European Journal of Pharmaceutics and Biopharmaceutics 137 (2019) 140–147

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Liquid jet breakup: A new method for the preparation of poly lactic-co-glycolic acid microspheres

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ARTICLE INFO

Keywords:
Jet breakup
Preparation
Microspheres
In vitro release
Drop-size control

ABSTRACT

The purpose of this study was to apply the phenomenon of liquid jet breakup to the preparation of sustained-release microspheres. The mechanisms of liquid jet breakup in different jet states were investigated and the single factor method was used to study the effect of each process parameter on the particle size and size distribution of microspheres. Meantime, the prepared microspheres were characterized by morphology, drug loading, encapsulation efficiency and *in vitro* release. The results indicated that the process of liquid jet breakup could have 5 different states. The laminar flow state dominated when the Reynolds number (*Re*) was low, and the prepared microspheres had larger particle sizes. When the *Re* was high, the turbulent state was dominant and the microspheres had smaller particle sizes. And during the transition state from the laminar flow to the turbulence, the microspheres had a wide particle size distribution. Different process parameters could affect the particle size and distribution of microspheres by changing the *Re*, surface tension coefficient and viscosity. The microspheres prepared by liquid jet breakup were smooth and round with the drug loading of 35% and the encapsulation efficiency of 88%. In addition, when the polymeric carrier materials were different, the microspheres could have various drug release models such as sustained release with a lag phase, sustained release with no lag phase, pulsed release and so on, which could be applied widespread in the future.

1. Introduction

The sustained release of drugs can be achieved by combining the biodegradable polymers and the bioactive ingredients to prepare the drug-loaded microspheres. Microspheres are commonly administered by intramuscular injection, and stay in local muscle tissue after administration. With the gradual degradation of polymers, drugs in microspheres will be slowly released into the blood and play a therapeutic effect.

There are many methods to prepare microspheres, among which solvent evaporation [1–3], coacervation [4] and spray-drying [5,6] are the most common methods. Due to the requirement of the preparation, the coacervation method often has a problem of residual flocculants, which makes it difficult to be used in the scale-up of microspheres [7]. The spray-drying method is simple in operation, has good production continuity and high yield, but it is not suitable for heat-sensitive drugs, and the particle size of microspheres is difficult to be controlled [8]. The solvent evaporation method requires neither to use flocculants nor to raise the temperature, so it has a wide applicability.

Microspheres preparation by solvent evaporation basically consists of two steps: (i) emulsification of the organic phase in a aqueous phase to form droplets with suitable particle sizes (emulsification, or droplets formation); (ii) evaporation of the organic solvent in droplets, so as to achieve the solidification and drying of microspheres (solvent removal) [9]. Emulsification of the organic phase is a key step in the preparation of microspheres, which determines the particle size and size distribution of the resulting microspheres. Common emulsification methods include stirring [10,11], static mixing [12,13], extrusion [14-16], dripping [17] and so on. In stirring method, droplets are batch-wise produced. The pumping effect of the stirrer creates large flow patterns and high-shear regions in the vessel. When the flow goes through high-shear regions, droplets break-up occurs. Unlike stirring, static mixing method can produce droplets in a non-stop sequence. In the working process, the static mixer creates a stronger shear mixing effect between fluids by changing the flow direction of internal fluids to realize the emulsification of the organic phase in the internal liquid, and the particle size is determined by the flow rate. Static mixing is convenient to scale up using several mixers in parallel flow [18]. However, stirring and static mixing are both poor in drop-size control [19]. Extrusion is the process that extruding the drug-loaded organic phase through a single pathway into the aqueous phase to form the droplets with suitable particle sizes, and the droplets are gradually solidified until

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https://doi.org/10.1016/j.ejpb.2019.02.021
Received 11 August 2018; Received in revised form 29 January 2019; Accepted 24 February 2019
Available online 25 February 2019
0939-6411/

microspheres are formed. When the extrusion method is used to produce microspheres, the size of droplets can be controlled by adjusting the diameter of the pathway or the flow rate of the aqueous phase, which lead to a good uniformity of microspheres. However, there is a disadvantage that the complex production equipment is required in this method, such as the high pressure device for extruding the organic phase [20].

Liquid jet breakup is widespread in engineering applications. When a stream of fluid (jet) flows into another immiscible fluid at a certain rate, the former will be broken up, and subsequently shrink and agglomerate to form the droplets [21–23]. The formation of jet breakup results from a combination of inertial force, viscous force, surface tension and interaction force of two phases [24]. The device for preparing microspheres by the liquid jet breakup is very simple, and it only needs to meet the requirement that allows one fluid to quickly flow into another fluid through a narrow pinhole, so the scale-up can be achieved by increasing the number of pinholes. In the process of preparation, the size distribution of microspheres is uniform, and the particle size of droplets can be adjusted by changing the inner diameter of needles. In addition, emulsification can be completed within a short time after the organic phase leaves the pinhole, which avoids the secondary emulsification other than the stirring and the static mixing. Therefore, liquid jet breakup is a potential and efficient method for microspheres preparation.

In this study, the liquid jet breakup method was first applied to prepare the sustained-release microspheres based on the self-designed device, which used Risperidone (RSP) as the model drug, Poly (D, L-lactide-co-glycolide) (PLGA) as the polymeric carrier material, dichloromethane as the organic phase, and Poly (vinyl alcohol) 1788 (PVA) solution as the aqueous phase. By adjusting each process parameter, we divided the jets into 5 working states, and analyzed the corresponding mechanisms of breakup and emulsification. Meantime, the single factor method was used to investigate the effect of different process parameters on the particle size and distribution of microspheres during the preparation. In addition, we characterized the prepared microspheres and determined the *in vitro* release so as to verify the feasibility of the liquid jet breakup method for the preparation of sustained-release microspheres.

2. Materials and methods

2.1. Materials

Risperidone (RSP) was purchased from Energy Chemical Company (Shanghai, China), with purity of greater than 99%. Poly (D, L-lactide-co-glycolide), with an average molecular weight of 20000, whose copolymer ratio of D, L-lactic acid and glycolic acid is 75:25 (abbreviated as PLGA 75:25–20000, intrinsic viscosity (ηi) = 0.18–0.25 dl/g), PLGA 75:25–100000 (ηi = 0.65–0.75 dl/g) and PLGA 50:50–50000 (ηi = 0.35–0.45 dl/g) were all obtained from Jinan Daigang Biomaterial Co., Ltd (Shandong, China). Poly (vinyl alcohol) 1788 (PVA 1788, 87–89% in alcoholysis degree, average Mw = 72.6–81.4 kDa) was provided by Shanghai Ying Jia Industrial Development Co., Ltd (Shanghai, China). Dichloromethane was obtained from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). All other reagents were of analytical grade.

2.2. Device for preparing microspheres

The device for preparing microspheres is shown in Fig. 1, which includes the emulsifying module and the drying module. The key component of the emulsifying module is a needle with a certain inner diameter, which is directly connected to the syringe on the syringe pump, and half of the needle tip is immersed in the aqueous phase below. The syringe pump can push the syringe at a constant speed, thus making the pre-filled organic phase in the syringe flow through the needle at a constant rate to form a liquid jet. The main part of the drying module is a temperature-controlled magnetic stirrer, and a beaker containing the aqueous phase is placed on the stirrer.

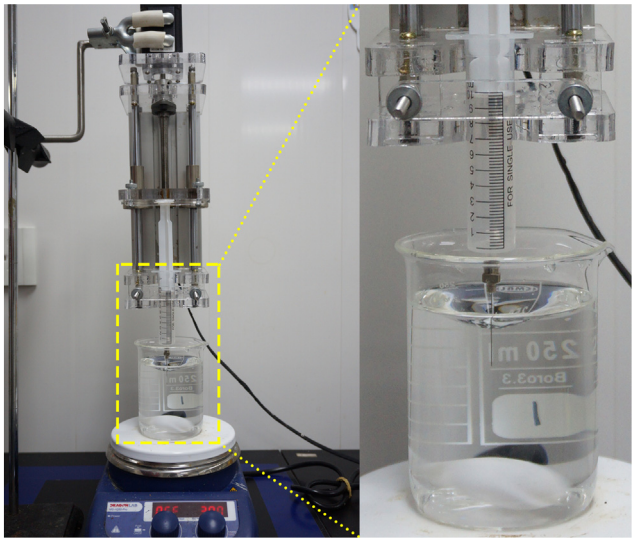

Fig. 1. Schematic of the device for preparing microspheres.

2.3. Preparation of microspheres

RSP and PLGA were mixed at a ratio of 4:6, and the mixture was dissolved in dichloromethane to prepare the drug-containing organic phase. A syringe was used to load the organic phase and was installed on the syringe pump after the inner gas was purged. A proper amount of PVA 1788 was heated to be dissolved in purified water, and the PVA solution (aqueous phase) with a certain concentration could be obtained after cooling. Then the needle was semi-immersed in the aqueous phase, and the stirring speed and temperature of magnetic stirrer were also set. When the temperature was stabilized, the syringe pump was opened to make the organic phase injected into the aqueous phase at a constant rate. After injection, the pump was closed and the aqueous phase was kept stirring for 24 h. The aqueous phase was filtered using a 2500 mesh sieve, and the prepared microspheres were washed with purified water at 25–30 °C for three times. Then the microsphere powder was obtained after the sample was lyophilized at −40 °C under vacuum for 24 h.

2.4. Mechanisms study of liquid jet breakup

The mechanisms of liquid jet breakup in different jet states were studied to guide the preparation of microspheres. In the experiment, the morphology of the organic phase jet and the process of breakup were observed under different process parameters as shown in Table 1 with PLGA 75:25-20000 as the polymeric carrier material. To prevent the flow disturbance for better observation, the stirring speed and the concentration of the aqueous phase were set to 0 rpm and 0% (m/v),

Table 1
Process parameters in the experiment of the observation of different liquid jet states.

State	A	B	C	D	E
Organic phase flow (μl/s)	15.63	31.25	125	125	250
Organic phase concentration (%)	6%	6%	6%	3%	3%
Stirring speed (rpm)	0	0	0	0	0
Aqueous phase concentration (%)	0	0	0	0	0
Needle inner diameter (μm)	130	130	130	130	130
Aqueous phase temperature (°C)	10	10	10	10	10

Table 2
Investigation of different process parameters in the preparation of microspheres.

Group	A	B	C	D	E	F
Organic phase flow (μl/s)	31.25, 62.5, 93.75, 125	125	125	125	62.5	62.5
Organic phase concentration (%)	6	2, 4, 6, 10	6	6	6	6
Stirring speed (rpm)	300	300	200, 300, 400, 500	300	300	300
Aqueous phase concentration (%)	0.5	0.5	0.5	0.1, 0.5, 1, 5	0.5	0.5
Needle inner diameter (μm)	130	130	130	130	80, 130, 170, 230	130
Aqueous phase temperature (°C)	10	10	10	10	10	1, 10, 20, 30

respectively. In the experiment, a camera was used to record the jet state, and the camera aperture value was f = 22, the exposure time was 1/160 s, ISO-400 and the focal length was 50 mm. The lens was focused on the needle part and the pictures were taken 3 s later after the syringe pump worked.

2.5. Effect of different process parameters on the preparation of microspheres

The single factor method was used to investigate the effect of different process parameters, including the organic phase flow, organic phase concentration (PLGA concentration), stirring speed, aqueous phase concentration (PVA concentration), needle inner diameter and aqueous phase temperature, on the particle size and size distribution of microspheres in the process. PLGA 75:25-20000 was used as the polymeric carrier material. The experiment was designed as Table 2, and the particle size and size distribution (span) were determined by the method as described below.

2.6. Characterization of microspheres

Three batches of lyophilized powder of drug-loaded microspheres were prepared with different polymeric carrier materials under the same process conditions (Table 3) to investigate the feasibility of the liquid jet breakup method for the preparation of sustained-release microspheres. Characterization studies of microspheres included the measurement of the particle size and size distribution, morphology observation and determination of drug loading and encapsulation efficiency in microspheres. In addition, the *in vitro* release of microspheres was also investigated in the experiment.

2.6.1. Measurement of the particle size and size distribution of microspheres

The lyophilized powder of microspheres was suspended in 500 ml of purified water and was measured three times by a laser diffraction particle analyzer (Mastersizer 2000, Malvern, UK), respectively. The particle size was expressed as the volume mean diameter in the apparatus. The span used to evaluate the distribution of microspheres was calculated as follows:

$$\text{Span} = (d_{90} - d_{10})/d_{50}$$

where d_{90}, d_{50} and d_{10} means the maximum particle diameters below 90%, 50% and 10% of the microspheres volume exists, respectively.

Table 3
Different parameters for preparing microspheres.

Batch no.	1	2	3
PLGA type	75:25-20000	50:50-100000	75:25-100000
Organic phase flow (μl/s)	62.5	62.5	62.5
Organic phase concentration (%)	6	6	6
Stirring speed (rpm)	300	300	300
Aqueous phase concentration (%)	0.5	0.5	0.5
Needle inner diameter (μm)	130	130	130
Aqueous phase temperature (°C)	10	10	10

2.6.2. Morphology of microspheres

The first batch of lyophilized powder of microspheres was dispersed in the purified water before placed on a glass slide, and the morphology of microspheres was observed under a microscope (XD-202, Jiang Nan, China) and the pictures were taken randomly.

2.6.3. Determination of the drug loading and encapsulation efficiency of microspheres

About 20 mg of lyophilized powder (n = 3) was dissolved in 5 ml of dichloromethane in 100 ml volumetric flask, and the volume was adjusted to 100 ml using ethanol. The solution was filtered through a 0.45 μm organic filter and determined with 20 μl via high performance liquid chromatograph (HPLC, Shimadzu, Japan), which was connected with an UV detector at 278 nm. And the analysis was conducted on an Intersil ODS-3 C_{18} column (250 mm × 4.6 mm, 5 μm particle size) using methanol-water-triethylamine (adjusting pH to 6.20 ± 0.02 by acetic acid) (80:19.5:0.5, v/v/v) as the mobile phase at a flow rate of 1.0 ml/min and a column temperature of 25 °C. The drug loading and encapsulation efficiency could be calculated as follows [25]:

$$\text{Drug loading (\%)} = (\text{weight of drug entrapped} / \text{weight of microspheres used}) \times 100\%$$

$$\text{Encapsulation efficiency (\%)} = \text{drug loading}/\text{theoretical loading} \times 100\%$$

2.6.4. In vitro release study of RSP microspheres

To investigate the *in vitro* release of drugs, about 10 mg of lyophilized powder (n = 3) was incubated in 35 ml phosphate buffer solution (PBS, pH7.4) and kept in a 37 ± 0.5 °C incubator shaker (SHZ-82A, Zheng Rong, China) under gentle agitation (100 rpm) for up to be completely released. 10 ml of media as a sample was withdrawn and superseded by fresh PBS of the same volume at each predetermined time point. Then the obtained samples were filtered and assayed via HPLC for the determination of drug release.

3. Results and discussion

3.1. Mechanisms of microspheres preparation by the method of liquid jet breakup

In order to guide the preparation of microspheres, the mechanisms of liquid jet breakup in different jet states were investigated. In the preparation, the organic phase was pressurized and rapidly flowed through a cylindrical channel (pinhole) to form an organic phase jet, which was then broken up by numerous sources including inertial force, viscous force, surface tension and so on. Then the broken organic phase shrunk and agglomerated to form spherical emulsion droplets based on the action of surface tension, and thus the most important step in the microspheres preparation, the emulsification of the organic phase, was completed.

The Reynolds number was introduced to describe the driving force for emulsification, which was calculated as follows:

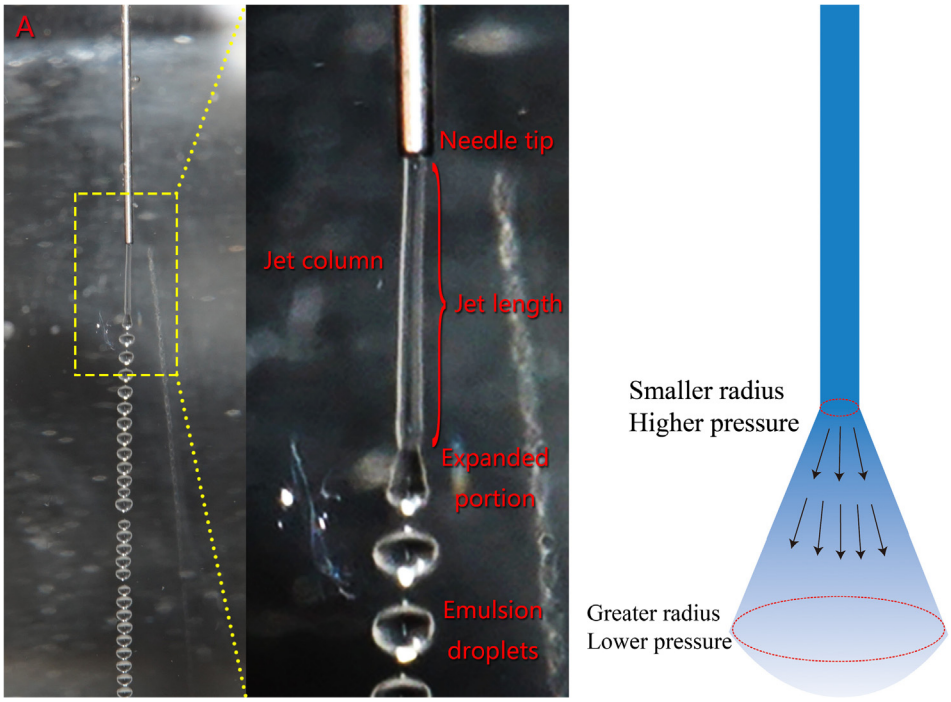

Fig. 2. Jet state of A. (The arrow indicated the liquid flow direction. The darker the color, the higher the pressure).

$$Re = \frac{\rho v d}{r}$$

where *Re* is the Reynolds number, ρ is the fluid density, d is the pipe diameter, v is the fluid flow rate in the pipe and r is the fluid viscosity. With the increase of the *Re*, the emulsified system gradually transitions from the laminar flow to the turbulence, the effect of the inertial force is increased [26], and the driving force for emulsification is enhanced. On the basis of above formula, five states (A, B, C, D, E) with different process parameters, corresponding to the *Re* from small to large, were designed as Table 1 by adjusting the organic phase flow and organic phase concentration, and then the states of liquid jet breakup were observed, respectively. The results are shown in Figs. 2–4. It should be noted that v was the fluid flow rate (the distance of liquid moving per unit time) in the formula, while the process parameter that could be directly controlled in the experiment was the organic phase flow (flow volume through the cross section per unit time). It was obvious that the flow rate was proportional to the flow when the inner diameter of the needle was constant. Similarly, the organic phase concentration was changed in order to alter the fluid viscosity (r) in the study.

As shown in Fig. 2, state A had the slowest flow rate and the highest viscosity so that the liquid jet had a low *Re*. The organic phase jet column first moved straight for a distance (jet length) in a single jet state after separated from the needle, and then was broken up at the end of the jet column to form a single stream of droplets but continued to move in the original direction. At this point, the distribution of emulsion droplets was non-uniform and the particle size was larger than the needle inner diameter. The lower *Re* allowed the organic phase to be jetted in a laminar flow manner. After the organic phase was pushed away from the needle, the kinetic energy of the jet column was gradually consumed due to the friction between the aqueous phase and the organic phase jet, so the jet speed was slowed down, which caused the liquid to accumulate at the end of the jet, forming an accumulation region with a greater radius. Based on the surface tension, the region with a greater curvature radius had a lower internal pressure, so the organic phase was squeezed from the higher-pressure region at the end of the jet column (the radius of this part was close to the radius of the needle) into the accumulation region with a lower pressure, and finally the jet column was broken into droplets [27,28]. Strictly speaking, the

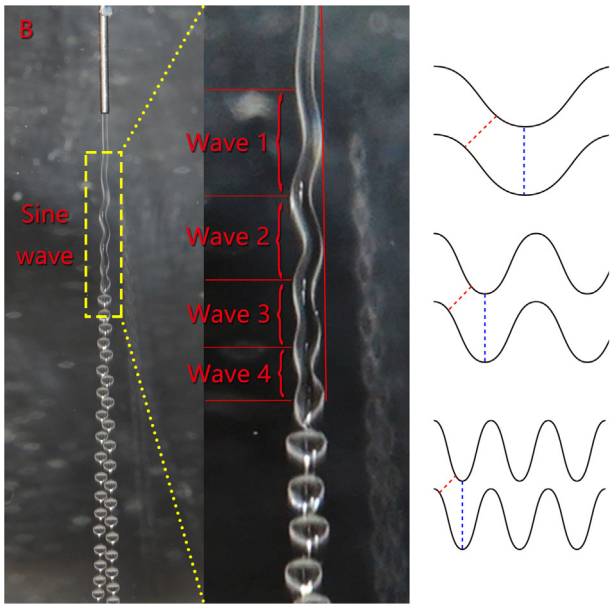

Fig. 3. Schematic diagrams of jet state B and the effect of the wavelength on the diameter difference of the jet column. (Red dotted line: the region with the smallest diameter. Blue dotted line: the region with the greatest diameter). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

internal pressure of the jet column was influenced by two separate curvature radius components. In this case, one was the curvature radius of the jet column in the horizontal section, which was already discussed, and the other was the curvature radius of the wave itself, which was in the vertical direction. It was observed that the pressure generated by the latter was always negative to the breakup process, and thus it wasn't considered here when analyzing the cause of liquid jet breakup. In state A, the pressure difference due to the surface tension was the main driving force for liquid jet breakup, the existence and development of which were closely related to the state of the

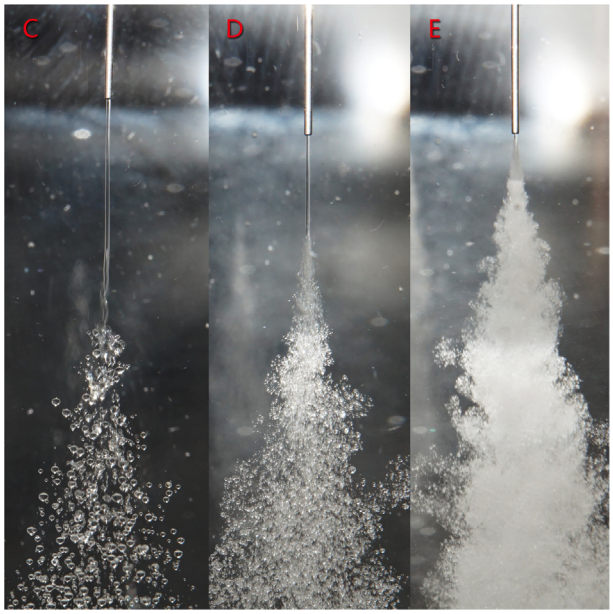

Fig. 4. Jet state of C, D and E.

accumulation region. Since the formulation of the accumulation region was easy to be influenced by any tiny perturbations in the system, the prepared droplets had a non-uniform particle size distribution.

In state B, the increase in the organic phase flow increased the *Re* and prolonged the jet length. As shown in Fig. 3, the end of the jet column showed a sinusoidal wave, and two streams of droplets with different directions were formed after the jet was broken up, of which the sizes were similar to the needle inner diameter and the distributions were uniform. In this case, the jet was also in the laminar flow state, but the higher flow rate of the organic phase enhanced the disturbance of the aqueous phase to the jet, so asymmetrical surface waves were generated on both sides of the jet column, which finally made the jet column act on the sinusoidal wave. The similar phenomenon could also be observed when a liquid jet moved in compressed air [29]. The sine wave had many characteristics. It continued to move along the direction of the jet column, and the amplitude of the wave was almost constant, but the wavelength was gradually reduced, causing the diameter of the thinnest region in the interior of the jet column to be gradually reduced (red dotted line in Fig. 3). According to the fourth wave far away from the needle, when the wavelength was shortened to be about twice the length of the initial diameter of the jet column, the difference in thickness between different regions in the interior of the jet column reached the maximum. Similar to state A, a pressure difference was generated because of the difference in the thickness of the column based on the surface tension, driving the organic phase into flowing from the thin region to the thick region and thus causing the jet to be broken up into droplets. However, different from state A, the pressure difference in state B was mainly induced by the reduction of the wavelength, which was less affected by the perturbations in the system, so the particle size distribution of microspheres was uniform. And the direction of droplets was gradually divided into two streams due to the different vibration directions of the sine wave.

In state C, D and E, the *Re* was gradually improved by increasing the organic phase flow or decreasing the organic phase concentration. According to Fig. 4, it could be observed that as the *Re* increased, the sinusoidal pattern of the jet gradually disappeared, the movement direction of the droplets was changed from the previous linear motion to a conical distribution, the jet length began to be shortened, the particle size was gradually reduced, and the jet column was atomized as soon as it left the needle at state E. From state C to state E, the jet gradually transitioned from the laminar flow to the turbulent flow, and the instability of the jet was sharply increased. Since the energy in the turbulent flow was much higher than that in the laminar flow, the particle size of droplets was significantly smaller than that in state A and B, and was independent on the needle inner diameter. In addition, the size distribution was non-uniform due to the uneven energy distribution in the transition state (state C).

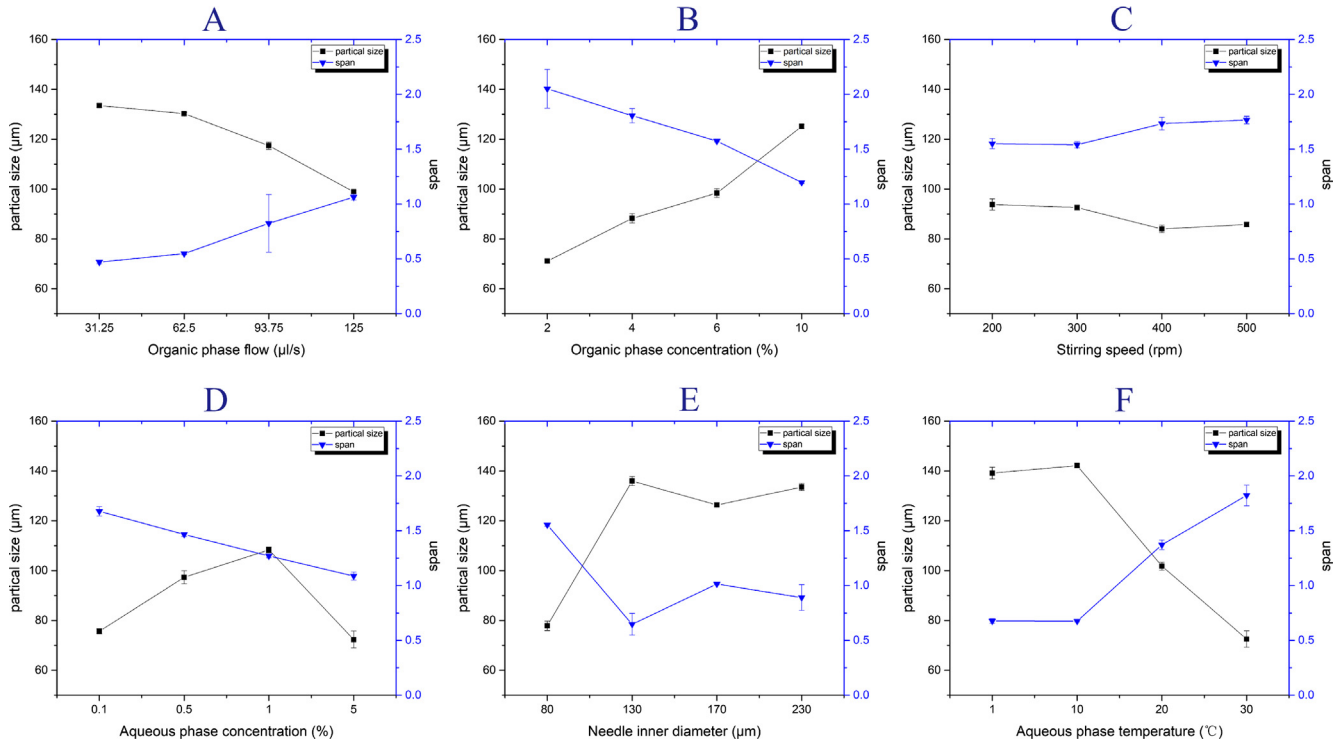

Fig. 5. Effects of each process parameter on the particle size and size distribution of microspheres. (A) Organic phase flow. (B) Organic phase concentration. (C) Stirring speed. (D) Aqueous phase concentration. (E) Needle inner diameter. (F) Aqueous phase temperature; n = 3, mean ± SD.

3.2. Effect of process parameters on the preparation of microspheres

The single factor method was used to investigate the effect of different process parameters on the particle size and size distribution of microspheres so as to guide the preparation of microspheres as Table 2. Results are shown in Fig. 5. Overall, increasing the organic phase flow, stirring speed and aqueous phase temperature would reduce the mean particle size, improve the span and decrease the distribution uniformity. Nevertheless, the opposite phenomena would be generated when increasing the organic phase concentration and needle inner diameter.

On the basis of the previous analyses, the *Re* could be regarded as the driving force for emulsification. According to the equation, the *Re* could be improved by increasing the fluid flow rate v, the pipe diameter d and the fluid density ρ, while it could be reduced after increasing the fluid viscosity r. In the process parameters, the organic phase flow would affect the flow rate v, and it was obvious that the higher the flow, the faster the flow rate when the needle inner diameter was constant. Similarly, the stirring speed also affected the fluid rate v, and the faster the aqueous phase moved, the higher the relative speed was. In addition, the needle inner diameter not only directly affected the pipe diameter d, but also affected the flow rate v. Since a constant flow was applied in the study, the flow rate v was inversely proportional to the square of the needle inner diameter, so that the *Re* was inversely proportional to the needle inner diameter. And in the range of the needle inner diameter from 130 μm to 230 μm, the reason for the stability of the particle size could consist of two parts. Microspheres had a particle size equal to the inner diameter of the needle when the liquid jet was in state B at 130 μm. While the liquid jet was in state A at 170 μm and 210 μm, where the particle size was mainly affected by the surface tension of the organic phase rather than the needle inner diameter, leading to the mean particle size of about 130 μm. However, after the inner diameter was reduced to 80 μm, the *Re* was significantly increased, and the jet state was transformed into state C, resulting in a decrease in the mean particle size and distribution uniformity.

Increasing the temperature of the aqueous phase could indirectly increase the temperature of the organic phase jet, and thus reduced the density and viscosity of the jet. However, the changes of organic phase density and viscosity due to the temperature were very limited in the adjustment range of parameters, which weren't enough to explain the significant changes in the particle size only from the perspective of the driving force for emulsification (*Re*). Therefore, the resistance to emulsification was introduced to make a reasonable explanation. When the organic phase began to be broken up under various external forces, the concave meniscus would be generated. And the excess pressure would be caused after the surface tension acted on the concave meniscus to resist the external force. If the external force was strong enough, the organic phase would be broken up and emulsified into droplets, whereas emulsification would not occur in turn. Therefore, the excess pressure could be considered as the resistance to emulsification, which was calculated by the Young-Laplace equation as follows:

$$Ps = \mathrm{r}\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$

where Ps is the excess pressure, r is the surface tension coefficient, and R_1 and R_2 are the principal radii of curvature (for the sphere, $R_1 = R_2$). According to Guggenheim et al. [30], when the temperature was increased, the surface tension coefficient of the organic phase was decreased, and thus the excess pressure was decreased. In consequence, the particle size of microspheres was reduced by the combination of the decrease in the resistance to emulsification and the increase in the driving force for emulsification.

The organic phase concentration directly affected the viscosity r and the fluid density ρ, which both affected the *Re*. The higher the concentration of the organic phase, the higher the viscosity and the fluid density. Obviously, the increase in viscosity was greater than that in density, causing the *Re* to be reduced and thus the driving force for emulsification to be reduced, too. Meantime, the increase in viscosity meant an increase in the frictional resistance between the molecules of the organic phase during the emulsification process, which increased the resistance to emulsification as a result. In summary, the particle size of microspheres was increased based on the above factors.

The effect of the aqueous phase concentration (PVA concentration) on the morphology of microspheres is shown as Fig. 5D. The particle size showed a tendency that it was gradually increased in the early period but then was decreased sharply in the later period when the aqueous phase concentration was gradually increased, but the uniformity of the particle size distribution was gradually improved at the same time. When the concentration of the stabilizer (PVA) in the aqueous phase was increased from 0.1% to 1%, it could be seen that the jet column was gradually shortened, the jet state gradually transitioned from C to B, and thus the particle size of microspheres was increased, which might be caused by the increased density of the aqueous phase and the increased flow resistance. When the aqueous phase concentration was increased to 5%, the viscosity of the aqueous phase was increased significantly, the inter-molecular friction and the resulting perturbance to the jet were both increased dramatically, which might lead to a sudden decrease in the particle size of microspheres.

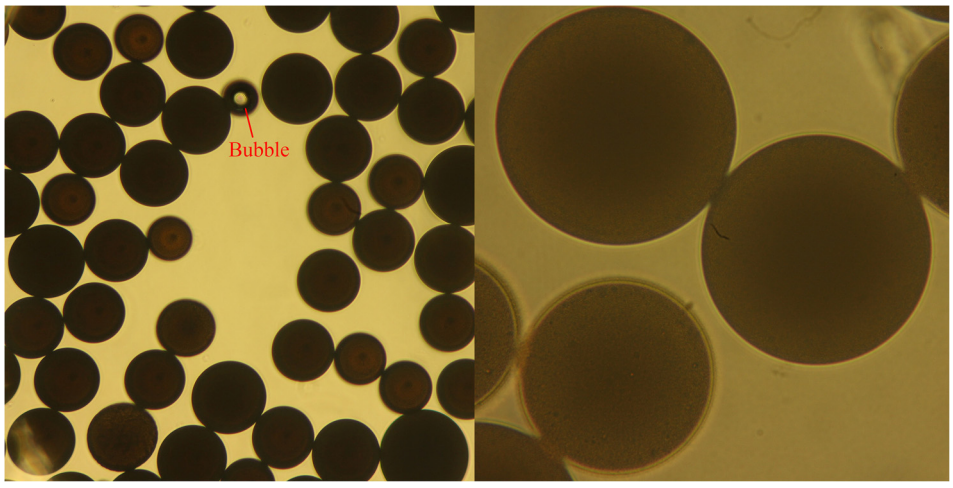

Fig. 6. Morphology of microspheres under the microscope (batch no. 1).

Table 4
Characterization results of microspheres.

Batch no.	Drug loading (%)	Encapsulation efficiency (%)	Particle size (μm)	Span
1	34.2 ± 0.4	85.4 ± 0.0	135.9 ± 1.8	0.65 ± 0.10
2	35.5 ± 0.2	88.7 ± 0.5	143.4 ± 6.6	0.64 ± 0.03
3	35.9 ± 0.8	89.8 ± 2.1	165.0 ± 5.4	0.42 ± 0.05

Results are presented as an average with standard deviation of 3 samples.

3.3. Characterization of microspheres

Three batches of microspheres were prepared using different polymeric carrier materials. And the morphology of the first batch of lyophilized powder was observed under the microscope. As shown in Fig. 6, microspheres prepared by the method of liquid jet breakup were presented as the slightly reddish and black solid spheres with the good roundness and the uniform size distribution under the microscope

The results of the drug loading and encapsulation efficiency of the three batches of microspheres are shown in Table 4. It could be seen that there was little difference between each batch with the mean drug loading of about 35% and the mean encapsulation efficiency of about 88%. The batch no. 1 had a particle size of 135.92 μm and a span of 0.65. While the batch no. 2 and batch no. 3 had the larger particle sizes, which were corresponding to 143.35 μm and 164.97 μm, and the spans were 0.64 and 0.42, respectively.

As Fig. 7, the *in vitro* release profiles of the three batches of microspheres indicated that the first two batches of microspheres could release for about 250 h, and the third batch could release for about 750 h. The release curves of batch no. 1 and batch no.2 were both "s"-shaped, except that the first batch had a release of drugs at the beginning, but the second batch had almost no drug release before 100 h. Until the mid-late stages of release (after 100 h), the microspheres of batch no. 2 began to release drugs at a fast rate, while the batch no. 1 still maintained a slow drug release rate. In particular, the microspheres of the third batch didn't release drugs until 300 h, and then the drug release rate was gradually increased with an "s"-shaped curve formed subsequently.

According to the results, the relatively high drug loading and encapsulation efficiency might benefit from the fact that the emulsification of organic phase was completed in a single and instantaneous manner in the liquid jet breakup method, reducing the loss of drugs when the organic phase was broken, and thus causing a less leakage of microspheres. Moreover, the viscosities of the organic phase were different due to the different carrier materials used in the three batches of microspheres, which resulted in the different particle sizes and size distributions of microspheres. Compared with PLGA 75:25-20000 used in the batch no. 1, the polymer (PLGA 50:50-50000) in the batch no. 2 had a higher molecular weight and thus a slower degradation rate, which led to a less drug release in the initial stage. However, due to the relatively high copolymer ratio of glycolic acid in PLGA 50:50, the degradation rate was higher than that of PLGA 75:25 at the same molecular weight. Therefore, the degradation rate of PLGA 50:50 was increased rapidly after the polymer was swelling in the mid-late stage of the curve, which resulted in a centralized release of drugs in the batch no.2 of microspheres. Similarly, the no. 3 batch of microspheres used PLGA 75:25-100000 as the polymeric carrier material, of which the molecular weight was so high that there was no drug being released until the polymer was swelling after 300 h.

It was worth noting that the *in vitro* release profile of microspheres in the batch no. 3 was similar to that of commercial drug Risperdal Consta® [31], and both of them had a lag phase after administration. A two-week oral administration after the injection was used by Risperdal Consta® to compensate for the problem that there was no drug being released in the first two weeks. In contrast, the microspheres of the batch no. 1 released drugs immediately after administration with no obvious lag phase, and the release trend was stable in about two weeks, which was more convenient for the treatment of diseases. The second batch had a centralized drug release trend and a fastest release rate that about 48% of drugs were released within 24 h, which was similar to the pulsed release and wasn't suitable for the treatment of chronic diseases considering the longer treatment period of microspheres. But it could be used for diseases requiring pulsed administration, such as local anti-infection treatment. Microspheres could be prepared when the model drug RSP was replaced with antibiotics, and the drugs could be pulsed released after a lag phase, resulting in a higher blood concentration to eliminate infection and reducing the bacterial resistance caused by continuous administration.

4. Conclusions

In the present study, the method of liquid jet breakup was applied to prepare microspheres. During the preparation of microspheres, the liquid jet had five working states. Both state A and state B were laminar and the particle sizes of prepared microspheres were greater than or close to the needle inner diameter. And the jet was "proactively" induced to be broken up by the surface wave when it was in state B, where the distribution of microspheres was the most uniform and the particle size could be easily controlled by the needle inner diameter. State D and state E were turbulent, and the particle sizes of prepared

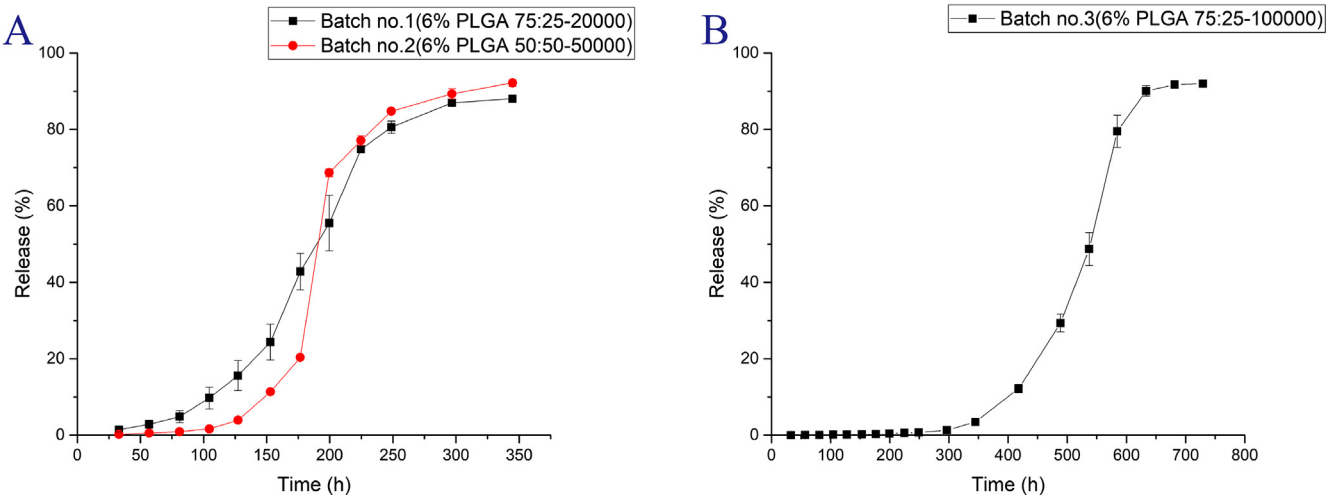

Fig. 7. *In vitro* release results. (A) Batch no. 1 and batch no. 2. (B) Batch no. 3; n = 3, mean ± SD.

microspheres were much smaller than the needle inner diameter, which was suitable for preparing microspheres with small particle sizes. State C was a transitional state between the laminar flow and the turbulence with a wide distribution of prepared microspheres, which should be avoided in the actual production. Different process parameters would affect the particle size and distribution of microspheres by changing the *Re*, surface tension coefficient and viscosity. In addition, the microspheres prepared by liquid jet breakup had better roundness, higher drug loading and higher encapsulation efficiency. Based on different polymeric carrier materials, the microspheres could have various drug release models such as sustained release with a lag phase, sustained release with no lag phase, pulsed release and so on, which could meet different demands for drug treatment.

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