Preparation of biodegradable polymer microcarriers by ultrasonic emulsification

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Abstract

The polymer material PCL (polycaprolactone) has good biodegradability and good performance on drug coating. For the long-term delivery drugs, the biodegradable polymers reduced drug side effects and enhanced the drug efficacy. This study combined emulsification and ultrasonic nozzle to prepare biodegradable polymer microspheres. The ultrasonic emulsification compared to other general emulsification methods had advantage of being stable and fast. In the process, the oil phase solution PCL flew through the ultrasonic nozzle at flow rate 0.5-5 ml/min. Through the standing wave energy on the nozzle surface, the PCL solution was sprayed into droplets. Then, the droplets were dripped into the aqueous phase solution PVA (Polyvinyl alcohol) to cure as the PCL microspheres. After the emulsification process was completed, the solution was poured into water to make the solvent of the emulsion solution volatilized. Finally, the solution was rinsed with water and the PCL microspheres were filtered by the filter. As a result, the PCL microspheres were able to be observed through an optical microscopy (OM) and a scanning electron microscopy (SEM).In order to measure the rate of degradation, the PCL microspheres were used for the experiment. The PCL microspheres at 2.8x1.2x1.4 cm^3 volume were poured into an amano Lipase PS solution at a concentration of 0.005 mg/ml. Then, the solution was under a constant temperature (50 ^oC) and stable shaking situation, and the weight changes of the PCL microspheres were recorded per day. The result shows that the PCL microspheres particle size was 30-75 μm and the PCL microspheres at 2.8x1.2x1.4 cm^3 volume were degraded fully in 7 days.

Key words: microsphere, polycaprolactone, ultrasonic, emulsification

Introduction

As foreign material was into biological environment, the protein of the biological environment attacked the material naturally. The biological defense system caused the inflammatory response, foreign body response, blood coagulation and thrombus formation. Therefore, biocompatibility was an important evaluation standard for biomaterials [1].

The biodegradable polymer polycaprolactone(PCL) selected in this study was first synthesized by the Carruthers team in the 1930s [2]. PCL was biodegradable semi-crystalline polyester. It was the polyester polymer which used the ring opening polymerization by the monomer espial-caprolactone. PCL could be dissolved in most of the organic solvents. The melting point of PCL was about 55-60 ^oC and the glass transition temperature was about 60 ^oC. Due to the good workability, PCL was a widely used biodegradable material [3-7].

The productive method of the polymer microspheres could be classified as the bead type and the particle growth type. The bead type was classified as the suspension polymerization, spray vibration and film emulsion. Then the particle growth type was classified as dispersion polymerization and emulsifier-free polymerization [8, 9]. Due to the emulsifier-free polymerization easily made microspheres and controlled the parameters, the method was chosen in this study [10].

Methods

A. Uniform experimental design

In this study, PCL microspheres with the best uniform particle size were selected as a goal. The uniform experiment method was used in this study. The $U_{16}^*(16)$ uniform design table was selected. The six factors were power of ultrasonic vibration, PVA concentration, pump feed rate, the height between ultrasonic nozzle and solution, magnet rotation speed, and temperature as shown in Table I. The upper and lower limits of each factor were set as shown in Table II. The factor level table combined with U_{16}^* uniform design table, we could get the corresponding experimental parameter table, as shown in Table III.

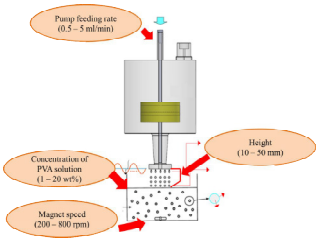

Fig.1 Device of emulsification

TABLE I
FACTOR CODE

Factor	Code
Power (W)	A1
Pump feeding rate(ml/min)	A2
Magnet speed (rpm)	A3
Concentration of PVA solution (wt%)	A4
Temperature (^{o}C)	A5
Height (mm)	A6

TABLE II
THE EXTREME VALUE OF THE FACTORS

Factor	Minimum	Maximum
A1	10	13
A2	0.5	5
A3	200	800
A4	1	20
A5	30	50
A6	10	50

TABLE III
TABLE OF FACTOR LEVEL

LEVEL.	A1	A2	A3	A4	A5	A6
1	10	0.8	400	12.43	47.29	47.24
2	10.2	1.2	640	3.54	43.3	41.92
3	10.4	1.6	200	16.24	39.31	36.6
4	10.6	2	440	7.35	35.32	31.28
5	10.8	0.7	680	20	31.33	25.96
6	11	1.1	240	11.16	50	20.64
7	11.2	1.5	480	2.27	45.96	15.32
8	11.4	1.9	720	14.97	41.97	10
9	11.6	0.6	280	6.08	37.98	50
10	11.8	1	520	18.78	33.99	44.58
11	12	1.4	760	9.89	30	39.26
12	12.2	1.8	320	1	48.62	33.94
13	12.4	0.5	560	13.7	44.63	28.62
14	12.6	0.9	800	4.81	40.64	23.3
15	12.8	1.3	360	17.51	36.65	17.98
16	13	1.7	600	8.62	32.66	12.66

B. Emulsification experiment by ultrasonic

This study used the ultrasonic nozzle to produce microspheres during emulsification process. The process of emulsification experiment as follow：

The oil phase solution was injected to the ultrasonic nozzle by the infusion pump. The ultrasonic nozzle sprayed the droplets to the aqueous solution below. After the droplets were sprayed into the aqueous solution, the shear force in the aqueous solution cut the droplets into smaller size. After the aqueous solution was stirred by the magnet for a while, the semi-cured microspheres would gradually cure as EA separated out. Finally, microspheres fixed shape after cleaning and drying.

During the experiment, the ultrasonic nozzle used a hollow expansion bar to control the frequency and the amplitude. It produced the maximum amplitude at the nozzle tip. Then the standing wave would happen on the nozzle surface. When the ultrasonic energy broke the surface tension, the droplets were sprayed out. The device of emulsification was shown in Fig.1.

C. Study on degradation rate of PCL polymeric materials

In the degradation rate experiment of PCL polymer, four different molecular weight of microspheres were weighed and placed in a biological environment for degradation experiments. The microspheres had been taken out for cleaning, drying and weighing at set intervals. The loss percentage of the original weight was calculated to quantify the data as the degradation rate of the material.

The size of the test piece was 11 mm x 9 mm x 1.4 mm. After weighing, the initial weight was W_a. The material was placed in a 20-mL vial and the amono Lipase PS solution at a concentration of 0.005 g/ml was added. The sample was shown in Fig.2.

The sample was placed in a constant temperature water tank. The temperature is set to 50 ^{o}C and the shaking speed is 175 rpm. The sample was taken away at the set interval time. Then the sample was washed with deionized water and dried in an oven. The sample was recorded for the weight W_b and placed in a vial again. The fresh amano Lipase PS solution was added and placed it back to the tank to continue experiment. Weight loss percentage W_{loss} was calculated as follows:

$$\%W_{loss}= [(W_a-W_b)/W_a] \times 100\%$$

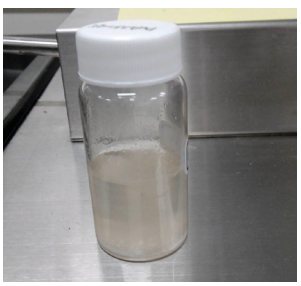

Fig. 2 The sample of degradation

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Results

A. Uniform experimental design

The result from the uniform experimental design used Kriging of Matlab software to build the model. The level normalization was shown in Table IV.

TABLE IV
LEVEL NORMALIZATION

LEVEL	A1	A2	A3	A4	A5	A6
1	0	0.2	0.333	0.601	0.864	0.931
2	0.066	0.466	0.733	0.133	0.665	0.798
3	0.133	0.733	0	0.802	0.465	0.665
4	0.2	1	0.4	0.334	0.266	0.532
5	0.266	0.133	0.8	1	0.066	0.399
6	0.333	0.4	0.066	0.534	1	0.266
7	0.4	0.666	0.466	0.066	0.798	0.133
8	0.466	0.933	0.866	0.735	0.598	0
9	0.533	0.066	0.133	0.267	0.399	1
10	0.6	0.333	0.533	0.935	0.199	0.864
11	0.666	0.6	0.933	0.467	0	0.731
12	0.733	0.866	0.2	0	0.931	0.598
13	0.8	0	0.6	0.668	0.731	0.465
14	0.866	0.266	1	0.200	0.532	0.332
15	0.933	0.533	0.266	0.868	0.332	0.199
16	1	0.8	0.666	0.401	0.133	0.066

Then the genetic algorithm from the optimization system of Matlab was used as the solver to find the optimized factor combination of parameters. The maximum particle size distribution percentage was set as the goal for the optimization. The optimal combination of factor parameters was discussed based on different factor parameters combinations.

The optimal factor parameters were used in emulsification method again. Then the PCL microspheres were observed and analyzed by SEM. The SEM figure showed that the PCL microspheres particle size evenly distributed. The range of particle size was in 50~100μm and the maximum particle size percentage was 83 % in Fig.3. So, the optimal factor parameters in emulsification had obvious effect on controlling the particle size. The entity of microspheres was shown in Fig. 4.

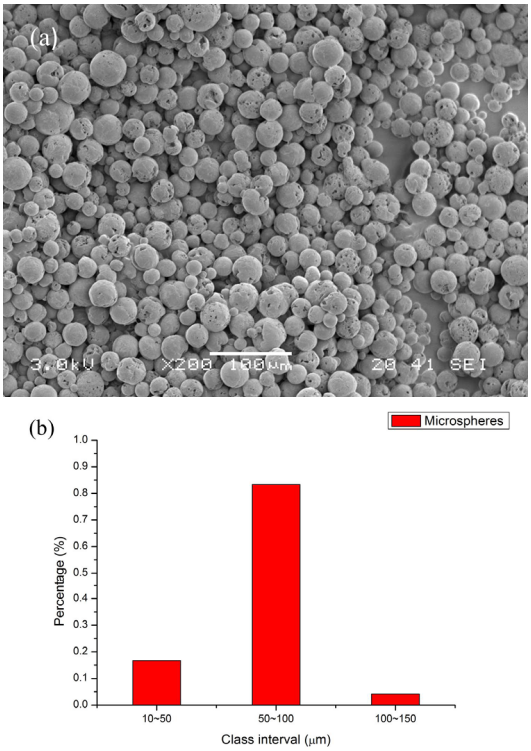

Fig. 3 Optimization of microspheres particle size (a) SEM (b) Percentage of particle size

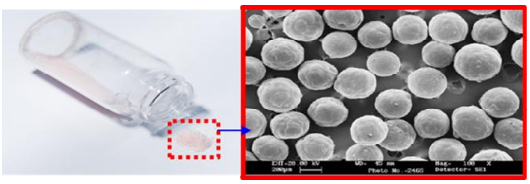

Fig. 4 Entity of microspheres

B. Study on degradation rate of PCL polymeric materials

Four groups of PCL samples with different molecular weights were tested and the effects of molecular weights on PCL samples were understood in the range of 36,000 to 96,000.

The weight of PCL microspheres in different time were plotted in Fig. 5. Expect for the sample A01, the other three samples showed the almost same weight loss percentage. The initial weight of sample A01 was 0.61 g. That was the heaviest in four samples. However, the sample A01 had high weight loss percentage, so the sample A01and sample A02 had same weight in the sixth day. The result showed that the sample with lower molecular weight had better discernment of degradation in this experimental condition. That meant the lower molecular weight had higher weight loss percentage.

The Fig. 6 showed that the weight loss percentage of PCL microspheres in different times. After six days of degradation experiment, the weight loss percentage of A01 and A02 was

99.2 % and 97.5 %, respectively. The slope of A01 and A02 degradation curves were larger relatively in first four days. Then the weight loss percentage of the sample A03 and A04 was 87.3 % and 84.1 %. The slope of A03 and A04 degradation curves remained gently in six days, which meant the rate of degradation changed small.

In this study, PCL microspheres were experimenting in harsh conditions. The weight loss percentages of four samples were more than 80% in six days. Because of that, the four PCL sample with different molecular weight could not distinction obvious with weight loss percentages. According to the result, the biodegradability of PCL material could be proved. The PCL of molecular weight between 53,000 and 63,000 were indeed significant differences in this experimental condition. By reducing the temperature of the sink from 50°C in degradation experiment, the temperature affected degradation rate effectively.

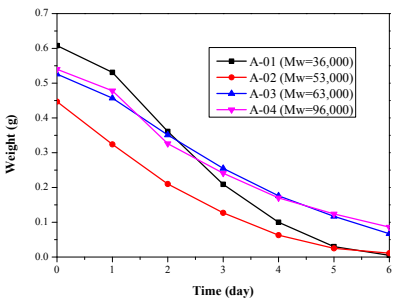

Fig. 5 Weight curve of PCL microspheres

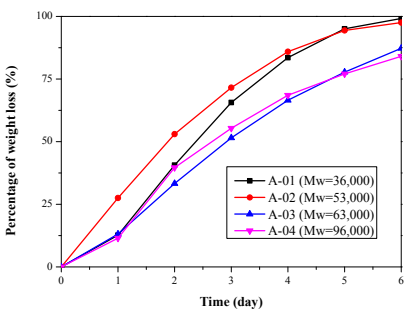

Fig. 6 Weight loss percentage curve of PCL microspheres

Conclusion

In this study, the ultrasonic nozzle combined with emulsification successfully. After the uniform experiment, the optimal combination of parameters was calculated. The result showed that the range of particle size was from 50 μm to 100 μm and the maximum particle size percentage was 83 %. The optimal factor parameters of the emulsification were considerably effective to the particle size. As for the PCL degradation experiment, the weight loss percentages of four PCL groups reached to 80% in six days. According to the result, the biodegradability of PCL material could be proved.

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