〈Original Article〉

Optimization of a novel method: surface liquid spraying to prepare poly (d,l-lactide-co-glycolide) microspheres using central composite design experiment

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Summary A novel approach which had foreground of industrialization, surface liquid spraying, was studied in this paper to prepare microspheres for controlled release drug delivery system. The biodegradable polymers poly-lactic/glycolic acid were used as wall materials in the preparation of microspheres containing the Vinpocetin. A novel surface liquid spraying method was devised for the preparation of MS containing this lipophilic compound. In this paper, studies on the formulation and using the central composite design--response surface methodology to analysis the main effects and interactions of three factors on preparation of the VIN-PLGA-MS. The optimum formulation could be achieved. Drug loading, entrapment efficiency, scanning electron microscopy and Differential Scanning Calorimetry were examined to discuss the physical, chemical and morphological characters of MS. The results indicated that the drug loading and entrapment efficiency of MS were high by preparing with this novel method. The orbicular shape and uniform particle size distribution were obtained. Thus, the novel method developed in our paper can give a promising way for industrialization.

Key words: Surface liquid spraying method, Microsphere, PLGA, Vinpocetin, Central composite design, Industrialization

1. Introduction

Poly (lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer, has received tremendous interest regarding the development of parenteral depot systems such as microspheres[1-3]. Encapsulation of drugs in PLGA matrices from which they are released at a relatively slow rate over a prolonged time allow less frequent administrations, thereby increasing patient compliance and reducing discomfort, protection of the therapeutic compound within the body, and avoidance of peak-related side effects by maintaining more-constant blood levels of the drug[4]. Furthermore, since microspheres can be administered by injection, one can also achieve localizeddrug delivery and high local concentration[5]. Particle size is the main effect in the drug release process. In this paper, the relationship between the particle size and the parameters of instrument was set up. Consequently, the particle size distribution

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could be controlled by the system parameters. The purpose of the present study is to investigate how the particle size of microspheres is influenced by different parameters of instrument in the microsphere production. Vinpocetine as the drug for central nervous system was used widely and needed for long time. Contained by microspheres, not only helpful for the therapy, but also convenience for the patients.

2. Materials and Method

1. Materials

Vinpocetine (VIN) (North East Pharmaceutical Corporation); PLGA (Boehringer Ingelheim, Germany, inherent viscosity 0.55-0.75, RG502H. 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).

2. Preparation technique

A technique for manufacturing microspheres has been developed in this paper of the 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 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 4h to evaporate the dichloromethane. After washing with water three times, MS were collected and desiccated under aspirator-reduced pressure.

3. Morphological analysis

The particles were sprinkled onto a doublesided tape and sputter-coated with gold. The morphology was characterised by scanning electron microscopy (AMRAY-1000B, Science Institution of China).

4. Observation by the Differential Scanning Calorimetry (DSC)

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.

5. Determination of the drug loading and entrapment efficiency

The dry VIN-PLGA-MS were analyzed at 274 nm by means of a Vspectrophotometer. Calibration curve was shown as Fig. 1. The drug loading (C) and entrapment efficiency (En) was calculated as Eq. 1 and Eq. 2.

$$\textit{Drug loading} = \frac{\textit{Drug (enc.)}}{\textit{Drug (tot.)} + \textit{Polymer}} \quad (1)$$

$$\textit{Encapsulation efficiency} = \frac{\textit{Actual drug loading}}{\textit{Theoretical drug loading}} \quad (2)$$

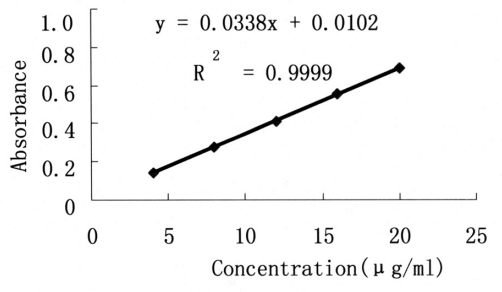

Fig. 1 The work curve of VIN in methanol.

6. Influence of spraying parameter

The property of MS was 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, and to prepare for the central composite design (CCD).

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.

7. Experimental design

A central composite design response surface method was created to study the main effects and interactions of three parameters of instrument on mean particle size. The factors investigated were air-pressure, flowing-speed and height between the liquid surface and nozzle (H-LSN). A class of five level central composite design for the estimation of parameters in a second order model was developed by Box-Hunter[6-8]. Their levels are listed in Table 1. Twenty-seven experiments were required and the combinations were performed in random order. We used the quadratic polynomial equation to analyse the relationship among the parameters as shown in Eq. 3.

$$Y=b_0+b_1 \cdot X_1+b_2 \cdot X_2+b_3 \cdot X_3+b_4 \cdot X_1^2+b_5 \cdot X_2^2+b_6 \cdot X_3^2+b_7 \cdot X_1 \cdot X_2+b_8 \cdot X_1 \cdot X_3+b_9 \cdot X_2 \cdot X_3. \quad (3)$$

3. Results

1. Influence of spraying 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.The results was shown in Table 2.

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. 4, Eq. 5, Eq. 6).

$$A/\rho = 4/3\,\pi\,(R_1 - R_2)^3 \cdot N \quad (4)$$

$$A=v \cdot t \quad (5)$$

$$N=S/2\,\pi\,R_1 \quad (6)$$

Table 1 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

Table 2 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

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. 7

$$v \cdot t/\rho = 4/3\,\pi\,(R_1 - R_2)^3 \cdot S/2\,\pi\,R_1 \qquad (7)$$

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. 8.

$$v \cdot t/\rho = 2R_1 \cdot S \qquad (8)$$

Then the relationship between the *S* and the R_1 was obtained (Eq. 9).

$$R_1 = v \cdot t/2\rho \cdot S \qquad (9)$$

As described above, we selected three parameters (flowing-speed, air-pressure and H-LSN) and five levels to optimize the formulation by CCD.

Table 3 Experimental design by central composite design (CCD)

Trail No.	X1	X2	X3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.732	0	0
10	1.732	0	0
11	0	-1.732	0
12	0	1.732	0
13	0	0	-1.732
14	0	0	1.732
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

2. Data analysis of the experimental designs

Using a central composite design we investigated the main effects and interactions of the relevant process factors on spraying area. The experimental design by central composite design-- response surface methodology (CCD) was shown in Table 3.

The actual design experiment is listed in Table 4. 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 polynomial equation as shown below (Eq. 10)

$$Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X3 + b_4 \cdot X_1^2 + b_5 \cdot X_2^2 + b_6 \cdot X_3^2 + b_7 \cdot X_1 \cdot X_2 + b_8 \cdot X_1 \cdot X_3 + b_9 \cdot X_2 \cdot X_3 \qquad (10)$$

Table 4 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

Table 5 Optimum range of the parameter from each graph

Figure No.	Optimum range		
	Air-pressure (MPa)	Flowing-speed (ml/min)	H-LSN (cm)
6	40-60	median	5-8.5
7	45-65	1.5-2.5	median
8	median	1-2.25	4.5-7

Table 6 Optimum parameters system of novel method

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

Table 7 Drug loading and entrapment efficiency

Content	Percentage
Drug loading (%)	15.9
Entrapment efficiency (%)	93.6

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 the Eq. (9).

The main effects and interactions of the factors investigated on the response variables were estimated by applying the statistical program SAS (SAS8.0 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[9]. 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. 11), and the R-Square was 0.9657.

$$Y=8.96037X_1+98.94529X_2+49.74825X_3-0.08483X_1^2-30.10484X_2^2-3.38354X_3^2 \quad (11)$$

Selected the one parameter as its median, 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. 2, Fig. 3 and Fig. 4 respectively. The optimum areas could be found from these graphs,

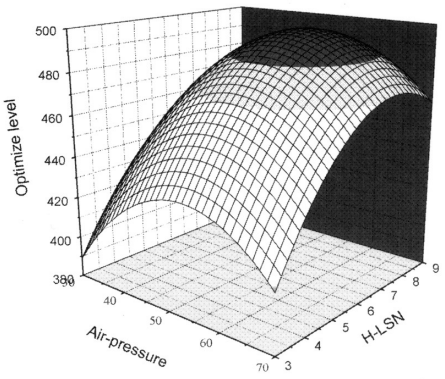

Fig. 2 Response surface plots of the interaction between air-pressure and H-LSN.

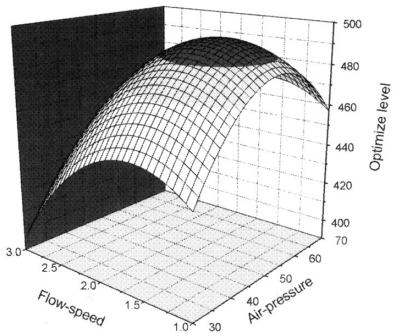

Fig. 3 Response surface plots of the interaction between air-pressure and flowing speed.

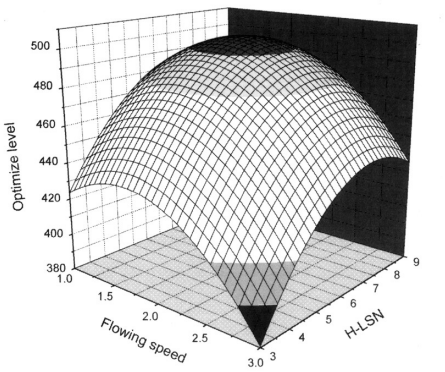

Fig. 4 Response surface plots of the interaction between flowing speed and H-LSN.

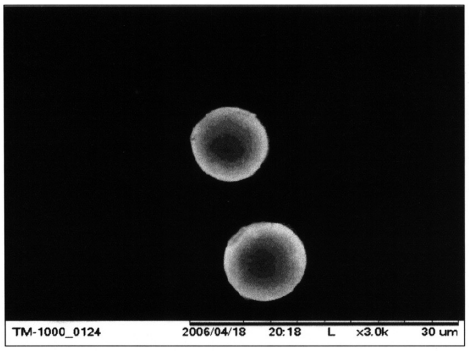

Fig. 5 Scan electron photomicrographs of VIN-PLGA-MS prepared by the surface liquid spraying method.

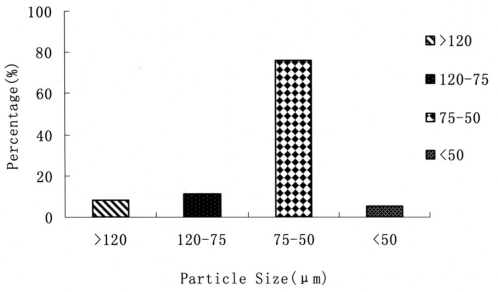

Fig. 6 Particle size distribution of microspheres prepared by surface liquid spraying.

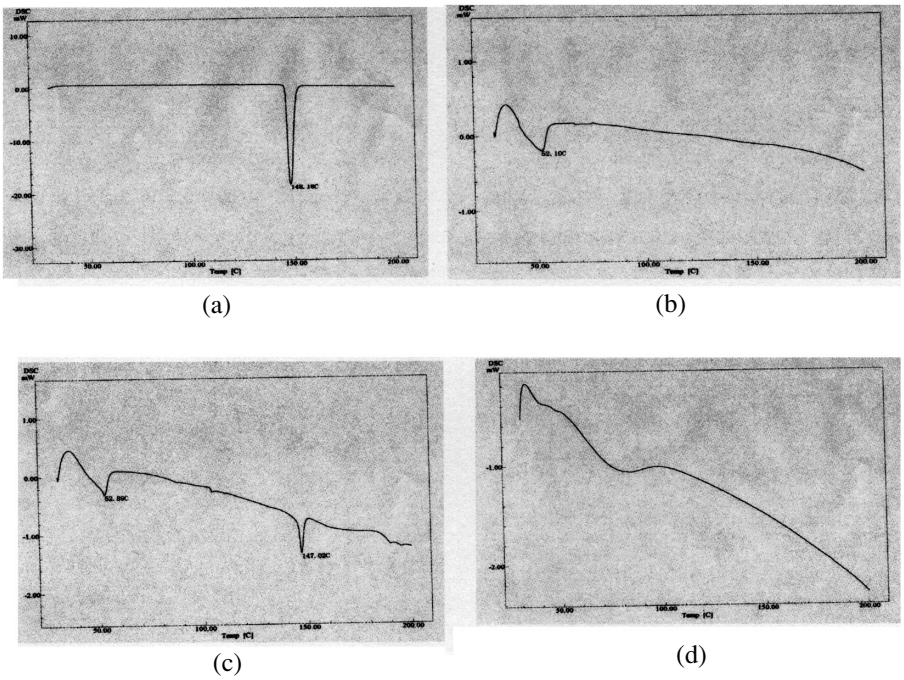

Fig. 7 The Differential Scanning Calorimetry (DSC) graphs of microspheres prepared by surface liquid spraying method.

and the optimum range of the parameter from each graph was shown in Table 5.

Thus the optimum parameter system of the novel surface liquid spraying method was achieved (Table 6).

3. Morphological analysis

Scanning electron photomicrographs of VIN-PLGA-MS were shown in Fig. 5.

4. Particle size distribution

Using the optimum formula the microspheres were prepared by the novel surface liquid spraying method. The VIN- PLGA-MS was sieved across a series of standard mesh and water was used as the flowing-assistants. After desiccation, the weigh of VIN-PLGA-MS on each mesh was weighted respectively. Microspheres which were smaller than 50 μm were used a microscope to observe the forming

process of MS 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 (Fig. 6).

5. Drug loading and entrapment efficiency

Drug loading and entrapment efficiency was shown in Table 7.

6. Observation by the DSC

The graphs of DSC were shown as Fig. 7. The DSC results of model drug, carrier material, physical mixture of drug with carrier material and the microsphere prepared by novel method were shown as a, b, c and d respectively.

4. Discussion

1. Influence of spraying parameters

From the Eq. 9, 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. This result could give us a potential method to foresee the particle size in the preparation of the MS with the novel method.

A central composite design was applied to study the main effects and interactions of three factors on spraying area.In this paper, we used CCD to optimize the formulation of the novel method to prepare the VIN-PLGA-MS. From the Fig. 2-4, we could find the deepest area is the range of optimize formulation.

2. Morphological analysis

From the Fig. 5, we could found, the microspheres which prepared by the novel surface liquid spraying method with the optimum formulation had spheric shape. There were a few crystals on the surface of microspheres. The reason might be as follow. 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 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.

3. Drug loading and entrapment efficiency

In this paper, we developed a novel mechanisation method to prepare the microspheres. The novel method was controlled by the equipment and its parameters. As a result, it could be controlled more easily than the others methods, and the artificial operation errors were avoided. Therefor, the high drug loading and entrapment efficiency could be obtained.

4. Observation by the DSC

As demonstrated in Fig. 7, graph a and b, the model drug had a strong peak at the 148.18 μm and the carrier had a peak at 52.1 μm, so the *Tg* of the drug and the carrier was 148.18 μm and 52.1 μm respectively. From the graph c, we can find easily that the physical mixture of drug with carrier material had two peaks at 148.18 μm and 52.1 μm 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.

5. Conclusion

The novel surface liquid spraying method used in the formulation of microsphere gives high encapsulation efficiency and mean particle size distribution.Three spraying parameters were investigated to set up the relationship between the parameters and the particle size of microspheres.

The central composite design- response surface methodology as a popular statistic method was used in this paper. The main effects and interactions of the relevant process factors on spraying area was investigated. The three factors and five levels experiment

was designed, then the quadratic polynomial equation which could indicate the relationship between parameters and the spraying area was obtained. From the graph of response surface, the optimum formulation of VIN-PLGA-MS was achieved.

From the result of this paper, because of the whole preparation process was determined by the mechanism parameters and easily to control 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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