Preparation of pesticide microspheres based on polylactic acid: optimized by response surface methodology

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Abstract. Polylactic acid, as a kind of environment-friendly material, has excellent biodegradability, biocompatibility and plasticity. In recent years, it has been widely used in medicine, packaging and other fields, but rarely in agriculture. In this study, the process of buprofezin - polylactic acid microsphere was optimized by response surface method. Changing parameters, especially the concentration of PLA and the PLA/drug ratio, can increase the encapsulation efficiency of buprofezin - PLA microspheres. Ultimately, the highest encapsulation efficiency reached to 96.47%. The result of FESEM and laser particle size distribution analyzer showed that the microspheres have smooth surface and good dispersion, with particle size of 34.2 μm.

1. Introduction

Due to the importance of sustainable development, non-petroleum based biodegradable materials have gained more and more attention. Polylactic acid (PLA) is a polyester obtained by polymerization of lactic acid, which mainly derived from the fermentation of starch (such as corn and rice) [1]. PLA is well known for its complete degradation into CO₂ and H₂O₂, and its good performance in biocompatibility, moldability, mechanical strength and thermoplastic [2,3]. As one of the most widely used biodegradable polymer, PLA has been applied in many fields such as medicine, manufacturing and food packaging [4,5,6]. Currently, PLA source products began to appear in agriculture, such as biodegradable plastic film and nursery tray like [7]. The application of non-petroleum based biodegradable materials (e.g. PLA), can help to protect the environment and alleviate the energy shortage.

Because of the photolysis, hydrolysis and the loss by rain/wind, conventional pesticide formulations exhibit very low efficiency on target [8]. To maintain the effect on pests and diseases, people have to increase the amount and frequency of the pesticide. Over-use of pesticides leads to serious environmental pollution and excessive pesticide residues [9]. Since the controlled release microsphere can reduce the total amount of pesticides and the frequency, the new formulation can be an effective solution. In this study, the PLA microspheres of buprofezin (a kind of easily degradable pesticide) was prepared by single emulsion solvent evaporation method.

For there’re several factors affecting the reaction, the experimental design is very important. The
response surface method (RSM) is a classic statistical method to evaluating the role of parameters and searching optimum process for the response. Recently, the method has been used in optimizing the preparation of sustained release microspheres \cite{10,11,12}. In order to reduce costs and improve the efficiency, we optimized the process with RSM to obtain conditions for a high encapsulation efficiency (E.E.) of the microspheres.

2. Materials and Methods

2.1. Materials
Polylactic acid was purchased from eSUN Industrial Corporation (Shenzhen, China), with a molecular weight (Mw) of 100,000. Buprofezin was purchased from Yinong agricultural Corporation (Changshu, China). Dichloromethane (DCM, AR) and polyvinyl alcohol (PVA, 87-89% hydrolyzed) was purchased from Aladdin Industrial Corporation (Shanghai, China).

2.2. Preparation of Buprofezin - PLA microspheres
PLA was dissolved in DCM to prepare the polymer solutions with different concentrations. Buprofezin was added into the polymer solution, and sonicated 3 min (oil phase). PVA was dissolved in distilled water by heating (water phase). Then the oil phase was slowly added to the water phase with rapid stirring to emulsify into small droplets. The emulsion was maintained stirring and heated to 45 °C to remove DCM from the droplet, so that the PLA can be solidified into microspheres. Then collected the microspheres by vacuum filtration. Washed with distilled water for three times to remove residual PVA. The microspheres were harvested after drying and stored at room temperature.

2.3. Encapsulation efficiency determination
The E.E.% was measured at a wavelength of 245 nm with an ultraviolet (UV) spectrometer. The standard curve was prepared using drug concentration ranging from 0.078 to 0.431 μg/mL and had a regression equation of $y = 28.719x + 0.1664$, with $R^2 = 0.9994$. The E.E.% of the drug loading was calculated using the equation (1) as follow:

$$\text{E.E.} (%) = \frac{\text{The actual drug amount}}{\text{The theoretical drug amount}} \times 100\%$$

(1)

2.4. Box-Behnken experimental design
Before design, a lot of preliminary experiments had been performed to choose the parameters and their levels. Four parameters were selected, including PLA concentration, PVA concentration, PLA/drug ratio and the stirring speed. In order to optimize the preparation parameters, 29 experiments were designed according to Design Expert® software (version 10.0.7), and the effects of four parameters on the E.E.% were evaluated. The four independent variables and their coded factors are listed in Table 1.

| Table 1. Variables and their levels used for the Box-Behnken design |
| --- |
| Independent variables | Levels |
| --- | --- |
| A = concentration of PLA (mg/mL) | Low, (-1) | High, (+1) |
| B = concentration of PVA (%) | 40 | 80 |
| C = ratio of PLA to buprofezin (m/m) | 0.5 | 1.5 |
| D = stirring speed (r/min) | 3 | 5 |
| | | 800 | 1200 |

3. Result and discussion

3.1. Design of experiments and model fitting
The results of actual and predicted response values as well as residuals of all the 29 experiments carried out were shown in Table 2.
Table 2. Response values and the residuals of encapsulation efficiency

| Run | A  | B  | C  | D  | Actual | Predicted | Residual |
|-----|----|----|----|----|--------|-----------|---------|
| 1   | 1  | 0  | 0  | 1  | 87.78  | 89.74     | -1.96   |
| 2   | 1  | 0  | 1  | 0  | 93.45  | 93.69     | -0.24   |
| 3   | 0  | 0  | 0  | 0  | 94.90  | 94.30     | 0.60    |
| 4   | 0  | 0  | 1  | 1  | 89.81  | 89.23     | 0.58    |
| 5   | -1 | 0  | 0  | 1  | 79.43  | 82.11     | -2.68   |
| 6   | 0  | 0  | -1 | 1  | 83.19  | 81.05     | 2.14    |
| 7   | 0  | 0  | 1  | -1 | 88.46  | 89.38     | -0.92   |
| 8   | 0  | 1  | 0  | 1  | 87.35  | 86.92     | 0.43    |
| 9   | 0  | -1 | -1 | 0  | 86.33  | 87.72     | -1.39   |
| 10  | 0  | 0  | 0  | 0  | 93.24  | 94.30     | -1.06   |
| 11  | 1  | 0  | -1 | 0  | 92.74  | 93.14     | -0.40   |
| 12  | -1 | 1  | 0  | 0  | 83.96  | 82.86     | 1.10    |
| 13  | -1 | 0  | 0  | -1 | 83.60  | 83.66     | -0.06   |
| 14  | 0  | -1 | 1  | 0  | 88.63  | 88.62     | -0.01   |
| 15  | 1  | -1 | 0  | 0  | 91.11  | 90.99     | 0.12    |
| 16  | 0  | 0  | 0  | 0  | 94.71  | 94.30     | 0.41    |
| 17  | 0  | 1  | 1  | 0  | 93.36  | 93.99     | -0.63   |
| 18  | 0  | 0  | 0  | 0  | 95.88  | 94.30     | 1.58    |
| 19  | -1 | 0  | 1  | 0  | 93.29  | 92.10     | 1.19    |
| 20  | 0  | 1  | -1 | 0  | 79.50  | 81.53     | -2.03   |
| 21  | 0  | 0  | -1 | -1 | 84.83  | 84.19     | 0.64    |
| 22  | 1  | 1  | 0  | 0  | 96.04  | 94.22     | 1.82    |
| 23  | -1 | -1 | 0  | 0  | 86.31  | 86.91     | -0.60   |
| 24  | -1 | 0  | -1 | 0  | 80.31  | 79.28     | 1.03    |
| 25  | 1  | 0  | 0  | -1 | 92.14  | 91.48     | 0.66    |
| 26  | 0  | -1 | 0  | 1  | 83.23  | 81.74     | 1.49    |
| 27  | 0  | -1 | 0  | -1 | 89.34  | 88.98     | 0.36    |
| 28  | 0  | 0  | 0  | 0  | 92.79  | 94.30     | -1.51   |
| 29  | 0  | 1  | 0  | -1 | 82.28  | 82.98     | -0.70   |

By analysing the data of the 29 runs in Table 2, the final empirical formula for E.E.% was shown in equation (2) as follow:

\[
Y = 94.30 + 3.86A - 0.20B + 3.34C - 0.82D + 1.82AB - 3.07AC - 0.048AD + 2.89BC + 2.79BD + 0.75CD - 1.98A^2 - 3.57B^2 - 2.77C^2 - 5.57D^2
\]  

(2)

where Y is the predicted response of E.E.%, the variables of A to D are the coded values of PLA concentration, PVA concentration, PLA/drug ratio and stirring speed, respectively.

3.2. Analysis of variance (ANOVA)

The significance and validity of the model were tested by ANOVA, which were showed in Table 3. The p-value less than 0.05 means significant. The results of ANOVA showed the model’s p value <0.0001, indicating that the model is highly significant and reasonable. The lack of fit is not significant, for the p-value = 0.2390. The correlation coefficient R² = 0.9443 and Adj. R² = 0.8887, indicating that the equation fitted well. Adeq Precision = 12.199 > 4, indicating the response signal was strong enough. So the regression equation mentioned (equation 2) can be used to predict and analyze the E.E.% of microspheres.
Table 3. ANOVA for encapsulation efficiency

| Source  | Sum of Squares | Degree of Freedom | Mean Square | F-Value | p-Value Prob.> F |
|---------|----------------|------------------|-------------|---------|-----------------|
| Model   | 696.56         | 14               | 49.75       | 16.97   | < 0.0001*       |
| A       | 179.10         | 1                | 179.10      | 61.07   | < 0.0001*       |
| B       | 0.50           | 1                | 0.50        | 0.17    | 0.6847          |
| C       | 134.00         | 1                | 134.00      | 45.69   | < 0.0001*       |
| D       | 8.10           | 1                | 8.10        | 2.76    | 0.1187          |
| AB      | 13.25          | 1                | 13.25       | 4.52    | 0.0518          |
| AC      | 37.64          | 1                | 37.64       | 12.83   | 0.0030*         |
| AD      | 9.025×10⁻³     | 1                | 9.025×10⁻³  | 3.077×10⁻³ | 0.9565 |
| BC      | 33.41          | 1                | 33.41       | 11.39   | 0.0045*         |
| BD      | 31.25          | 1                | 31.25       | 10.66   | 0.0057*         |
| CD      | 2.24           | 1                | 2.24        | 0.76    | 0.3974          |
| A²      | 25.56          | 1                | 25.56       | 8.71    | 0.0105*         |
| B²      | 82.90          | 1                | 82.90       | 28.27   | 0.0001*         |
| C²      | 49.68          | 1                | 49.68       | 16.94   | 0.0010*         |
| D²      | 201.60         | 1                | 201.60      | 68.74   | < 0.0001*       |
| Residual| 41.06          | 14               | 2.93        |         |                 |
| Lack of Fit | 34.63      | 10               | 3.46        | 2.15    | 0.2390N         |
| Pure Error | 6.43         | 4                | 1.61        |         |                 |
| Cor Total | 737.62        | 28               |             |         |                 |

R² = 0.9443, Adj. R² = 0.8887, Pred. R² = 0.7160, Adeq Precision = 12.199

* Significant (“p-Value Prob. > F” less than 0.05).
N Non-significant.

The analysis demonstrated that A, C, AC, BC, BD, A², B², C², D² are significant model terms. The PLA concentration (A), PLA/drug ratio (C) and the quadratic term (D²) had a highly significant effect (P < 0.001) at the E.E.%. The PLA concentration - PLA/drug ratio interaction term (AC), PVA concentration - PLA/drug ratio (BC) and PVA concentration - stirring speed (BD) had a significant effect (P < 0.05), and also the quadratic terms (A², B² and C²).

3.3. Process optimization
The 3D response surfaces and the 2D contours for the effects of the variables on the E.E.% of the microspheres are shown in Figure 1.
Figure 1. The 3D response surfaces and 2D contours for the interactions of variables on E.E.%: (a) PLA concentration - PLA/drug ratio (AC); (b) PVA concentration - PLA/drug ratio (BC); (c) PVA concentration - stirring speed (BD).

The results depicted the interaction between PLA concentration (A) and PLA/drug ratio (C), PVA concentration (B) and PLA/drug ratio (C), PVA concentration (B) and stirring speed (D), respectively. Consistent with ANOVA analysis, the elliptical contours demonstrate that there are significant interaction between variables.

The maximum E.E.% (96.47%) is calculated by Design Expert Software with the optimal condition as: PLA concentration = 78.24 (mg/mL), PVA concentration = 1.16%, PLA/drug ratio = 4.27 (m/m), stirring speed = 1004 (r/min). Under the condition, the experimentally result was found to be 96.73±0.52%, which is highly consistent with the model prediction.

3.4. Appearance and particle size of the microspheres
The field emission scanning electron microscopy (FESEM) was used to show the appearance of buprofezin - PLA microspheres. The micrograph demonstrated that the microparticals were smooth spherical, with a good dispersion. The particle size was measured by a laser particle size distribution analyzer. Both of the results were shown in Figure 2.
4. Conclusions
The utilization of pesticides is about 10%, which lead to excessive application. The over-using polluted the environment seriously and increased the pesticide residues. Application of sustained release formulations of pesticide can play a positive role on sustainable development, the environment protection and human health. We prepared the buprofezin - PLA microspheres by evaporation emulsification method. Since cost is an important factor limiting application, we optimize the encapsulation efficiency by RSM. After optimization, the conditions for obtaining the maximum E.E.% (96.47%) were PLA concentration at 78.24 (mg/mL), PVA concentration at 1.16%, PLA/drug ratio at 4.27 (m/m), stirring speed at 1004 (r/min). The results of FESEM and laser distribution analyzer showed that the size of the microspheres is about of 34μm, with a smooth surface and good dispersion.

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