—Regular Articles—

Application of a Novel Approach to Prepare Biodegradable Polylactic-co-glycolic Acid Microspheres: Surface Liquid Spraying

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A novel approach which had foreground of industrialization, surface liquid spraying, was studied in this paper to prepare biodegradable polylactic-co-glycolic acid (PLGA) microspheres for controlled release drug delivery system. To compare with the normal methods, the microspheres prepared by this approach were characterized by particle size distribution and photograph of microscope. The relationship between the particle size and the instrument parameters of novel method was set up for the first time. The central composite design (CCD) was applied to study the main effects and interactions of three instrument factors on preparation of microspheres. The particle size of microspheres was below 200 μm and the shape of microspheres was spherical in nature evidenced by microscope photographs. Vinpocetine was used as the model drug to prepare the vinpocetine PLGA microspheres (VIN-PLGA-MS), and then drug loading, entrapment efficiency, scanning electron microscopy (SEM), Differential Scanning Calorimetry (DSC) and in vitro drug release behavior were examined. The results indicated that the drug loading and entrapment efficiency were increased using the novel method. The drug released slowly more than 30 days. The release behavior was fit for four kinds of kinetic model. The result indicated that release behavior was fitted by Zero-order kinetic model before release 72 hours, and was fitted with First-order kinetic model after release 72 hours. The novel method developed in our paper can give a promising way for industrialization, and the foreground was also proved by the scale-up batch experiment.

Key words——surface liquid spraying method; polylactic-co-glycolic acid; vinpocetine; microsphere; central composite design; industrialization

INTRODUCTION

Microspheres (MS) made of PLGA as parenteral depots were popular because they can eliminate the inconvenience of repeated injection, level the blood drug concentration, need no surgical procedure for implantation and removal.[1] They can be made of polylactic-co-glycolic acid (PLGA), a biodegradable and biocompatible polymer with the advantage of being degraded and eliminated from the body once it has achieved its goal.[2]

The preparation methods of MS are various, for example, the emulsion solvent evaporation, double-emulsion solvent evaporation, multiple emulsions and drying spray method, which can be chosen according to the properties of drug and the requirements.[3] Tarun. K Mandal and Srini used the emulsion solvent evaporation method to prepare the Zidovudine microspheres.[4] Ywu-Jang Fu and Shin-Shing used the spray drying method to prepare 5-FU microspheres.[5] But only a few methods can be appropriate for industrialization, which are not controlled easily to some extent. In our experiment, we proposed a novel method which was appropriate for both lab and industrialization, because it could be controlled easily. The reason was that it could set up the relationship between the particle size and the instrument parameters. The particle size distribution and microscope photographs were investigated. Compared with the normal methods, the uniform particle size distribution was got using the novel method. Vinpocetine (Fig. 1) was chosen as a lipophilic model drug and it was loaded into PLGA-MS by a novel surface liquid spraying method. The drug loading, entrapment efficiency, scanning electron microscopy

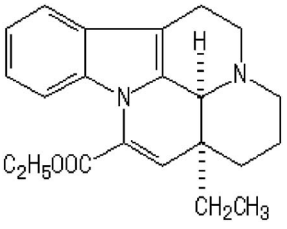

Fig. 1. Chemical Structure of Vinpocetine

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(SEM), Differential Scanning Calorimetry (DSC) and in vitro property of MS were studied. Compared with the normal method, the high entrapment efficiency was obtained using the novel method. The most important equation which could connect the particle size with the spraying-area was set up for the first time. The central composite design was applied to study the main effects and interactions of three instrument factors on preparation of microspheres, thereafter the optimum formula was obtained. In vitro experiment indicated that MS prepared by the novel method had a better release behavior than that made by the normal one. In order to investigate the drug release behavior, different kinetic models were fitted. Before 72 hours, drug release behavior was fitted by Zero-order kinetic model, after that point of time, drug release behavior was fitted by First-order kinetic model. For the parameters of instrument in the experiment could be controlled easily, the surface liquid spraying method can be used for industrialization. In this paper, the scale-up batch experiment was also carried out to prove the foreground for industrialization of this method. Compared with the lab scale, MS prepared by the scale-up batch had the approximate results. It was indicated that this novel method had a good reproducibility even in the scale-up batch. So this novel method could give us a promising way for industrialization.

EXPERIMENTAL

Materials and Instruments Vinpocetine (VIN) (North East Pharmaceutical Corporation); PLGA (Boehringer Ingelheim, Germany, inherent viscosity 0.55–0.75, LA : GA 75 : 25); Polyvinyl alcohol (PVA-1788) (Beijing Organic Chemical Plant); Dichloromethane and the others reagents were all analytical grade from Shenyang Chemical Agent and water used was distilled. Fluidized Bed (FU-MP-01 Powrex Corporation OSAKA TOKYO JAPAN); Scanning Electron Microscope (AMRAY-1000B, Science Institution of China); UV Spectrophotometer (Shanghai Cany Precision Instrument Co., Ltd); Thermostatic Waterbath Shaker (Shanghai S.R.D Scientific Instrument Co., Ltd); Differential Scanning Calorimetry (TA-60, DSC-60, SHIMADZU).

METHODS

Preparation of Blank PLGA-MS Blank PLGA-MS were prepared by surface liquid spraying method. Briefly, the organic phase was 10% PLGA in dichloromethane solution, the aqueous phase was 2% PVA aqueous solution. The resultant organic solution was sprayed into the PVA aqueous solution (The ratio of oil to water phase was 1 : 10) under moderate magnetic stirring (600 rpm) at room temperature to form an oil-in-water (O/W) emulsion. At the same time, the suitable parameters of the spray instrument from fluidized bed were chosen such as the flowing-speed, air-pressure, height between the liquid surface and nozzle (H-LSN) to prepare the microspheres. Then stirring was kept (400 rpm) at room temperature for 4 h to evaporate the dichloromethane. After washing with water three times, MS were collected and desiccated under aspirator-reduced pressure.

The normal emulsion solvent evaporation method was also used to prepare blank PLGA-MS.[6,7] PLGA were dissolved in CH_2Cl_2 by vortex mixing to prepare the 10% polymer oil phase. The aqueous phase consisted of a 2% PVA solution. Then, the oil phase was added to 2% PVA solution (The ratio of oil to water phase was 1 : 10) under moderate magnetic stirring (600 rpm) to form an oil-in-water (O/W) emulsion. The solvent evaporation step was performed by continuous stirring for 4 h under room temperature. After evaporation of methylene chloride, the microspheres were washed three times and desiccated under aspirator-reduced pressure.

Measurement of Physicochemical Properties of Blank PLGA-MS The blank PLGA-MS was sieved across a series of standard mesh and water was used as the flowing-assistants. After desiccation, the weigh of PLGA-MS on each mesh was weighted respectively. Microscope was used to observe the forming process of blank PLGA-MS which were smaller than 50 μm and their appearance. More than 300 particles of each batch were observed, and the particle size was calculated, thereafter, the particle size distribution graph was made.

Influence of Spraying Parameter The property of MS was also influenced by instrument parameters such as flowing-speed, air-pressure and height between the liquid surface and nozzle (H-LSN). In this paper, we used the single factor analysis to find the rule among the three parameters.

Keeping the flowing-speed and air-pressure constant, three levels of H-LSN was selected to prepare the PLGA-MS and the area of the rotundity was investigated, which sprayed from the nozzle.

The same method has been done with the other parameters.

Determination of the Drug Loading and Entrapment Efficiency The dry VIN-PLGA-MS prepared by two methods were dissolved in methanol, and were analyzed at 274 nm by means of a UV spectrophotometer. The drug content percentage (C) and entrapment efficiency (E_n) was calculated as Eq. 1 and Eq. 2.

$$C\% = \frac{\text{The amount of drug in the MS}}{\text{The total amount of MS}} \times 100 \quad (1)$$

$$E_n\% = \frac{\text{The amount of drug in the MS}}{\text{The amount of drug fed into the system}} \times 100 \quad (2)$$

Observation of VIN-PLGA-MS by SEM and DSC Compared with the normal emulsion solvent evaporation method, the dried VIN-PLGA-MS of the final product were observed by SEM to examine their morphology and surface characteristics.

To verify the drug loaded property, the microspheres were also analysised by DSC. The DSC of model drug, carrier material, physical mixture of drug with carrier and the microspheres prepared by novel method were shown as a, b, c and d respectively. Thermal characterization of microspheres was performed with a DSC. The equipment was calibrated with indium. The sample (approximately 5 mg) was heated twice from 0 to 200℃ at 20℃/min in a nitrogen atmosphere. The melting temperature (Tm) was determined from the endothermic peak of the DSC curve recorded in the first heating scan. The glass transition temperature (Tg) was determined from the DSC curve recorded in the second heating scan. Reported glass transition temperatures are midpoint values.

***In Vitro* Release Behavior of VIN-PLGA-MS** Many different methods were used to evaluate the in vitro release of microspheres, such as dialysis, membrane diffusion, fixation sampling and phase separation method.[8] Generally considering, fixation sampling method was selected to evaluate the in vitro drug release behavior in our experiment.

The MS (10 mg) were suspended in 20 ml of pH 6.8 PBS in the ampule which was shaken in the thermostatic waterbath shaker at 37℃. Every different formula had been divided into more than 30 bottles. At the first day they were sampled at 1, 4, 6, 8, 12 and 24 hours, in the following days they were sampled every 24 hours. At each time point, series bottles had been taken out and assayed by the UV method mentioned above. After a certain days the drug content retained within the MS was assayed to verify the release characteristics.

Prepared the Scale-up Batch with the Novel Surface Liquid Spraying Method To verify the perspective of industrialization, the scale-up batch was made by using the novel surface liquid spraying method. It was enlarged as one hundred times as the lab formulation, the operation was the same as predescribed.

RESULTS AND DISCUSSION

The blank PLGA-MS was prepared by two different methods. The particle size (μm), distribution, shapes and surface characteristics were detected.

Particle Size Distribution The particle size distribution of PLGA-MS prepared by two methods was shown in Fig. 3. The particle size distribution of normal method was shown in graph a and that of the novel method was shown in graph b.

As shown in Fig. 2(a), to some extent, though the particle size was almost between 120 and 50 μm, the distribution was not as good as we expected. The reason might be as follow. Using the magnetic stirring could not give enough shearing stress or the shearing stress might be decreased as the distance increased from the stirring center. So the particle size was maldistribution. This problem might also influence the industrialization of MS.

As shown in Fig. 2(b), the particle size between 75 and 50 μm was nearly 80%. The particle size distribution was suitably. For using the novel method, oil-phase which was sprayed from the nozzle, was atomized to the small and symmetrical fogdrop. So the emulsion which had small particle size was prepared. After evaporation of the dichloromethane, the smaller particle size and symmetrical distribution of MS could be got.

For these two graphs were the weight distribution of particle size, we could find though the large particle size had nearly 10%, the amount of these particles was just a little.

The particle size distribution and spherical shape of the blank PLGA-MS prepared by two methods were also evidenced by microscope photographs (Fig. 3).

From these figures, we can see the particle size distribution of blank PLGA-MS prepared by novel method was better than that made of the normal

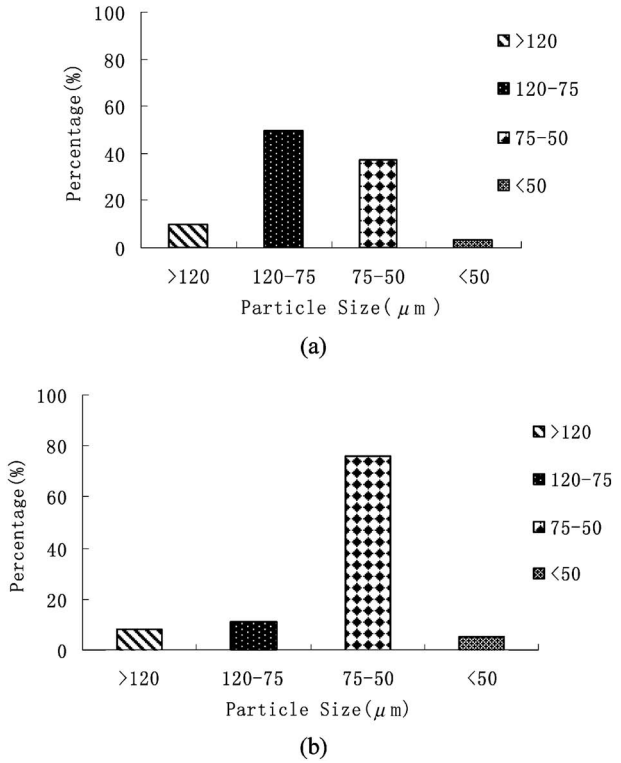


Fig. 2. Particle Size Distribution of Normal Emulsion Solvent Evaporation Method and Novel Surface Liquid Spraying Method

(a) Particle distribution of normal emulsion solvent evaporation method. (b) Particle distribution of novel surface liquid spraying method.

method.

Influence of Spraying Parameters The spraying area could be formed when the oil-phase sprayed from the nozzle. We presumed the droplet of atomization was rotundity and the flowing-speed was constant. Then the equations about the conditions of the novel method could be obtained. (Eq. 3, Eq. 4, Eq. 5)

$$A/\rho=\frac{4}{3}\pi(R_1-R_2)^3\times N \tag{3}$$

$$A=v\times t \tag{4}$$

$$N=S/2\pi R_1 \tag{5}$$

The weight of the carrier material was shown as A. The density of the carrier materials was shown as ρ. The outside and inside radius of the MS was shown as R_1 and R_2 respectively. The flowing-speed and the time of spraying were shown as v and t. The number of the MS was shown as N and the spraying area was shown as S.

From these equations above, we can see that the weight of the carrier material could be calculated by the flowing-speed and the time of spraying. The number of the MS could be calculated by the spraying area. As a result, Eq. 3 could be changed into Eq. 6.

$$v\times t/\rho=\frac{4}{3}\pi(R_1-R_2)^3\times S/2\pi R_1 \tag{6}$$

The ektexine of the MS was usually a gauzy film, so R_1 was nearly equal to R_2. For the reason above, we could use the surface area instead of the volume area. The equation was shown as Eq. 7.

$$v\times t/\rho=2R_1\times S \tag{7}$$

Then the relationship between the S and the R_1 was obtained. (Eq. 8)

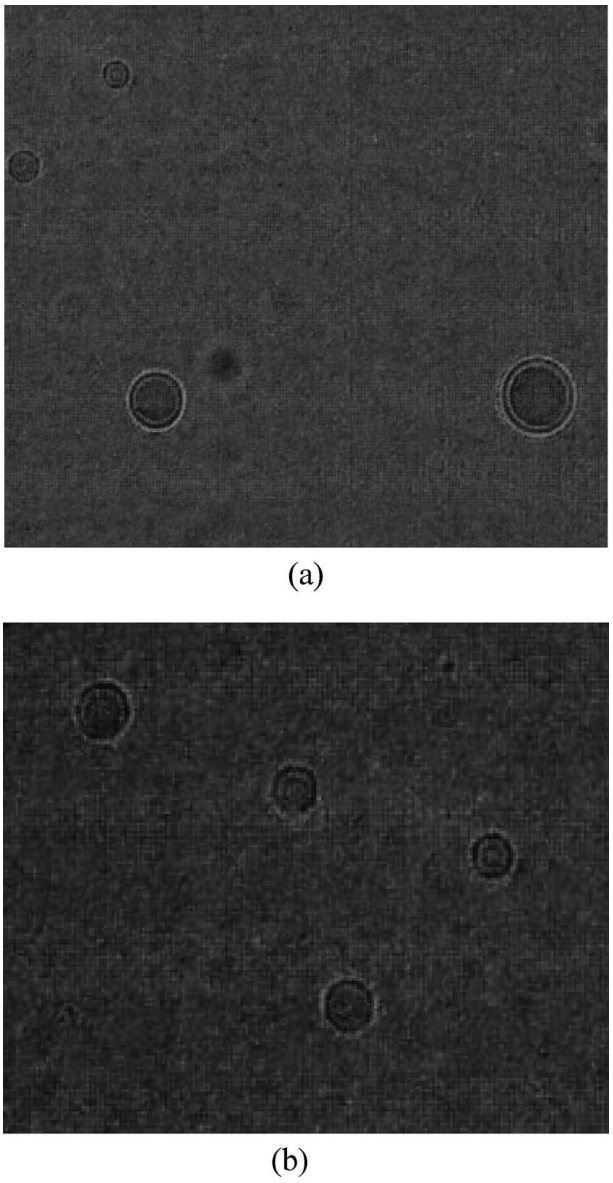


Fig. 3 Microscope Photographs of MS Prepared by the Two Methods

(a) Microscope photograph of MS prepared by the normal emulsion solvent evaporation method. (b) Microscope photograph of MS prepared by the novel surface liquid spraying method.

$$R_1 = v \times t/2\rho \times S \quad (8)$$

From this equation, we can find easily that with the S increased, the particle size decreased. If we presumed the time of spraying from the nozzle was one second. Then A was weight in one second. Therefore, the particle size would be increased as the flowing speed increased.

As described above, we selected three parameters (flowing-speed, air-pressure and H-LSN) and three levels to optimize the formulation as followed (Table 1).

A. Keeping the flowing-speed and air-pressure constant, three levels of H-LSN were chosen to prepare the VIN-PLGA-MS, then the area spraying from the nozzle was discussed.

As illustrated in Table 1, different H-LSN had much influence on the spraying-area. At the same condition, the spraying-area increased as the H-LSN increased.

B. Keeping the H-LSN and air-pressure constant, three levels of flowing-speed were chosen to prepare the VIN-PLGA-MS, then the area spraying from the nozzle was discussed.

From Table 1, we can see, different flowing-speed could not have much effect on the spraying-area. Oppositely, high flowing-speed increased the amount of unit area, which may weaken the effect of the spraying, hence, the particle size of MS increased.

C. Keeping the H-LSN and flowing-speed constant, the three levels of air-pressure were chosen to prepare the VIN-PLGA-MS, then the area spraying from the nozzle was discussed.

As demonstrated in Table 1, as the air-pressure increased from 30 to 50 MPa, the spraying-area increased. However, as the air-pressure increased from 50 to 70 MPa, the spraying-area was decreased. It might be explanation as follow: at the first step during which air-pressure increased from 30 to 50 MPa, the high pressure could enhance the spraying effect, so the spraying-area could be enlarged. Reversely, the pressure increased from 50 to 70 MPa, which was too high to keep the spraying normal, so the spraying-area would be diminished.

Table 1. Effect of the Different H-LSN Levels on Spraying-area

H-LSN (cm)	Air-pressure (MPa)	Flowing-speed (ml/min)	Spraying-area (cm^2)
3	30	2	4π
6	30	2	9π
9	30	2	16π
6	30	2	9π
6	50	2	10.56π
6	70	2	9π
6	30	2	10.24π
6	30	4	12.25π
6	30	6	12.25π

From Table 1 above, keeping the other parameters constant, and just discussing the height between the liquid surface and nozzle (H-LSN), flowing-speed and air-pressure, respectively, we could find easily that the spraying-area increased as the H-LSN increased and decreased as the air-pressure increased over 50 MPa, meanwhile it kept constant as the flowing-speed increased.

Experiment Designed by Central Composite Design (CCD) A central composite design was applied to study the main effects and interactions of three factors on spraying area. A class of five level central composite design for the estimation of parameters in a second order model was developed by Box-Hunter.[9–11] Several factors influence preparation of microspheres, of which air-pressure, flowing-speed and height between the liquid surface and nozzle (H-LSN) have a significant effect. Hence, these critical variables were chosen and designed as X_1, X_2, and X_3, respectively, in our model. The low, middle and high levels of each variable were designated as -1.732, 0 and $+1.732$, respectively, and the corresponding actual values for each variable are listed in Table 2. The actual design experiment is listed in Table 5. Twenty experiments were required and the combinations were performed in random order. The three significant independent variables X_1, X_2, and X_3, and the mathematical relationship of the response Y on these variables can be approximated by the quadratic (second-degree) polynomial equation as shown below (Eq. 9).

Table 2. Factors and Levels Investigated in the Preparation of Microspheres

Factor	Level				
	-1.732	-1	0	1	1.732
Air-pressure (MPa)	30	38.5	50	61.5	70
Flowing-speed (ml/min)	1	1.4	2	2.6	3
H-LSN (cm)	3	4.3	6	7.7	9

$$Y=b_0+b_1\times X_1+b_2\times X_2+b_3\times X_3+b_4\times X_1^2+b_5\times X_2^2+b_6\times X_3^2+b_7\times X_1\times X_2+b_8\times X_1\times X_3+b_9\times X_2\times X_3 \quad (9)$$

A CCD shown in Table 3 allows the development of mathematical equations where each response variable y is assessed as a function of air-pressure (X_1), flowing-speed (X_2) and height between the liquid surface and nozzle (H-LSN) (X_3), and calculated as the sum of a constant, three first-order effects (terms in X_1, X_2, and X_3), three interaction effects (terms in $X_1 \cdot X_2$, $X_1 \cdot X_3$ and $X_2 \cdot X_3$) and three second-order effects (X_1^2, X_2^2 and X_3^2) according to (Eq. 9).

The main effects and interactions of the factors investigated on the response variables were estimated by applying the statistical program SAS (SAS 8.0 for Windows, SAS Institute Inc.). Analysis of variance (ANOVA) was used to test the statistical significance of each source of variation in the spraying area.[12] The sums of insignificant ($p>0.05$) effects and interactions were added to the experimental error. Summarising equations for the response variables were obtained as below (Eq. 10), and the R-Square was 0.9657.

$$Y=8.96037X_1+98.94529X_2+49.74825X_3-0.08483X_1^2-30.10484X_2^2-3.38354X_3^2 \quad (10)$$

Table 3. Actual Design of the CCD for Microspheres

Experiment No.	Air-pressure (MPa)	Flowing-speed (ml/min)	H-LSN (cm)	Spraying area (cm^2)
1	38.45	1.42	4.27	36.48
2	61.55	1.42	4.27	40.42
3	38.45	2.58	4.27	26.48
4	61.55	2.58	4.27	36.24
5	38.45	1.42	7.73	72.55
6	61.55	1.42	7.73	74.24
7	38.45	2.58	7.73	72.24
8	61.55	2.58	7.73	76.55
9	30	2	6	28.64
10	70	2	6	64.24
11	50	1	6	50.24
12	50	3	6	50.24
13	50	2	3	19.62
14	50	2	9	80.24
15	50	2	6	83.58
16	50	2	6	83.58
17	50	2	6	83.58
18	50	2	6	83.58
19	50	2	6	83.58
20	50	2	6	83.58

One parameter was chosen. The response surface plots of the interaction between air-pressure and H-LSN, air-pressure and flowing speed, H-LSN and flowing speed on spraying area were shown in Fig. 4 (a), (b) and (c) respectively. The optimum areas could be found from these graphs.

Thus the optimum parameter system of the novel surface liquid spraying method was achieved. (Table 4)

Drug Entrapment Efficiency From Table 5, compared with the traditional emulsion solvent evaporation method, the novel surface liquid spraying could increase the entrapment efficiency of the drug. The entrapment efficiency of vinpocetine was increased from 78.2% to 93.6%.

In this paper, we thought that the novel method was controlled by the equipment and its parameters. As a result, it could be controlled more easily than the traditional methods, and the artificial operation errors were avoided.

Determination of Drug Content Drug content percentage was dependent on two aspects. One was the drug content of the final product, the other was the total weight of the final product.

As shown in Table 6, to some extent, it was not much more different between the two methods. Though the novel surface liquid spraying method could enhance the drug content in the final product, it also increased the weight of the final product.

From this table, we could see that the novel surface liquid spraying method not only enhanced the drug content, but also increased the yield.

Observation of VIN-PLGA-MS by SEM and Differential Scanning Calorimetry (DSC) Scanning electron photomicrographs of VIN-PLGA-MS which were prepared by the two methods were shown in Fig. 5.

From these two figures, we can see though microspheres made by two different methods had approximate shapes, the VIN-PLGA-MS prepared by normal method had much drug crystals that existed on the surface of MS. The reason might be as follow. Using the normal method to prepare the MS, the larger particle size could be obtained during the dropping process. On one hand, the larger particle size might prevent the system from becoming the O/W emulsion. On the other hand, the dichloromethane solution can not be evaporated fast under the condition of the smaller surface area caused by the larger particle

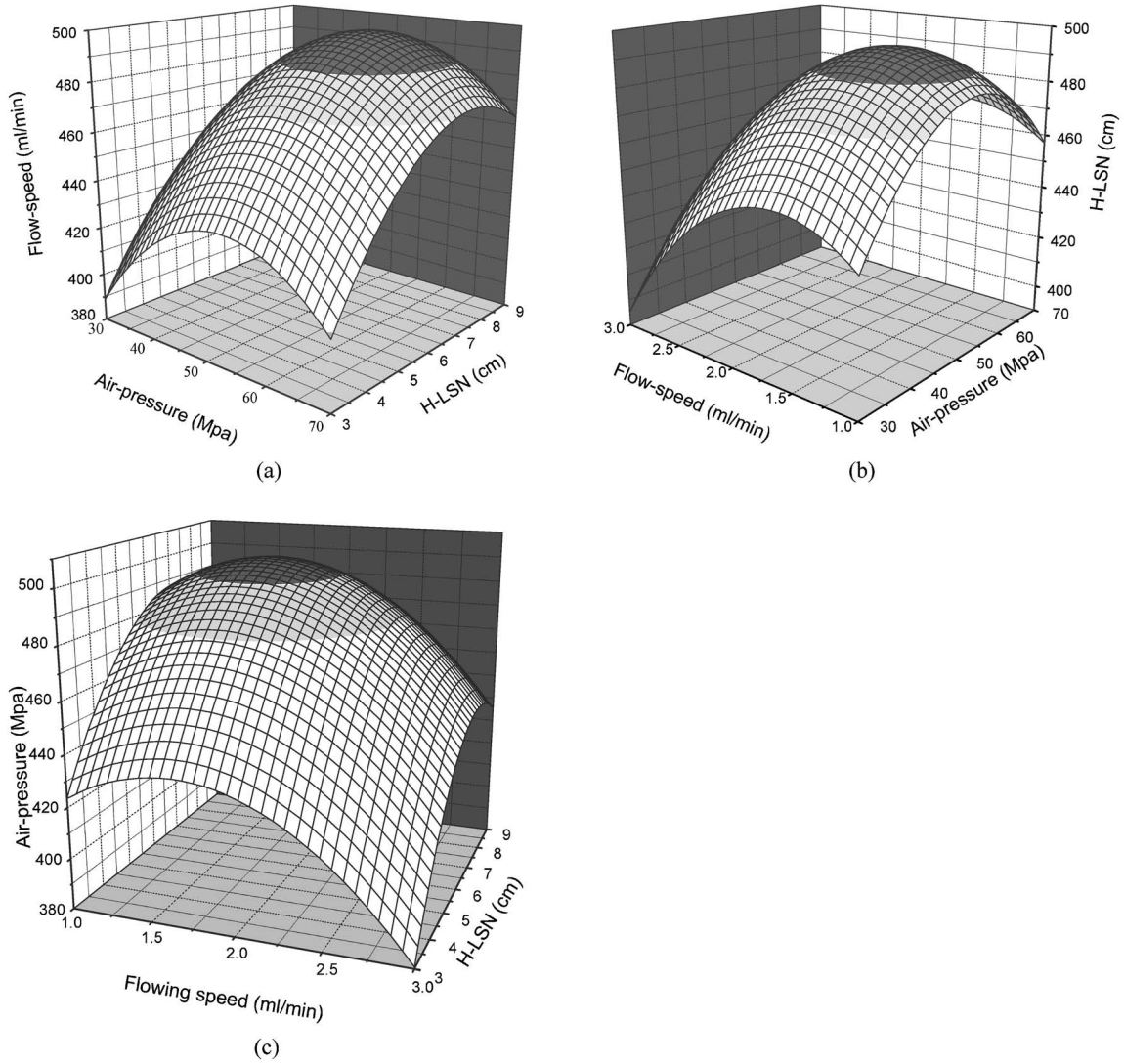


Fig. 4 Response Surface Plots of the Interaction among the Three Parameters

(a) Response surface plots of the interaction between the Air-pressure and the H-LSN. (b) Response surface plots of the interaction between the Air-pressure and the Flow-speed. (c) Response surface plots of the interaction between the Flow-speed and the H-LSN.

Table 4. Optimum Parameters System of Novel Method

Parameter	Optimum result
H-LSN (cm)	6
Air-pressure (MPa)	50
Flowing-speed (ml/min)	2

Table 5. Entrapment Efficiency of Different Preparation Methods of VIN-PLGA-MS

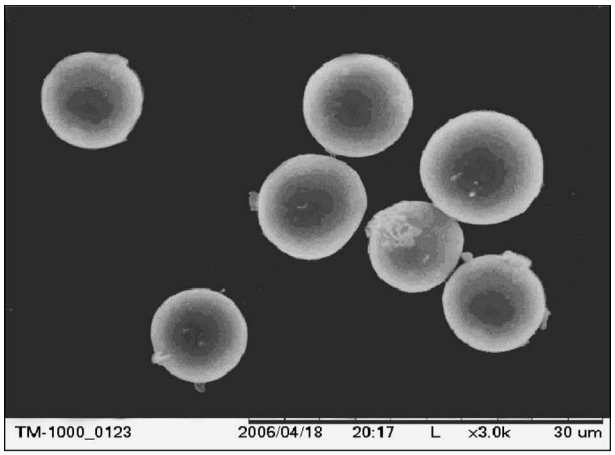
Method	Entrapment efficiency (%)
emulsion solvent evaporation	78.2
novel surface liquid spraying	93.6

Table 6. Drug Content Percentage of Different Preparation Methods of VIN-PLGA-MS

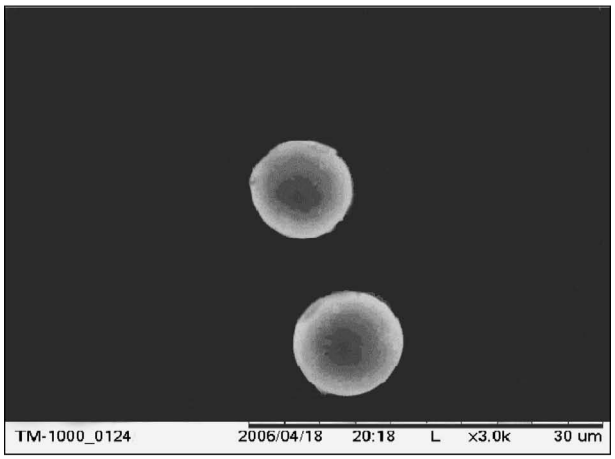
Method	Drug Content Percentage (%)
emulsion solvent evaporation	14.3
novel surface liquid spraying	15.9

size. In addition, Vinpocetine was lipophilic drug, which hardly dissolved in water phase. As a result, the drug crystals were all existed on the surface of MS.

Microspheres which were prepared by novel method had smaller particle size. The smaller particle size of the fogdrop, the larger the surface area. And the larger the surface area, the faster the evaporation



(a)



(b)

Fig. 5 Scan Electron Photomicrographs of Final Product of VIN-PLGA-MS Prepared by the Two Methods

(a) Scan electron photomicrograph of VIN-PLGA-MS prepared by normal method. (b) Scan electron photomicrograph of VIN-PLGA-MS prepared by novel method.

speed. Simultaneously, the system could become O/W emulsion easily because of the smaller particle size. The dichloromethane solution had been evaporated before the drug diffused out of the inner phase. As a result, there were a few crystals on the surface of the VIN-PLGA-MS.

The graphs of Differential Scanning Calorimetry (DSC) were shown as Fig. 6. The DSC results of model drug, carrier material, physical mixture of drug with carrier material and the microspheres prepared by novel method were shown as (a), (b), (c) and (d) respectively.

As demonstrated in the graphs (a) and (b), the model drug had a strong peak at the 148.18℃ and the carrier had a peak at 52.1℃, so the Tg of the drug and the carrier was 148.18℃ and 52.1℃ respectively. From the graph (c), we can find easily that the physical mixture of drug with carrier material had two peaks at 148.18℃ and 52.1℃ respectively. This results indicated that their properties were kept in physical mixture. According to the graph (d), the two peaks which represent the properties of the model drug and the carrier material disappeared. The reason should be explained that the micropheres were formed and the drug was trapped by the carrier material.

***In Vitro* Release Behavior of VIN-PLGA-MS**

MS were prepared as mentioned above, and the *in vitro* drug profiles of MS were evaluated. The result was shown in Fig. 7.

Seen from the profiles above, VIN-PLGA-MS prepared by these two methods had much more different release behavior. The release behavior of microspheres prepared by novel surface of liquid spraying method was slower at the beginning and then faster than that made by the normal method. The initial burst of microspheres was solved to some extent. Obviously, the surface structure of the microsphere was almost dependent on the evaporation speed of the dichloromethane solution. When the novel method was used to prepare the MS, the smaller particle size could be obtained during the spraying process. The smaller the particle size was, the larger the surface area was. And the larger the surface area was, the faster the evaporation speed was. So the evaporation speed could be increased. The smaller particle size could also keep the O/W emulsion steady. The model drug could not diffuse out of the inner phase easily. As a result, there were not a lot of crystals on the surface of MS which had been discussed above. Diffusion was the main motivation of drug-release at the beginning. So the release could be faster at the beginning for a lot of crystals on the surface of MS. Thus, the microspheres made by the novel method exhibited a slow release behavior at the beginning.

Drug release from the MS usually had the two mechanisms.[12] One was the diffusion, the other was the bioerosion. The initial burst release was presumably through diffusion due to the little residue of VIN in or near the surface of MS formed in the process of preparation, the long and slow release behavior was achieved through complex diffusion from the increasingly permeable matrix due to the bioerosion of PLGA. This release behavior was accorded with zero order kinetics.[13] Other factors such as molecular

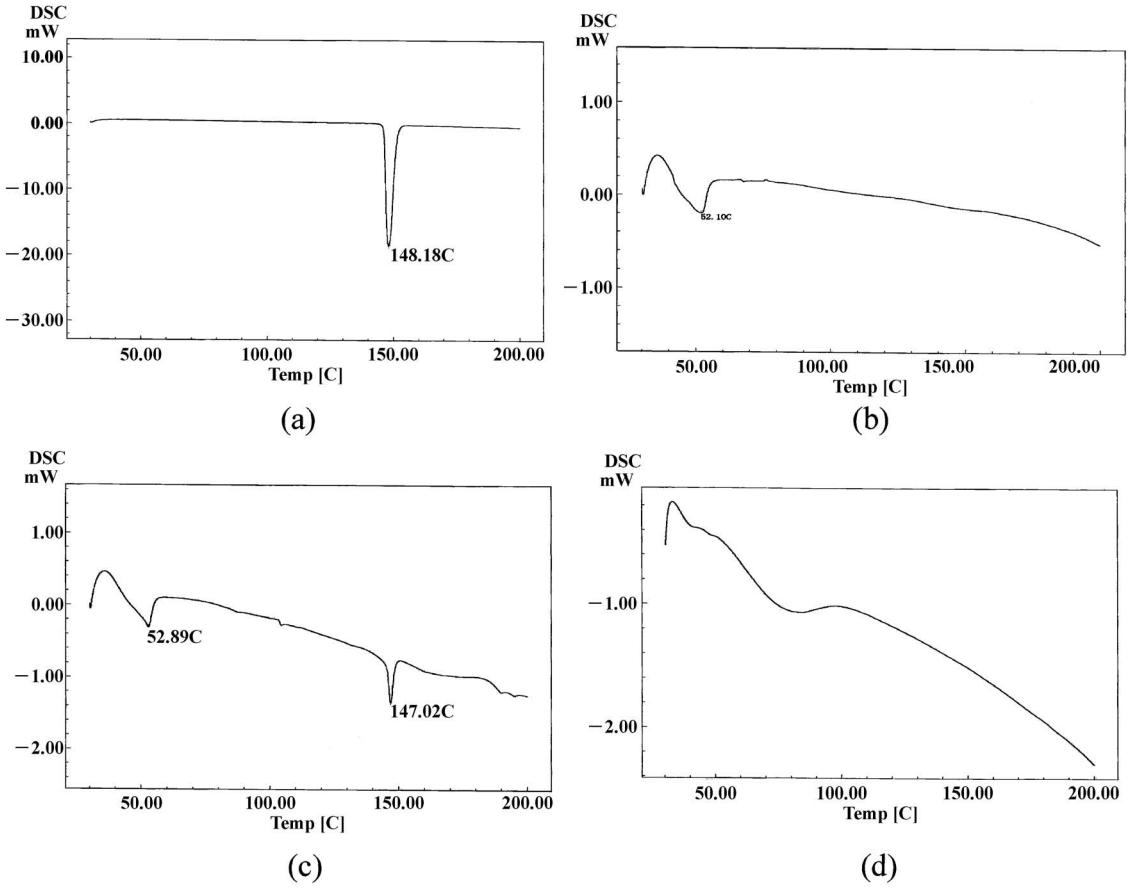


Fig. 6 The Differential Scanning Calorimetry (DSC) Graphs of Microspheres Prepared by Novel Method
(a) The DSC results of the model drug. (b) The DSC results of the carrier material. (c) The DSC results of the physical mixture of drug with carrier material. (d) The DSC results of the microspheres prepared by novel method.

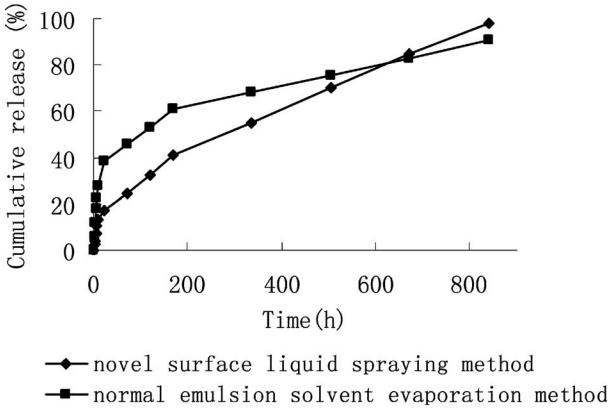


Fig. 7 Drug Release Profile of Two Different Preparation Methods

weight, molecular weight distribution as well as sterilization may also alter the degradation rate of the biodegradable polyesters in microspheres.[14,15]

From the profiles (Fig. 7), we can also see that at the beginning, because of the diffusion mechanism, the MS prepared by the normal methods had faster release behaviors. With the drug release continued, the bioerosion became the main mechanism. When water permeated into the MS, MS prepared by the novel method became faster than that made by the normal method.[16]

It was clear that the release of Vinpocetin from PLGA microspheres is a combination of diffusion and bioerosion.[17] During the diffusion stage, the release of Vinpocetin occurs by diffusion through aqueous channels in microspheres. These aqueous channels could be the external pores on the surface or were generated via the leaching of the drug at or near the surface, leading to initial burst release. After that the release profiles exhibit a plateau period and during this period microspheres swell and internal porosity decreases sharply. During this period of time, the polymer matrix presents itself as a dense netlike structure and fewer drugs could diffuse out due to entanglement between the polymer and the drug. The second release stage involves the degradation of PLGA and is associated with generation of micropores in the

degrading polymer. It is known that during the degradation of PLGA, acidic oligomers and monomers will be produced leading to an acidic microenvironment in the aqueous pores of the matrix. Since the degradation process of PLGA is catalyzed by protons and different internal morphology of microspheres will probably influence the distribution of protons in the microclimate, it was assumed that different morphology of microspheres might lead to different degradation speed and therefore different microstructure of eroding microspheres.[18,19]

Because of the reason mentioned above, microspheres which had the smaller particle size could be wetted easily. As a result, it could be eroded easily than which had the larger particle size. Therefore drug release from the MS prepared by the novel method could be faster than that made by normal method after the period of burst release.

To explain the drug release behavior more clearly, three mathematical models were chosen to describe the release patterns on the basis of the known physical geometry of the particles and the fact that the matrices under investigation are not disintegrating systems. So, for empirical analysis, the following mathematical models were used.[20,21]

Zero-order-model:

$$W = k_0 \times t \tag{11}$$

First-order-model:

$$\ln (100 - W) = \ln 100 - k_1 \times t \tag{12}$$

Higuchi square root of time model:

$$W = k_2 \times t^{1/2} \tag{13}$$

Hixson and Crowell cube-root model:

$$(100 - W)^{1/3} = 100^{1/3} - k_3 \times t \tag{14}$$

Where W is precentage drug release at time t, and k_0, $k_1(h^{-1})$, $k_2(h^{-1/2})$ and $k_3(h^{-3})$ are release rate constants.

After the dissolution studies, the estimated amount of drug released from the microspheres in time t and the observed results have been compared to one another using Eqs. (11–14). Statistical analyses were performed using Statistical Analysis System (SAS) 8.0 for windows. The result is shown in Table 7.

From Table 7, we can see, drug release behavior of microspheres could not be fitted by only one kinetic model because of the bad R-square. The reason had been discussed above. Drug release from the microspheres usually had two mechanisms. One was diffusion at the beginning which displayed Zero-order kinetic model, the other was the bioerosion which had been verified as First-order kinetic model. Because of that, drug release behavior of microspheres should be fitted by two kinetic models together. Thus, we used the zero and first order kinetic models to fit the drug release behavior. The result is shown in Table 8.

Table 7. Result of the Statistical Analyses

Kinetic model	Average Release rate constant (k)	R-Square
Zero-order model	0.5825	0.6163
First-order model	0.0067 h^{-1}	0.3138
Higuchi model	2.843 $h^{-1/2}$	0.3929
Hixson and Crowell cube-root model	0.00974 h^{-3}	0.3832

Table 8. Result of the Drug Release Behavior Fitted by Two Kinetic Models

Kinetic model	Release time	Average Release rate constant (k)	R-Square
Zero-order model	before 72 hours	0.966	0.9793
First-order model	after 72 hours	$3.24 \cdot 10^{-2}$ h^{-1}	0.9955

Table 9. Physicochemical Properties of MS Prepared in the Scale-up Batch

Properties	Result
Drug Entrapment Efficiency	95.5±0.8%
Drug Content Percentage	8.84±1.1%
Particle Size	60.5±10.3 μm

So the developed equation of release was stated below (Eq. 15):

$$Y = k_0 \times t_1 + e^{k_1 \times t_2} \tag{15}$$

Where, Y was percentage drug release

k_0 was average release rate constants under Zero-order kinetic model

k_1 was average release rate constants under First-order kinetic model

t_1 was drug release time before 72 hours

t_2 was drug release time after 72 hours

Prepared the Scale-up Batch with the Novel Surface Liquid Spraying Method The physicochemical properties of the scale-up batch was shown in Table 9.

To verify the perspective of industrialization, the scale-up batch was prepared in the lab, which used the novel surface liquid spraying method. The same process was used as pre-described. In brief, the organ-

ic phase was 10% PLGA in Dichloromethane solution, the aqueous phase was 2% PVA aqueous solution, and the ratio of the weight of VIN to Polymer fed was 1 : 10. The resultant organic solution was sprayed into the aqueous PVA solution (the oil/water phase volume ratio was 1 : 10) under moderate magnetic stirring (600 rpm) at room temperature to form an oil-in-water (O/W) emulsion. Then keep stirring (400 rpm) at room temperature for 4 hours, to evaporate the dichloromethane. After washing with water three times, MS were collected and desiccated under aspirator-reduced pressure.

CONCLUSION

Compared with the normal method, the blank PLGA-MS, which were prepared by the novel surface liquid spraying method, had a good spherical form and uniform particle size distribution. Three spraying parameters were investigated to set up the relationship between the parameters and the particle size of microspheres for the first time. Compared with the normal methods, VIN-PLGA-MS was prepared by novel surface liquid spraying method had a higher drug loaded efficiency which increased from 78.2% to 93.6%. *In vitro* drug release done with constant temperature water bath shaker showed that the novel method could give us a good release behavior compared with the traditional methods. By controlling the technology and the parameters of the spraying system, the sustained-release VIN biodegradable microsperes with proper particle size and drug content could be obtained.

The differences between the normal method and the novel method were explained as follows:

a. The mechanism of glomeration is different. The 'normal' method was conglobated by the force of shearing which came from the stirrer. But the 'novel' method was conglobated by the force of air pressure which produced from air compressor.

b. The parameters which could control preparation process are different. The preparation process of 'normal' method was controlled by the adding speed (Ex. The speed of oil phase drops into the water phase) and the stirring speed. The preparation process of 'novel' method was controlled by the parameters of spraying instrument. Such as flowing-speed, air-pressure and the height between the liquid surface and nozzle.

The novel surface liquid spraying method was used to prepare the microspheres for the first time. It could use not only for the lab, but also for the industrialization.

Vinpocetine as the drug for central nervous system was used widely and needed for a long time. If it was prepared as biodegradable microspheses, it must be convenient for both therapy and patients.

From the result of the big batch, it could be found obviously that the novel method could be used for the industrial manufacture and the industrialization of MS prepared in this way will come true. So the novel surface of liquid spraying method will have a bright foreground.

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