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Preparation of 5-Fluorouracil Loaded Polylactide-co-glycolide-co-methoxy Poly(ethylene glycol) (PLGA-mPEG) Nanoparticles *via* High Speed Shearing

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Abstract 5-Fluorouracil(5-FU) loaded nanoparticles(NPs) were prepared by a high speed shearing double emulsion method with polylactide-co-glycolide-co-methoxy poly(ethylene glycol)(PLGA-mPEG) as loading material. The prepared NPs possess a negative *zeta* potential and their loading efficiency is about 15%(mass fraction). The result of *in vitro* release shows that the release behavior of 5-FU from NPs is coincident with Zero-level release from the second day.

Keywords 5-FU; PLGA-mPEG; Nanoparticle; *Zeta* potential

1 Introduction

During the past decade, considerable attention has been paid to the preparation of drug loaded biocompatible polymer spheres because polymeric spheres can effectively protect the drug from adverse external conditions and control drug release behavior[1—3]. 5-Fhorouracil(5-FU) as one of the oldest and best antineoplastic chemotherapy drugs has been used in clinical practice for decades. Recently, Yin *et al.*[4] prepared 5-FU loaded PLA-PEG microparticles of about 3 μm diameter that showed a well control release property. Compared with microspheres, nanoparticles(NPs) have their own advantages, such as longer circulation time, lower bio-barrier, *etc.*[5].

In this study, we chose polylactide-co-glycolide-co-methoxy poly(ethylene glycol)(PLGA-mPEG) as sphere formation material to prepare 5-FU loaded NPs *via* high speed shearing W/O/W double emulsion method. We investigated the influences of the concentration of polymer, preparation process, and volume ratio of different phases on the obtained NPs' size, *zeta* potential, and drug loading efficiency. Then, the drug loaded NPs were prepared under the optimal conditions. The resulted NPs' surface morphology and *in vitro* release behavior were investigated.

2 Experimental

2.1 Materials

PLGA-mPEG(PLGA, M_n=35000, 50:50; PEG, M_n=2000) was bought from Tsinan Daigang Bioscience Corporation; poly vinyl alcohol(PVA, M_n= 13000—23000, 87%—89% hydrolyzed) was bought from Aldrich; 5-FU was bought from Changzhou Jianhu Dongfeng Corporation; all solvents were produced by Beijing Chemical Industry Factory and were used without further purification.

2.2 Preparation of Drug Loaded NPs

5-Fu loaded polymer nanopartilcles were prepared by a double emulsion method(Scheme 1)[6]. Briefly, a certain amount of the polymer was added into 5 mL of dichloromethane. Then, a certain amount of 5%(mass fraction) NaOH solution containing 100 mg/mL 5-FU was slowly injected into the former solution under 26000 r/min high speed shearing(Fa-25, Fluko). After all 5-FU solution was injected, a whitish,

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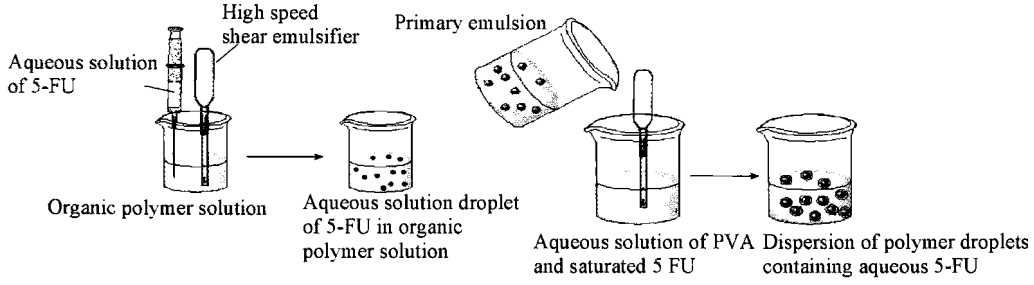
slightly transparent emulsion was obtained. This primary emulsion was then poured into 10 mL of 5-FU saturated solution containing 50 mg/mL PVA under 26000 r/min high speed shearing for a certain time that was repeated 3 times at intervals of 30 s for each stirring to obtain the double emulsion(W/O/W). Then, 90 mL of 5-FU saturated aqueous solution was added into the double emulsion, and mildly stirred for 30 min. Later, the solvent evaporation was carried out under vacuum at room temperature on a rotating evaporator(RE-85A rotating evaporator, Henan Yuxin Instrument Corporation). NPs were recovered by centrifugation at 12000 r/min, washed with 5-FU saturated aqueous solution 5 times and distilled water 3 times, and then lyophilized.



Scheme 1 Process of preparing W/O/W double emulsion

2.3 NPs' Morphology

The morphology of the NPs was investigated by a JSM-6700F Scanning Electron Microscopy(JEOL, Japan).

2.4 Particle Size Distribution and *Zeta* Potential

Particle size distribution and *zeta* potential were determined by Zetasizer(Nano-ZS, Malvern). Each sample of nanoparticles preparation was analyzed in duplicate with 30 readings for per nanoparticle sample suspended in distilled water.

2.5 5-FU Loading Efficiency

2.5.1 TGA Method

5-FU loading efficiency was determined by thermogravimetric analysis STA449C(NETSCH). A certain amount of dry NPs was heated at a heating rate of 10 °C/min under nitrogen atmosphere.

2.5.2 UV Method

Drug-loaded NPs of 20 mg was dispersed in 10 mL of HCl(0.1 mol/L). After 2 h of vigorous stirring, this solution was centrifuged at 12000 r/min for 0.5 h, and then the UV-Vis spectrum was detected at 260 nm with UV-PC2501.

2.6 *In vitro* Release of 5-FU from Polymer NPs

To determine the *in vitro* pharmaco-kinetics, 50 mg of 5-FU NPs was dispersed in 10 mL of PBS(pH=7.4). This solution was added into a dialysis bag, which was put into 90 mL of PBS(pH=7.4), sealed, and agitated(~75 r/min) at 37 °C. A sample of 10 mL was collected at specified time intervals from outer PBS and supplement equal amounts of fresh PBS.

3 Results and Discussion

Table 1 shows the relationship between the preparation conditions and the properties of the resulted NPs. For the purpose of obtaining smaller NPs, we investigated some factors that mainly influence the diameters of particles in the preparation process. The most important improvement compared to the other studies is the use of high speed shearing. This method possesses the advantages of being powerful and efficient. Compared with the NPs prepared by mechanical stirring having a mean diameter of 1—50 μm[1—4], the NPs prepared with high speed shearing have a mean diameter of 300—800 nm. We chose PLGA-mPEG rather than PLA-mPEG because compared to PLA-mPEG, PLGA-mPEG with glycolide introduced into polymer backbone exhibits a longer solvent evaporation time and solidification time, and the surface tension makes the polymer sphere's diameter smaller than that made by the same MW PLA-mPEG. Also, the absence of a CH_2 pendent group of glycolide compared to lactide leads to a lower steric hindrance, which also helps to form smaller spheres. Meanwhile, a longer dispersing time used in formation emulsion leads to a smaller particle diameter. Furthermore, decreasing inner water phase volume is an efficient method to decrease particle diameter. As the volume of the inner water phase reduces, a more dispersive primary emulsion is formed, which can help us to obtain