Preparation of 5-Flurouracil Loaded Microspheres

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Abstract

Polylactide (PLA) is a kind of biodegradable and biocompatible material. In recent years, polylactide (PLA) and its copolymers have received significant attention from researchers. 5-Fluorouracil (5-Fu) is an antimetabolite of the pyrimidine analog type and is widely used for cancer treatment. As many other kinds of hydrophilic medicines, 5-Fu is difficult to be encapsulated by a water-in-oil-in-water emulsion solvent evaporation technique and the drug content is affected by the volume of the internal phase the increase in which resultes in a decrease in the yield of microspheres. In this study, 5-Fu loaded PLA microspheres which has quite high yield were prepared by a particular oil-in-water evaporation method. During the process of microspheres preparation, nano-silica was used in order to achieve high drug content. The size and morphology of the microspheres were observed by the scanning electron microscope (SEM). According to the results, these microspheres have smooth, spherical surface structure, with no apparent evidence of collapsing which can ensure the even release of the drug. Their size depends on the concentration of dispersed phase and gelatin and the size of nano-silica particles. The nano-silica content was determined by thermogravimetry analyzer (TGA) and by a spectrophotometer, the 5-Fu content in the microspheres could be calculated. It was shown that the PLA microspheres containing lower amount of nano-silica have higher ability to incorporate 5-Fu.

Introduction

Biodegradability and biocompatibility are the fundamental requirements that determine the possible therapeutic and surgical applications of a polymeric biomaterial. In recent years, polylactide (PLA) and its copolymers have received significant attention from researchers because they have the above mentioned characteristics, as well as good mechanical properties [1-4].

5-Fluorouracil (5-Fu) is an antimetabolite of the pyrimidine analog type that is widely used alone or in combination with different chemotherapeutical agents for treatment of advanced gastrointestinal tract cancer, breast cancer and several other types of cancer [5]. Because of its short biological half-life and its poor oral absorption, it is an appropriate candidate for microencapsulation [6-10].

As many other kinds of hydrophilic medicines, 5-Fu is difficult to be encapsulated. In this study, PLA microspheres containing nano-silica were prepared in order to find a new carrier which can achieve high drug content.

These microspheres were prepared by an oil-in-water evaporation method [11-15] using an aqueous solution of gelatin as the continuous phase and dichloromethane as the organic solvent. We have studied the effect of processing parameters on the properties of microspheres. Each sample was evaluated for the following parameters: yield of microspheres, particle size and morphology. The content of 5-Fu and nano-silica in microspheres has been also determined.

Experimental

Materials

PLA with average molecular weight 67,000 Da was purchased from Cargill-dow (USA). Nano-silica was purchased from Nantong General Pharmaceutical Factory (China). Gelatin and dichloromethane were purchased from Sinopharm Group Chemical Reagent Co. Ltd (China).

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Preparation of Microspheres

PLA microspheres containing nano-silica were prepared using an oil-in-water single-emulsion solvent evaporation technique. Briefly, PLA was dissolved in dichloromethane, and nano-silica was added into the organic phase. The mixture was sonicated using a 200-W probe-type sonicator (JHN-M-4E, Shanghai Jump Ultrosonica Equipment Co., China) until the nano-silica was homogeneously suspended. Then it was poured into the aqueous solution of gelatin whilst stirred at maximum speed with a magnetic stirrer (8I-2, Shanghai Sile Instrument Co., China). Then the resulting oil-in-water emulsion was stirred at a slow speed for 4 hours at room temperature to allow solvent evaporation and microspheres formation. The microspheres were collected by filtration, washed three times with distilled water and dried in a vacuum oven at 37°C for at least 24 hours.

Preparation of 5-Fu loaded Microspheres

The microspheres containing nano-silica were immersed in the aqueous solution of 5-Fu for 2 hours, washed with distilled water and dried in a vacuum oven at 37°C for 24 hours.

Determination of nano-silica content

TGA PERKIN-ELMER 7 was used to measure the nano-silica content (sample 3) in the nitrogen atmosphere, at the constant heating rate 10 °C/min.

Determination of 5-Fu Content

The calibration curve for determination of 5-Fu content in solution was obtained by examining the correlation between solution absorbance and concentration. The absorbance of 5-Fu solution used in the preparation of 5-Fu loaded microspheres was measured at 265 nm by a spectrophotometer (UV754, Shanghai Precision & Scientific Instrument, China) after the 5-Fu loaded microspheres were prepared. Based on the change of the drug concentration, in the outside solution calculated using the calibration curve the 5-Fu content in the microspheres was calculated.

Evaluation of PLA microspheres containing nano-silica

Yield of Microspheres

Microspheres containing nano-silica recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymer nano-silica added during the preparation of microspheres.

Particle Size and Morphology

The surface morphology of the polymeric microspheres was examined by scanning electron microscopy (SEM) (JXA-840, JEOL) after the samples were coated with gold. For each sample the diameter of at least 100 microspheres was measured and averaged.

Results and Discussion

Yield

Table 1 shows that the yield of the microspheres was quite high for all component ratios. Rajesh H. Parikh *et al.* [10] reported that an increase in the volume of the internal phase of the primary emulsion resulted in a decrease in the yield of microspheres prepared by a water-in-oil-in-water emulsion solvent evaporation technique. So a single-emulsion solvent evaporation technique used in this study could achieve a high yield of the microspheres but should not affect the drug content.

Table 1
The yield of microspheres containing nano-silica

	Concentration of PLA, (mg/ml)	Concentration of gelatin, (mg/ml)	Diameter of nano-silica, (nm)	% Yield
1	40	15	10	86.3
2	40	15	30	91.8
3	40	15	100	77.8
4	30	25	10	95.1
5	60	25	10	78.6
6	90	25	10	71.3
7	40	10	30	86.0
8	40	25	30	83.7
9	40	40	30	70.4

Morphology and Particle Size

Figure 1 shows the SEM photographs of PLA microspheres containing nano-silica prepared by an oil-in-water single-emulsion based on the solvent

evaporation method. The resulting microspheres have smooth, spherical surface structure, with no apparent evidence of collapsing which can ensure the even release of the drug.

Table 2 shows the effects of PLA concentration, the concentration of gelatin and the diameter of nano-silica on the size of microspheres. It can be seen that an increase in the concentration of PLA leads to an increase in the particle size of the microspheres. This is likely due to the increase in viscosity of dispersed phase following the increase of the PLA concentration, which leads to lower dispersion ability at the same shear rate. This result is in good agreement with the data obtained by Guo Yingzhi *et al.* [16].

It is also seen that an increase in the concentration of gelatin, which provided good protection during the preparation of microspheres, leads to a decrease in the particle size of the microspheres.

Furthermore, the increasing in nano-silica size results in the bigger diameter of microspheres.

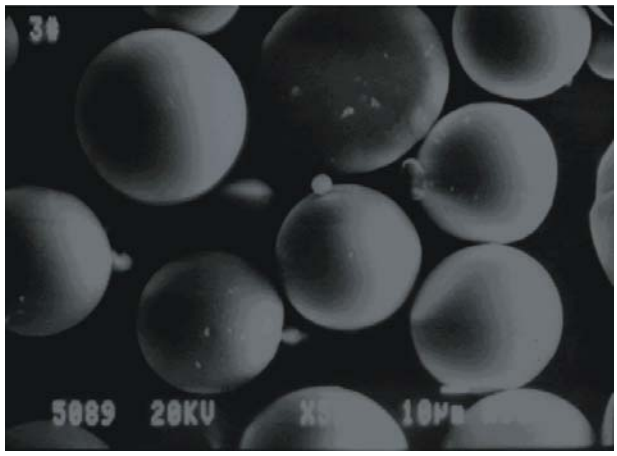

Fig. 1. The SEM photographs of PLA microspheres containing nano-silica.

Table 2

Effect of the concentration of PLA, the concentration of gelatin and the diameter of nano-silica on the size of microspheres

Affecting Factor	Concentration of PLA, (mg/ml)	Concentration of gelatin, (mg/ml)	Diameter of nano-silica, (nm)	Average diameter of microspheres, (μm)
Concentration of PLA	30	25	10	13.25
	60	25	10	27.50
	90	25	10	35.30
Concentration of gelatin	40	10	30	32.25
	40	25	30	27.13
	40	40	30	23.85
Diameter of nano-silica	40	15	10	21.59
	40	15	30	30.42
	40	15	100	40.01

Nano-silica content

Figure 2 shows PLA microspheres containing nano-silica lost weight at the temperature of 310°C because PLA degrades by ester interchange processes and decarboxylation from the chain ends [17-20]. In evidence, nano-silica content of sample 3 was low, only 6.0455%.

5-Fu Content

The absorbance equation of 5-Fu, A=92.42347C+ +0.05412 (C: mg/ml) was obtained. By calculated, the 5-Fu content of sample 3 is 50%. It was higher than that was reported [9,21]. This result indicates that the lower amounts of nano-silica could lead to high drug content.

Conclusions

The PLA microspheres containing nano-silica have smooth, spherical surface structure. The concentration of PLA and gelatin and the diameter of nano-

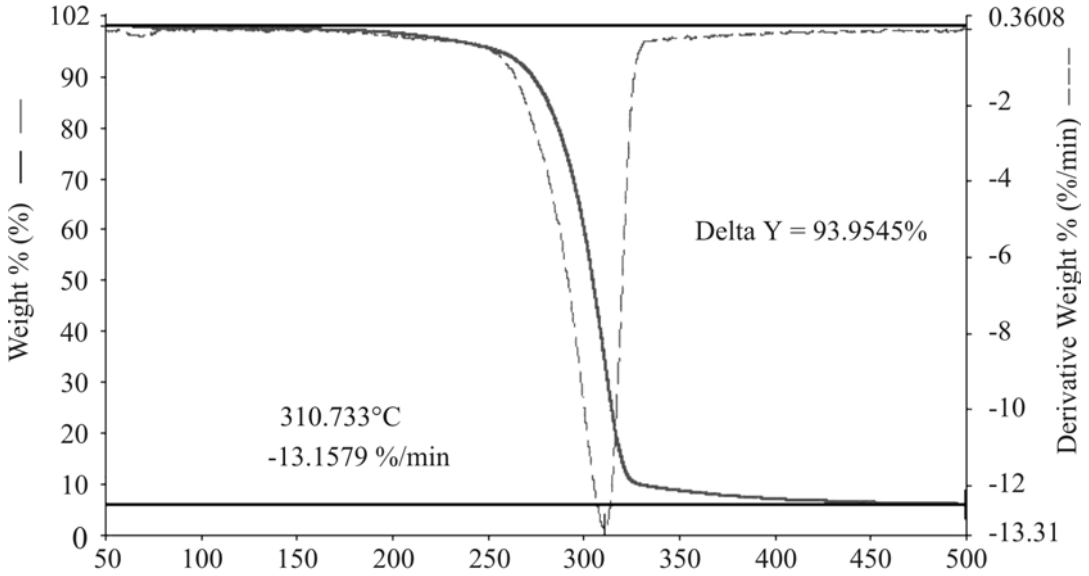

Fig. 2. The TGA curve of sample 3.

silica affect the particle size of the microspheres. The 5-Fu loaded microspheres, which contain lower amount of nano-silica can achieve high drug content.

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