morphology. The mean particles size and size distribution of the nanoparticlers was determined by dynamic laser light scaterring on a Beckman coulter particle size analyzer. Morphological examination of the nanospheres was carried out using a JOEL (Japan) JEM-100S transmission electron microscope (TEM). Drug release was determined by the method described by Zeng et al [14].

3- RESULTS AND DISCUSSION

Three samples of amphiphilic block tripolymers based on PEO–PPO–PEO block copolymer (Pluronic) and poly(caprolactone) were synthesized by bulk polymerization using different concentrations of pCL as described in table 1.

Fig. 1 presents FT-IR spectra of Pluronic, ζ-caprolactone monomer and Pluronic /PCL block tripolymers.. A new and strong carbonyl band appears at 1734 cm attributed to the formation of block tripolymer of PCL and Pluronic. Overlapping of the aliphatic CH stretching band of PCL at 2943 cm and of Pluronic at 2881 cm was also observed.

Nanoparticulate drug delivery systems composed of amphiphilic block copolymers have been extensively explored due to the many advantages they provide such as prolonged circulation, passive targeting and enhanced solubilization of hydrophobic drugs. Systems formed include micelles, nanospheres, nanocapsules and polymersomes which all display distinct structural and physicochemical properties. Formation of these various nanoparticles has been demonstrated to be highly dependent on the composition, molecular geometry and relative block lengths of the constitutive copolymers as well as the methods of preparation [15]. In the literature it has been shown that as the ratio of the molecular weights of the hydrophilic and hydrophobic blocks changes, the method of preparation to obtain particular nanoparticle formulations needs to be correspondingly altered [16].

When the molecular weight of the hydrophilic block exceeds that of the hydrophobic block, the copolymer is easily dispersed in water and will self-assemble into small, relatively monodisperse micelles. However, when the molecular weight of the hydrophobic block approaches or exceeds the molecular weight of the hydrophilic block, the copolymer becomes progressively more water insoluble and therefore will not self-assemble into a nanoparticle through direct dissolution or film casting methods, but rather dialysis, emulsification or in some cases nanoprecipitation techniques must be employed [15]. So, in this work as the molecular weight of PCL greater than that of Pluronic, the nanoparticles were prepared by solvent evaporation method and the polymer precipitates in the form of nanospheres.

The relative ratio of the hydrophobic to hydrophilic block length not only has an effect on the physical state of the nanoparticle, but has also been shown to have profound effects on the nanoparticle morphology. The morphology of prepared amphiphilic block copolymer nanoparticles is typically spherical, particularly if the molecular weight of the hydrophilic block exceeds that of the hydrophobic block thus forming aggregates in which the corona is larger than the core (so-called star micelles). If the copolymer is considerably more hydrophobic the immobile hydrophobic blocks will be sequestered into solid-like particles (what we term nanospheres) [15].

In order to characterize the morphology of the PCL copolymer nanoparticles, TEM measurement was carried out. **Fig.2-5** shows the photographs of PCL nanoparticles. It could be seen that most of the copolymer nanoparticles had a regular spherical. The solvent evaporation method followed for the synthesis of PCL NPs allowed the formation of well-stabilized spherical NPs. Particle size of drug unloaded PCL-PLU-PCL block tripolymeric nanospheres were in the range of 101-115 nm and the size of nanospheres increased with the molecular weight of PCL-PLU-PCL block tripolymerics described in table 1. Our results were in accordance with the results obtained by kim et al [17].

It is well known that drug encapsulation efficiency and drug loadingm efficiency are also crucial factors in the development of nanoparticles for drug delivery. In this study, 5-FU as a model drug was loaded onto these nanoparticles by the adsorption method to investigate the drug release behavior. From TEM and DLS results, the 5-FU loaded nanoparticles preserve spherical shape (Fig 6) and are on a nano-scale (Table 1). The drug loading content could reach as high as 14.1 % and the encapsulation efficiency is 54.88 %. Thus, 5-FU solubility in the aqueous nanoparticle suspension would be greatly improved. These results were confirmed the results obtained by Zhang et al [13].

5-Fluorouracil (5-FU), a pyrimidine analogue that interferes with thymidylate synthesis, has a broad spectrum of activity against solid tumors. However, 5-FU has limitations that include a short biological half-life, incomplete and non-uniform oral absorption, toxic side effects on bone marrow and the gastrointestinal tract, and non-selective action against healthy cells. In order to prolong the circulation time of 5-FU and increase its efficacy, numerous researchers have attempted to modify its

delivery by use of polymer conjugates or by incorporation of 5-FU into particulate carriers [18, 19].

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. This was in accordnce with our results that showed faster drug relese from cuc-6.

Sample	Particle size of drug free particles (nm)	Particle size of 5- FU-loaded particles (nm)	DLE (%)	EE (%)
Cuc-6	101	131	14.10	54.88
Cuc-7	109	133	11.77	38.68
Cuc-8	115	185	10.74	25.3

Table I: Prepared nanoparticles in terms of particle size of unloaded and particles loaded with 5-flurouracil, drug loading efficiency (DLE) and encapsulation efficiency (EE).

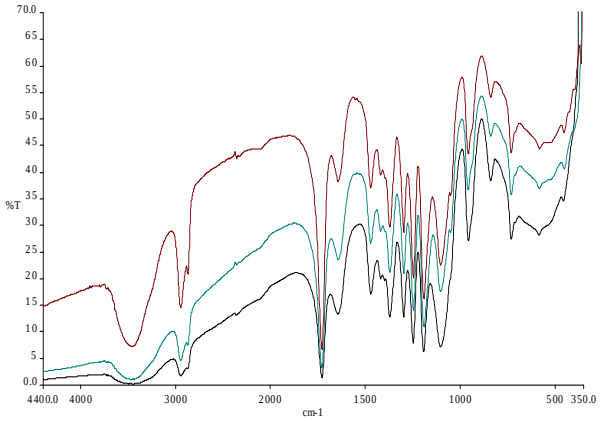

Fig. 1 FT-IR spectra of the CUC (CUC6 to CUC8) triblock copolymers as well as their homopolymers Pluronics and PCL

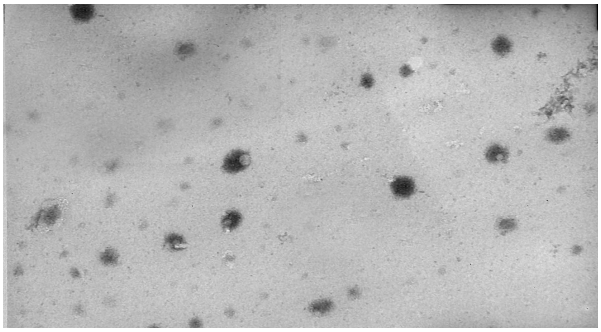

Fig 2: TEM photograph showing spherical naoparticles prepared from CUC-6

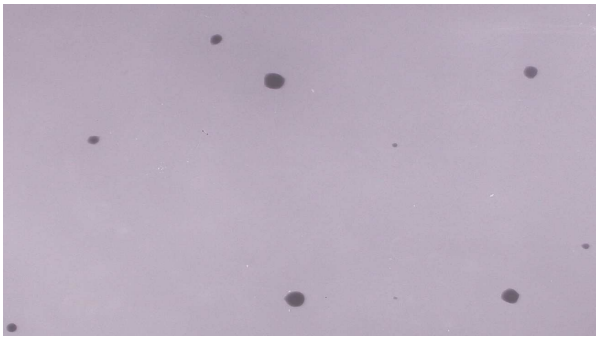

Fig 3: TEM photograph showing spherical naoparticles prepared from CUC-7

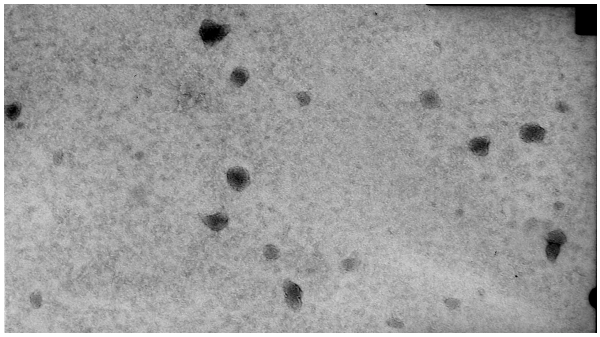

Fig 4: TEM photograph showing spherical naoparticles prepared from CUC-8

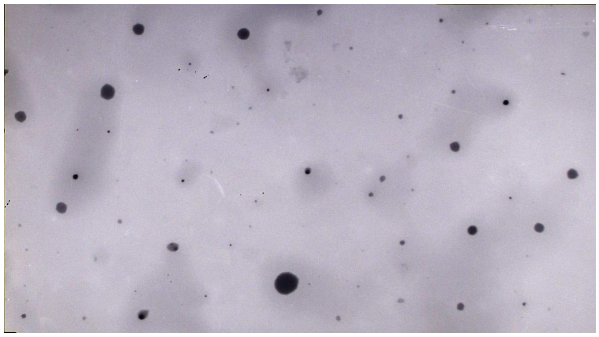

Fig5: TEM image of 5-FU loaded (PCLPluronic- PCL) nanoparticles

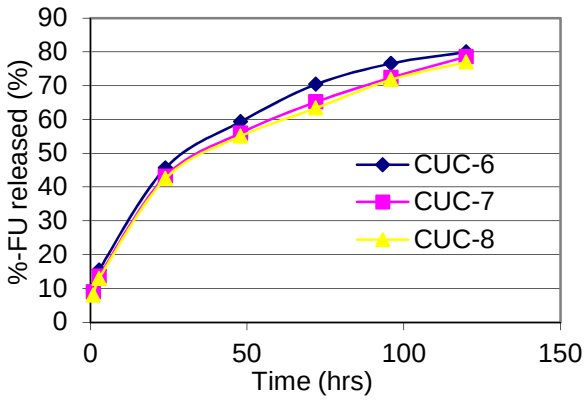

Fig 6: % of drug release with time.

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