Send Orders for Reprints to reprints@benthamscience.ae

The Open Biomedical Engineering Journal, 2015, *9*, 115-120

Open Access

Research of Amoxicillin Microcapsules Preparation Playing Micro-Jetting Technology

Huaiyuan Sun[1,2,*], Qingqing Gu[1],Yuehua Liao[2], and Chenjie Sun[1]

[1]*College of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai, 200093, China*

[2]*Shanghai Medical Instrumentation College, Shanghai, 200093, China*

Abstract: With polylactic-co-glycolic acid(PLGA) as shell material of microcapsule, amoxicillin as the model, poly(vinyl alcohol) and twain as surfactant, amoxicillin-PLGA microcapsules were manufactured using digital micro-jetting technology and a glass nozzle of 40μm diameter. The influences of the parameters of micro-jetting system on the mean grain size and size distribution of amoxicillin-PLGA microcapsules were studied with single factor analysis and orthogonal experiment method, namely, PLGA solution concentration, driving voltage, jetting frequency, stirrer speed, etc. The optimal result was obtained; the form representation of microcapsule was analyzed as well. The results show that, under certain conditions of experimental drug prescription, driving voltage was proportional to the particle size; jetting frequency and stirrer speed were inversely proportional. When the PLGA concentration for 3%, driving voltage for 80V, the jetting frequency for 10000Hz and the stirrer speed for 750rpm, the particles were in an ideal state with the mean grain size of 60.246μm, the encapsulation efficiency reached 62.39% and 2.1% for drug loading.

Keywords: Micro-jetting; Amoxicillin; PLGA; Microcapsule preparation.

1. INTRODUCTION

Micro-jetting is an important part of micro fluidic controlling system. Its principle is based on the nozzle internal volume changes generated by the transducer to generate a pressure wave in the body leads to the liquid chamber, the pressure wave was transmitted into the nozzle hole as fluid velocity, thus making the droplets ejected from the nozzle [1-3]. With the development and practice of microsystems and microfluidics digital technology, digital micro-jetting technology, as a new technique, has gained some development and preliminary application in medicine, bio-manufacturing engineering, pharmaceutical engineering and other fields [4].

Amoxicillin is a semi-synthetic β-lactam antibiotic, which has been applied widely since it can be administered orally. It is also a broad spectrum antibiotic. In recent years, it is commonly used in triple therapy for treatment of Helicobacter pylori (HP)-induced gastrointestinal ulcers [5], however the rate of ulcer recurrence is much higher [6, 7]. Some studies show that this is due to the short residence time of amoxicillin in the body; it cannot go deep into the affected area directly, thereby affecting the efficacy [8]. Polylactic acid-glycolic acid copolymer (PLGA) is a high-molecular compound formed by polymerization of lactic acid and glycolic acid. It has not only good biocompatibility, but also favored encysted and film formation properties as well as non-toxic. What's more, the degradation rate can be controlled. With all those advantages, PLGA has been widely used in the field of biomedical engineering [9].

Many different kinds of methods have been used to manufacture polymer microspheres, such as emulsion drying method, the spray drying method, membrane emulsification method and micro-channel method [10, 11], etc. The former two methods can hardly meet the special requirement in the biomedical field. And the latter two methods cost too much.

Micro-jetting technology is a new method to fabricate microspheres. Compared with above methods, the particle size can be controlled more precisely with micro-jetting. Under the room temperature and normality, the droplet resolution can reach 10^{-15}L to 10^{-12}L, i.e., the size of a single droplet can be controlled in submicron to micron grade size [12], 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 verified the feasibility of applying the micro-jetting technique to manufacture microcapsule by using digital microcapsule manufacturing system [13, 14].

Based on the principle of micro-jetting, this article used PLGA as shell material, amoxicillin as the main drug to obtain the particles with uniform grain size as well as controllable. Using single factor and orthogonal experimental methods, the relationships between the

*Address correspondence to this author at the No. 334 JungongRoad, Shanghai, China. Postcode: 200093; Tel: 18301781791;
E-mail: shy62123@163.com

1874-1207/15 2015 Bentham Open

parameters of micro-jetting system with microspheres particle size and its distribution have been studied. And the nature of microcapsules is examined.

2. MATERIALS AND METHODOLOGY

2.1. Reagents and Materials

PLGA(LA:GA=50:50 , DURECT Corporation), PVA (Shanghai RunJie chemical reagent co., LTD.), Amoxicillin original drug (DingHui Chemical Co., content of 99.7%, batch number 120511), Amoxicillin reference substance (China Pharmaceutical Group Chemical Reagent Co., content of 99.9%, batch number 111211), methylene chloride, Twain 80, distilled water.

2.2. Methods

a. Device and principle

Micro-jetting system which is used for manufacturing microcapsules was shown in Fig. (**1**). It is 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 a computer. The micro-jetting control unit is the core of the whole system; it was constituted by micro nozzle assembly, sprayer installation mechanism and electric controller. The system used 40μm inside diameter glass nozzles produced in MicroFab company in United States, the working principle of which is based on the inverse piezoelectric effect. Volume change produced by the nozzle internal piezoelectric transducer results to the liquid pressure wave in cavity body and the fluid within the tube moving at the same pace, then the pressure wave goes to the nozzle hole and transformed into the fluid velocity that makes the droplet spray from the nozzle [1-3]. A variety of complex waveform signal can be provided to the nozzle with Electric controller (CT-M3-02 controller).

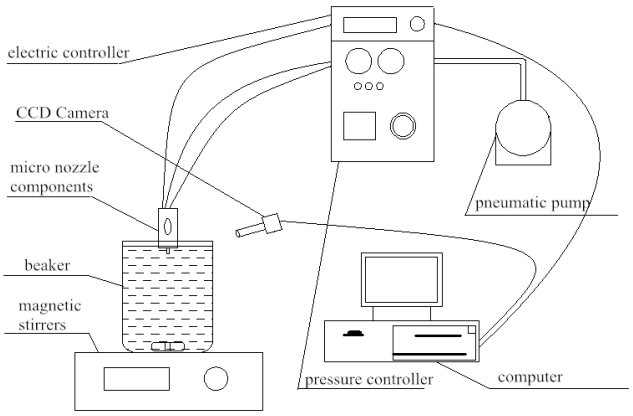

Fig. (1). Micro-injection preparation microcapsule system structure schematic drawing.

b. Preparation of amoxicillin-PLGA microcapsules

Taking certain amount of PLGA with a precision electronic balance and dissolving it into 2ml of methylene chloride to obtain the PLGA-methylene chloride solution. Took precise 40mg amoxicillin original drug separately and put it into 50ml of water until dissolved completely. Then took 2ml solution out of it and slowly dropped into the above PLGA-methylene chloride solution stirred with high velocity. The w / o colostrums would be formed after 60 seconds' emulsion. Injecting the colostrums into storage tube of the micro-jetting system, and the nozzle was inserted in aqueous solution of 1% PVA-Twain 80. Both PVA and twain play the role of surfactant. Micro-jetting was controlled by the piezoelectric controller, and meanwhile magnetic stirrer was mixing the solution with a certain speed to avoid adhesion between microcapsules sprayed. Magnetic stirrer continued to work for 4-5 hours after micro-jetting was completed to volatilize methylene chloride. Then microporous filter membrane of 0.22μm was used for vacuum filtration and microcapsules collection. Washing the microcapsules with deionized water 3 times and dried under vacuum for 24 hours.

c. Orthogonal experiment optimization and process validation

Considering the effects many factors have on the formation and quality of microcapsules, this article select four main factors affecting the microcapsules preparation as subjects of the study on the basis of pre-test. Namely, PLGA concentration (A), driving voltage (B), jetting frequency (C) and stirrer speed (D). From each three levels of each factor (as shown in Table **1**, with microcapsule particle size and homogeneity (i.e. relative standard deviation, RSD) as index, tests are arranged according to the L9(3^4) orthogonal table to choose the best technical parameters (based on a minimum RSD).

Table 1. Orthogonal experimental design factor level table.

level	factor			
	A%	*B*/V	*C*/Hz	*D*/r·min^{-1}
1	1	80	5000	500
2	2	90	7500	750
3	3	100	10000	1000

Seen from the data of orthogonal test of microcapsules preparation technology (as is shown in Table **2**), we can conclude that the impacts of the four factors on the microcapsule size and uniformity are as follows: B> A> D> C, the optimum technological condition is $A_3B_1C_3D_2$, i.e., PLGA concentration for 3 %, the driving voltage for 80V, jetting frequency for 10kHz and stirrer speed for 750rpm.

d. Particle size test and form representation

(1) The test of amoxicillin-PLGA microcapsules particle size distribution

Amoxicillin-PLGA microcapsules were dispersed in water. Particle size and distribution were analyzed with laser particle analyzer (Winner2005, Jinan Weina instrument co.,LTD.). Particle size and homogeneity of the

Table 2. Orthogonal experimental design and results.

Test No.	Factors and levels				Average particle size	relative standard deviation
	A	B	C	D	$\bar{d}$ (μm)	RSD%
1	1	1	1	1	55.334	3.51
2	1	2	2	2	57.421	3.25
3	1	3	3	3	61.923	5.34
4	2	1	2	3	58.543	2.21
5	2	2	3	1	63.671	3.43
6	2	3	1	2	68.223	2.64
7	3	1	3	2	60.246	1.87
8	3	2	1	3	64.782	2.35
9	3	3	2	1	70.246	3.51
I_j	174.678	174.113	188.339	189.251		
II_j	190.437	185.874	186.210	185.900		
III_j	195.284	200.392	185.850	185.248		
k_j	3	3	3	3		
I_j/k_j	58.226	58.044	62.780	63.084		
II_j/k_j	63.479	61.958	62.070	61.967		
III_j/k_j	65.095	66.797	61.950	61.749		
D_j	6.869	8.753	0.830	1.334		

microspheres were showed by average grain diameter $\bar{d}$ and relative standard deviation (RSD).

(2) The form representation of amoxicillin-PLGA microcapsules.

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

e. Determination of amoxicillin-PLGA microcapsules drug loading and encapsulation efficiency

(1) Determination method

Test with liquid chromatograph of LC20AT type(Shimadzu Corporation, Japan). Chromatographic conditions are: chromatographic column for C18 (4.6mm × 150mm, 5μm); mobile phase for acetonitrile-0.05mol/L potassium dihydrogen phosphate buffer (adjusted pH with 3mol/L potassium hydroxide solution to 6.0 ± 0 1) (4 : 96); flow rate for 1ml per minute; detection wavelength for 226nm; injection volume for 20μl. Typical chromatograms are shown in Fig. (**2**), Fig. (**3**).

Establish a standard curve: Precision 45mg amoxicillin reference was taken, dissolved it in phosphate buffer of 0.1mol/ L and pH 6.0 and diluted to 50ml as stock solution. Standard Solution with Series concentrations were prepared to be determined separately. Linear regression is made with the peak area (A) as Dependent Variable and concentration

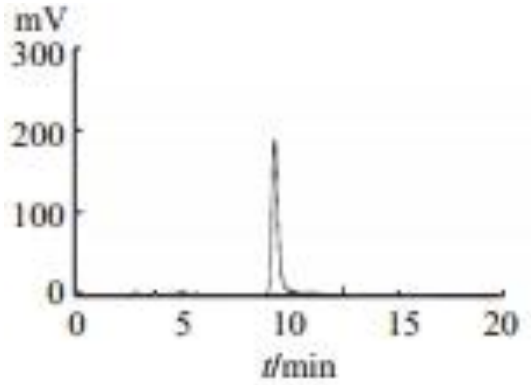

Fig. (2). Typical chromatogram of reference solution (0.1mg/ml).

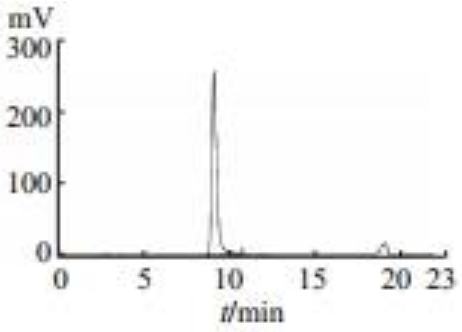

Fig. (3). Typical chromatogram of sample solution.

(c) as independent variable, the standard curve equation was obtained as follows: $A=2.36\times10^5c-1.89\times10^5$, $R^2=0.999\ 8$. The linear range is $50\sim600\ \mu$ g/ml.

(2) Determination of entrapment efficiency and drug loading

Took appropriate amount of amoxicillin-PLGA microcapsules into a measuring flask of 100ml. Moderate phosphate buffer with pH 6.0 was added and then dissolved by ultrasonic for metering volume. Filtering with filter membrane of 0.45μm and took the subsequent filtrate as sample solution to be determined, repeated the test for three times.

Calculation method of Encapsulation efficiency:

$$\text{Encapsulation efficiency} = \left(\frac{\text{content of amoxicillin in the microcapsule}}{\text{content of amoxicillin}}\right)\times100\%$$

Calculation method of Drug loading:

$$\text{Drug loading} = \left(\frac{\text{content of amoxicillin in the microcapsule}}{\text{weight of microcapsule}}\right)\times100\%$$

3. RESULTS

3.1. The Particle Size Influencing Factor Analysis

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

The influence of driving voltage on amoxicillin-PLGA microcapsules particle size

In experiments, keeping other conditions unchanged (jetting frequency was 5000 Hz, stirrer speed was 300rpm, PLGA concentration was 3%, PVA-Twain aqueous solubility was 1%), only changed the driving voltage value and got the result as shown in Fig. (**4**).

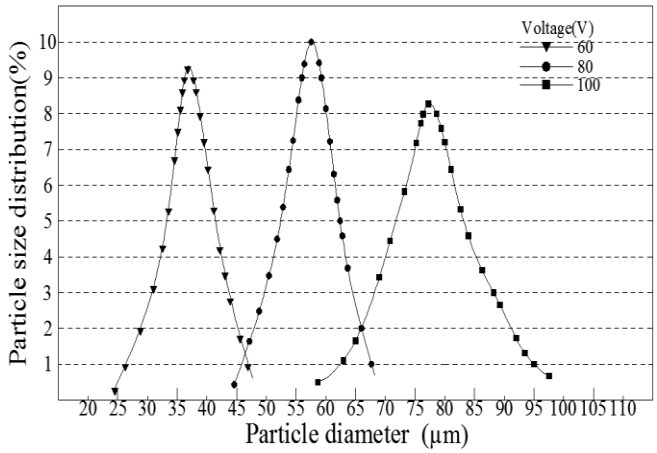

Fig. (4). Different driving voltage of the preparation of the amoxicillin-PLGA microcapsules particle size distribution.

It showed that, as the driving voltage was 60V,80V and 100V respectively, the corresponding average microcapsules particle size was 35.783μm, 53.969μm, 78.602μm, and the polydispersity index(PDI) was 0.032, 0.048, 0.165 with the increase of driving voltage, the amoxicillin-PLGA microcapsules particle size was increased and grain size uniformity was deteriorated.

The influence of jetting frequency to amoxicillin-PLGA microcapsules particle size

Jetting frequency refers to the interval between two continuous jetting. In experiments, keeping other conditions unchanged (driving voltage was 80V, stirrer speed was 300rpm, PLGA concentration was 3%, PVA-Twain aqueous solubility was 1%), only changed the jetting frequency and got the result as shown in Fig. (**5**).

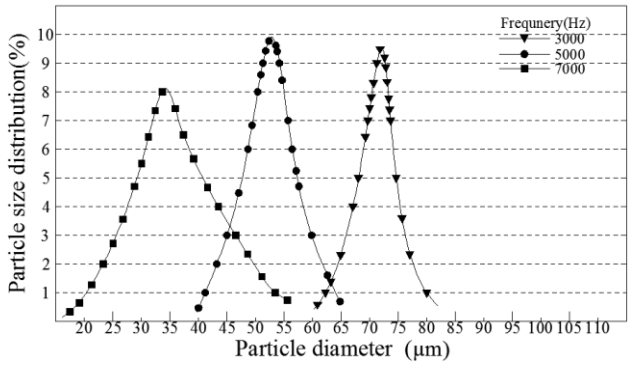

Fig. (5). Different jetting frequency of the preparation of the amoxicillin-PLGA microcapsules particle size distribution.

It showed that, as the jetting frequency was 3000Hz, 5000Hz and 7000Hz respectively, the corresponding average amoxicillin-PLGA microcapsules particle size was 73. 786μm, 55.564μm and 35 561μm, and the polydispersity index was 0.024, 0.041, 0.271. That is to say, with the jetting frequency increasing, the amoxicillin-PLGA microcapsules particle size was decreased and grain size uniformity was deteriorated.

The influence of stirrer speed on amoxicillin-PLGA microcapsules particle size

In experiments, keeping other conditions unchanged (driving voltage was 80V, jetting frequency was 5000 Hz, PLGA concentration was 3%, PVA- Twain aqueous solubility was 1%), only changed the stirrer speed to adjust water phase velocity and got the result as shown in Fig. (**6**).

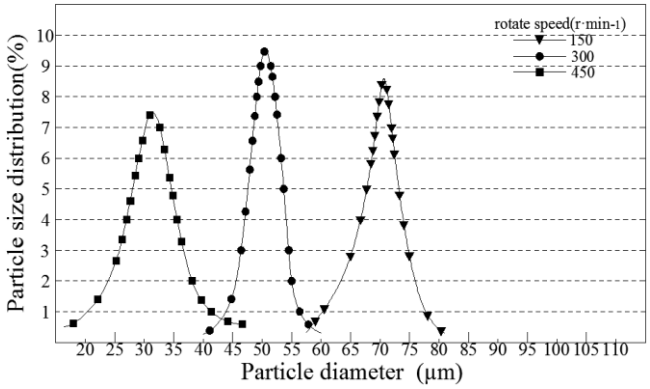

Fig. (6). Different stirrer speed of the preparation of the amoxicillin-PLGA microcapsules particle size distribution.

It showed that, as the stirrer speed was 150rpm, 300rpm and 450rpm respectively, the corresponding average microspheres particle size was 72.145μm, 51.784μm, 30.892μm, and its polydispersity index was 0.026, 0.039, 0.205. That is to say, with the increase of magnetic stirrer speed, the amoxicillin-PLGA microcapsules particle size was reduced and grain size uniformity was deteriorated.

3.2. Particle Size Distribution and Surface Morphology of Amoxicillin-PLGA Microcapsules

Three batches of amoxicillin-PLGA microcapsules are made according to the optimum preparation techniques. The average particle size is (60.246 ± 1.082)μm under the guide of a laser particle analyzer and relative standard deviation (RSD) is 1.87%. The particle size distribution curve is shown Fig. (**7**). A certain stability of preparation technology can be seen.

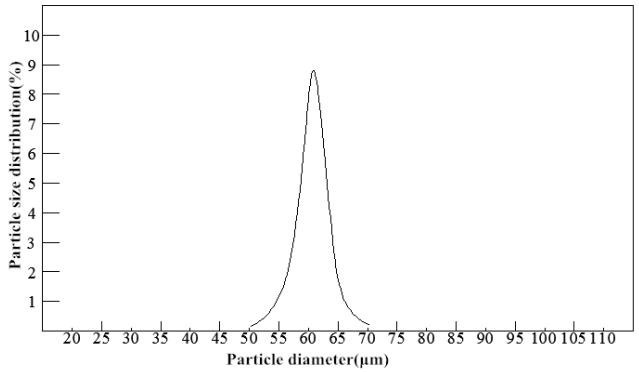

Fig. (7). Amoxicillin-PLGA microcapsules particle size distribution diagram.

When methylene chloride had volatilized completely from the oil phase, amoxicillin-PLGA microcapsules can be collected after solidifying, molding and filtering. Observed it with a Scanning Electron Microscope after drying, as is shown in Fig. (**8**) and Fig. (**9**), we can see that microcapsules are solid spheres; there is no adhesion between microcapsules; particle size uniformity is good; surface is smooth, round and highly integrated [15].

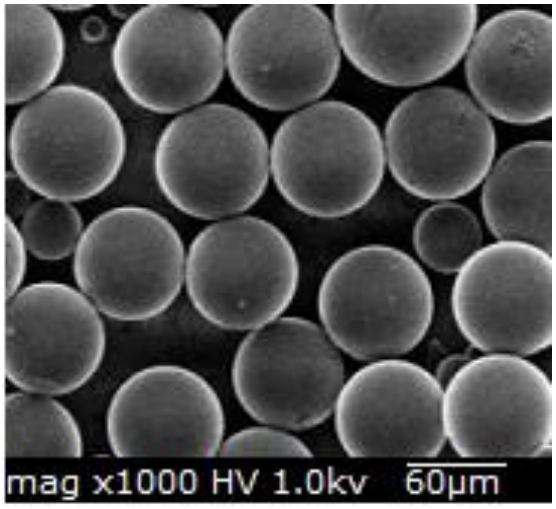

Fig. (8). The dried amoxicillin-PLGA Microcapsules.

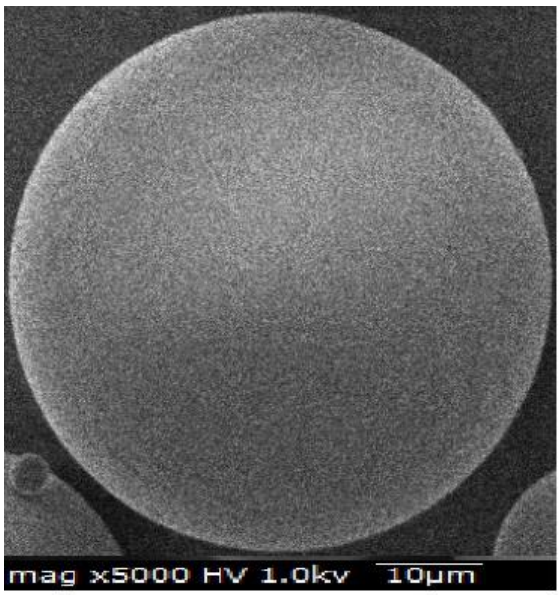

Fig. (9). Surface of amoxicillin-PLGA Microcapsule.

3.3. Drug Loading and Entrapment Rate Analysis of the Amoxicillin-PLGA Microcapsules

Amoxicillin-PLGA microcapsules prepared according to the optimal conditions were dissolved by ultrasonic and analyzed by high performance liquid chromatography. The entrapment rate calculated was (62.4 ± 3.9) %, and (2.1 ± 1.4720)%(n=3) for loading rate. Amoxicillin-PLGA microcapsules prepared with solvent evaporation method in literature [16] have an entrapment rate of 60%, showing that the use of digital micro-jetting technology for preparing microcapsules of drugs is feasible. Its drug encapsulation efficiency and drug loading is also more desirable compared with microcapsules prepared by other methods.

CONCLUSIONS

Based on the micro-jetting technology, the microsphere preparation system with micron grade piezoelectric nozzle and its controller as the core was adopted. We can make the following conclusions through experiments:

(1) Amoxicillin-PLGA microcapsules with controllable particle size, high uniformity as well as spherical and round were manufactured successfully using digital micro-jetting technology in this paper.

(2) Applying the single factor method, the relationships between the technical parameters (driving voltage, jetting frequency and stirrer speed) with amoxicillin-PLGA microcapsules mean grain size as well as the distribution of the particle size were well known: ①Driving voltage was proportional to the particle size and inversely proportional to the size uniformity. When the driving voltage is 100V, the average particle diameter is 70.246 μ m; much adhesion was observed between microcapsules under the microscope, particle size uniform was deteriorated, showing that the microcapsule particle size is proportional to the driving voltage, and the effect of driving voltage on the particle size is significant. ②The jetting frequency was inversely proportional to the microsphere particle size and the size

uniformity. The influence of the stirrer speed was the same as the jetting frequency.

The amoxicillin-PLGA microcapsules manufactured with micro-jetting provide a new method for the preparation of drug microcapsule. And that the microspheres grain size and particle size distribution could be controlled in dozens of microns by changing the corresponding system parameters on-demand, and high uniformity could also be kept. In addition, this paper verified the feasibility of using digital micro-jetting technology to prepare microcapsules with surface integrity and good uniformity, providing a beneficial reference for further study with microcapsules preparation using new technology.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

This work is funded by Shanghai education committee innovative research Program under Grant 12YZ195.The fabrication work was performed at Shanghai Medical Instrumentation College with the guidance of Professor Sun. Special thanks to former researchers those who have done a lot of exploratory work with Microjets application.

REFERENCES

[1] S. C. Lin, F. G. Tseng, H. M. Huang, Y. F. Chen, Y. C. Tsai, C. E. Ho, C. C. Chieng, "Simultaneous immobilization of protein microarrays by a micro stamper with back-filling reservoir," *Sensors and Actuators B: Chemical*, vol. 99, pp. 174-185, April 2004.

[2] D. Radulescu, N. Schwade, D. Wawro, "Uniform Paclitaxel-Loaded Biodegradable Microspheres Manufactured by Ink-Jet Technology", *Proc. Recent Adv. in Drug Deliv. Sys*., pp.1-5, 2003.

[3] L. Zhu, L. Y, Hou, and W. Y. Zhang, "Digital microinjection technique driven by piezoelectric actuator," *Mach. Tool Hydraul.. J.*, vol. 36, no. 9, pp. 17-20, Sep. 2008.

[4] H. Y. Sun, Y. D. Zhou, Y. H. Liao, and L. Y. Yang, "Development of microinjection technique in biomedicine field," *Sci. Technol. Rev. J.*, vol. 28, no.20, pp. 97-100, Oct. 2010.

[5] G. M. Buzas, and E. Szekely, "Eradication of Helicobacter pylori in peptic ulcer patients", *Orv Hetil*, vol. 140, no. 3, pp. 121-124, 1999.

[6] C. K. Lin, P. I. Hsu, K. H. Lai, G. H. Lo, H. H. Tseng, C. C. Lo, N. J. Peng, H. C. Chen, H. S. Jou, W. K. Huang, J. L. Chen, P. N.Hsu, "One-week quadruple therapy is an effective salvage regimen for Helicobactere pylori infection in patients after failure of standard triple therapy", *J. Clin. Gastroenterol*., vol. 34, no. 5, pp. 547-551, May-Jun 2002.

[7] E. Kawabami, S. K. Ogata, A. C. Portorreal, A. M. Magni, M. L. Pardo, F. R. Patrício, "Triple therapy with clarithromycin,amoxicillin and omeprazole for Helicobacter pylori eradication in children and adolescents", *Arq Gastroenterol.*, vol. 38, no. 3, pp. 203-206, Jul-Sep 2001..

[8] Z. P. Liu, J. Pan, and W. Y. Lu, "Amoxicillin mucoadhesive microspheres stability and adhesion studies," *Chin. Pharm. J.*, vol.39, no.9, pp. 676-679, 2004.

[9] X. L. Zhang, L.Y. Hou, and W. Y. Zhang, "Digital micro-jet with inline process of micro-nozzle," *Opt. Precision Eng. J.*, vol. 16, no. 11, pp. 2222-2226, 2008.

[10] S. M. Joscelyne, G. Tragardh, "Membrane emulsification-a literature review", *Membr. Sci.*, vol. 169, no. 1, pp. 107-117, 2000.

[11] S. Okushima, T. Nisisako, T. Torii, T. Higuchi, "Controlled production of monodisperse double emulsions by two-step droplet breakup in microfluidic devices", *Langmuir*, vol. 20, no. 23, pp. 9905-9908, Nov. 2004.

[12] T. J. Liu, L.Y. Hou, and W. Y. Zhang, "Experimental study on production of microcapsul based on micro-injection," *China Mech. Eng. J.*, vol. 17, no. 23, pp. 2499-2502, Dec. 2006.

[13] X. L. Zhang, L.Y. Hou, and W. Y. Zhang, "Preparation method and experimental apparatus of microcapsules by using microfluids digitalization technology," *J. Chem. Indust. Eng. (China)*, vol. 58, no. 8, pp. 2134-2137, Aug. 2007.

[14] Q. Li, L. Y. Hou, H. C. Wang, and W. Y. Zhang, "Preparation of fish roe microcapsules by using micro-fluidics digitalization," *J. Chem. Indust. Eng. (China)*, vol. 62, no. 4, pp. 1042-1047, Apr. 2011.

[15] M. L. Sun, J. F. Ban, and S.Y. Huang , "factors and control of PLGA microspheres drug loading and encapsulation efficiency, " *J. Guangdong College Pharmacy*, vol. 27, no. 6, pp. 1-6, 2011.

[16] Z. P. Liu, "amoxicillin mucoadhesive microspheres adhesion studies," *Shanghai, Fudan Univ.*, 2004.

Received: August 27, 2014 Revised: November 21, 2014 Accepted: December 19, 2014

© Sun *et al.*; Licensee *Bentham Open.*

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.