35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Research of PLGA Microspheres Preparation Based on the Micro-jetting Technology*

Huaiyuan Sun, Chenjie Sun, and Gang Zheng

Abstract— **Micro-jetting is a new method to manufacture microcapsule. According to the principle of micro-jetting technology, applying micro-jetting system to manufacture polylactic acid-glycolic acid copolymer(PLGA) microspheres; with PLGA for shell material on the ball, poly(vinyl alcohol) and twain as surfactant, using single factor analysis method to study the influence of driving voltage, jetting frequency and stirrer speed to the PLGA microspheres mean grain size and size distribution. The inside diameter of glass nozzles used in the experiments were 40μm. The results show that, in the conditions of experimental drug prescription certain, the driving voltage was proportional to the particle size, jet frequency and stirrer speed were inversely proportional. When the driving voltage for 60V~100V,the jet frequency for 1000Hz~5000Hz and the stirrer speed for 150~450rpm, the particle size distribution was in an ideal state.**

I. Introduction

Polylactic acid-glycolic acid copolymer(PLGA) is random polymerization form of the lactic acid and the glycolic acid, and it is a kind of degradable polymer organic compound. It has good biological compatibility, encystment and film performance as well as non-toxic. PLGA has been widely used in pharmaceutical, medical engineering materials and other fields. This kind of polymer has been used for decades in the medicine field (such as biodegradable suture line, microspheres preparation, etc.). Nowadays, Food and Drug Administration of the United States(FDA) has approved eight drugs of microsphere that can be injected[1].But in our country, only the leuprorelin microsphere preparation that is imitation has been reported to the State Food and Drug Administration(SFDA),others are limited in the laboratory research stage[2].

There are many kinds of methods to manufacture polymer microspheres such as emulsion drying method, the spray drying method, membrane emulsification method and micro-channel method[3-5], etc. The former two methods need to be in high temperature conditions and the microcapsule particle size distribution is width, and it is difficult to satisfy the requirements of keeping the biological activity in the fields of medical industry and biotechnology. The latter two methods that can be used for the preparation of monodisperse microspheres, but they need special devices that lead to high cost, such as hard porous glass membrane[6-8].

Micro-jetting technology is a new method to manufacture microspheres[9]. In the normal temperature and normality, the droplet resolution has reached 10^{-15}L to 10^{-12}L, that is the size of a single droplet can be controlled in submicron to micron grade size[10], so as to establish a foundation that micro-jetting technology would be used to manufacture microspheres. In recent years, Nanjing University of Science and Technology has used digital microcapsule manufacturing system and verified the feasibility of applying the micro injection technique to manufacture microcapsule[11-13].

Based on the principle of micro-jetting, this article used PLGA as the material of microspheres skeleton, and had a research of the relationships between the parameters of micro-jetting system with microspheres particle size and its distribution, applied it in the preparation of PLGA microspheres and obtained the particles with uniform grain size as well as controllable.

II. Materials and Methods

A. Materials

PLGA(LA：GA=50：50, DURECT Corporation),PVA (Shanghai RunJie chemical reagent co., LTD.),methylene chloride, Twain 80, distilled water.

B. Methods

a. Device and principle

Micro-jetting system to manufacture microspheres was shown in figure 1, and it was mainly composed of micro-jetting control unit, magnetic stirrer(GL-3250C, Haimen Qi LinBeiEr instrument manufacturing co., LTD.), CCD imaging equipment, pressure controller (CT-PT-01, MicroFab company), pressure pump(GM-0.33A, Tianjin JinTeng laboratory equipment co.,LTD.) and computer. The micro-jetting control unit was the core of the system components, and it was constituted by micro nozzle assembly, sprayer installation mechanism and electric controller. The system used 40μm inside diameter glass nozzles of MicroFab company in United States, its working principle is that, using the inverse piezoelectric effect, the nozzle internal piezoelectric transducer produce volume change and result the liquid pressure wave in cavity body which drive fluid in tube movement with it, then the pressure wave go to the nozzle hole place and translate the fluid velocity that make the droplet spray from nozzle[14-16]. Electric controller was CT-M3-02

*Research supported by Shanghai Scientific Research Innovation Foundation (12YZ195).

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controller, which can provide a variety of complex waveform signal to the nozzle.

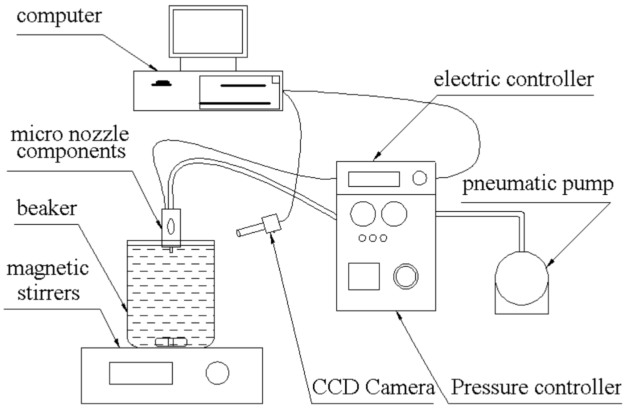

Figure 1. The structure schematic of micro-jetting manufacturing microspheres system

b. Manufacturing Microsphere

Taking a certain amount of PLGA with a precision electronic balance, dissolved it in 2ml methylene chloride and made into the PLGA-methylene chloride solution which concentration was about 3%. After it dissolved completely, injected the liquid into storage tube of the micro-jetting system, and then inserted the nozzle in aqueous solution of 1% PVA-Twain 80.The PVA and twain play the role of surfactant. Micro-jetting was controlled by the piezoelectric controller, and meanwhile magnetic stirrer was mixing with a certain speed. The stirring purpose was to avoid microspheres sprayed adhesion together. When the micro-jetting was completed, magnetic stirrer need to working 4-5 hours to remove methylene chloride, then used microporous filter membrane for vacuum filtration and collected microspheres, washed 3 times with deionized water, and vacuum dried 24 hours.

c. Particle size distribution and form representation

(1)The test of PLGA microspheres particle size distribution

PLGA microspheres were dispersed in water, tested the particle size and distribution of them with laser particle analyzer (Winner2005,Jinan Weina instrument co.,LTD.). The particle size and homogeneity of the microspheres were showed by average grain diameter and polydispersity coefficient.

(2)The form representation of PLGA microspheres

Taking a small amount of microspheres from the beaker and placing in a petri dish, watched with inverted optical microscope (LWD200-37T, Shanghai Cewei photoelectric technology co., LTD.), its micrometer minimum scale was 10μm. When methylene chloride had volatilized completely, filtrated and collected PLGA microspheres, then removed the moisture in vacuum oven. Last, observed the surface form of PLGA microspheres with scanning electron microscope (Quanta x50 FEG, FEI company).

III. RESULTS AND DISCUSSION

A. *The particle size and its distribution of PLGA microspheres*

The experiments by single factor method studied the effects of driving voltage, jet frequency, stirrer speed three factors on PLGA microspheres mean grain size and size distribution, and acquired optimum process parameters micro-jetting manufacturing PLGA microspheres.

a. The influence of driving voltage to microspheres particle size

The driving voltage was one of the most important parameters for micro-jetting, was the direct reason produced micro-jetting process. It affected directly the micro spray droplet size and was an important influencing factor to microspheres particle size and uniformity. In experiments, keeping other conditions unchanged (jet frequency was 5000 Hz, stirrer speed was 300rpm, PLGA-methylene chloride solution concentration was 3%, PVA-Twain aqueous solubility was 1%), changed the driving voltage value and got the result as shown in figure 2. It showed that, with the increase of driving voltage, the PLGA microspheres particle size was increased and grain size uniformity was deteriorated. As the driving voltage was 60V,80V and 100V, the corresponding average microspheres particle size was 35.783μm, 53.969μm,78.602μm, and the polydispersity index(PDI) was 0.032, 0.048, 0.165. This is because that, with the increase of the driving voltage, the pressure wave increased accordingly, it brought about eruptive droplet becoming bigger and the microspheres particle size increased.

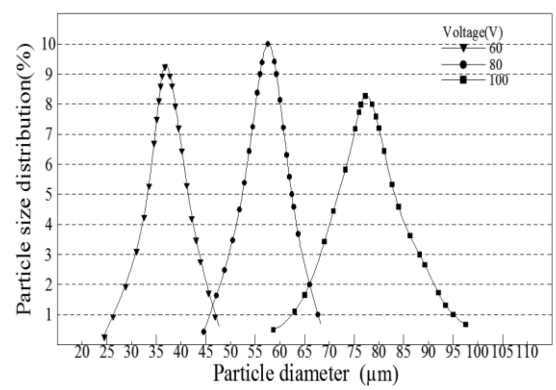

Figure 2. The particle size distribution of PLGA microspheres manufactured under different driving voltage

b. The influence of jet frequency to microspheres particle size

Jet frequency is refers to the rate system continuous jetting. When the oil phase was injected into water phase, its injection rate could be control by adjust jet frequency. In experiments, keeping other conditions unchanged (driving voltage was 80V, stirrer speed was 300rpm, PLGA-methylene chloride solution concentration was 3%, PVA-Twain aqueous solubility was 1%),changed the jet frequency and got the result as shown in figure 3.It showed that, with the increase of jet frequency, the PLGA microspheres particle size was reduced and grain size uniformity was deteriorated. As the jet frequency was 3000Hz, 5000Hz and 7000Hz, the corresponding average microspheres particle size was

73.786μm, 55.564μm and 35.561μm, and the polydispersity index was 0.024, 0.041, 0.271. This is because that, under the condition of the certain driving voltage, the shear force droplet born increased with the increase of the jet frequency, that brought about eruptive droplet becoming smaller and the microspheres particle size decreased.

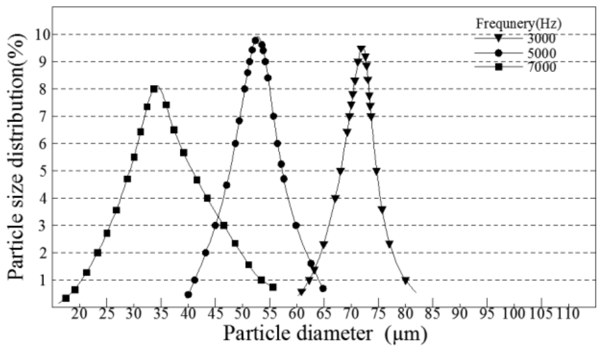

Figure 3. The particle size distribution of PLGA microspheres manufactured under different jet frequency

c. The influence of stirrer speed to microspheres particle size

Stirrer speed is related closely with water phase flow rate. In experiments, keeping other conditions unchanged (driving voltage was 80V, jet frequency was 5000 Hz, PLGA methylene chloride solution concentration was 3%, PVA-Twain aqueous solubility was 1%), changed the stirrer speed to adjust water phase velocity and got the result as shown in figure 4.It showed that, with the increase of magnetic stirrer speed, the PLGA microspheres particle size was reduced and grain size uniformity was deteriorated. As the stirrer speed was 150rpm, 300rpm and 450rpm, the corresponding average microspheres particle size was 72.145μm, 51.784μm, 30.892μm, and the polydispersity index was 0.026, 0.039, 0.205.

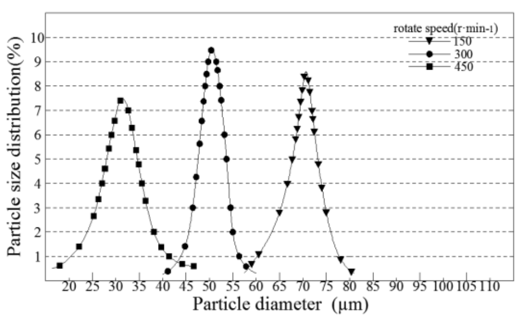

Figure 4. The particle size distribution of PLGA microspheres manufactured under different stirring speed

B. The form of PLGA microspheres

Through the inverted optical microscope, PLGA particles in water phase were spherical, as shown in figure 5. When the methylene chloride of oil phase had volatilized completely, filtrated and collected PLGA microspheres. After drying, the microspheres and their surface could be observed with scanning electron microscope (SEM) As shown in figure 6 shows, PLGA microspheres were solid spherical, without adhesion each other, and particle size uniformity was fine. Partial surface of microspheres were uneven (as shown in figure 7 shows), the probable cause was solvent volatilization.

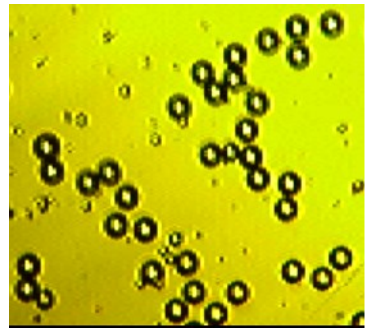

Figure 5. PLGA spherical particles in water phase

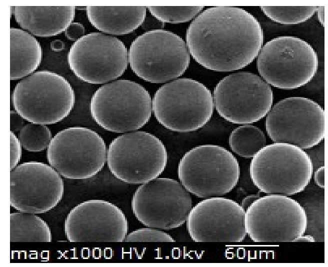

Figure 6. The dried PLGA solid microspheres

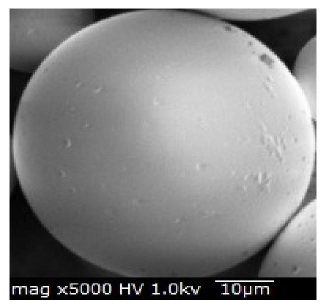

Figure 7. Surface of PLGA microspheres

IV. CONCLUSION

(1)Based on the micro-jetting technology, the microsphere preparation system with micron grade piezoelectric nozzle and its controller as the core was designed. And using the Polylactic acid-glycolic acid copolymer for shell material, PLGA spherosome particles with good uniformity were manufactured successfully.

(2)Applying the single factor method, knew well the relationships between the technical parameters (driving voltage, jet frequency, stirrer speed) with PLGA microspheres mean grain size as well as the distribution of the particle size. The driving voltage was proportional to the microsphere particle size and inversely proportional to the size uniformity. The jet frequency was inversely proportional to the microsphere particle size and the size uniformity. The influence of the stirrer speed was the same as the jet frequency.

(3)The microspheres manufactured with micro-jetting could be used for some slow and controlled release drugs. This method provided a new way for the preparation of drug microsphere, and that according to the need, the microspheres grain size and particle size distribution could be controlled in dozens of microns by changing the corresponding system parameters, and also could keep high uniformity. In addition, the equipment used in this method was simple, the yield would be improved with the increase of the nozzles. So it would be a kind of promising application technology.

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