

## Optimization of the Preparation of Nalmefene-Loaded Sustained-Release Microspheres Using Central Composite Design

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Nalmefene-loaded poly(lactic-co-glycolic acid) microspheres were prepared by O/O emulsification/solvent evaporation method. The central composite design-response surface methodology was used to optimize and predict the preparation microspheres. Effects of three independent variable variables *i.e.*, Span80 concentration in outer phase, poly(lactic-co-glycolic acid) concentration in inner phase and theoretical drug content were evaluated on a number of response variables. Response variables selected in this study were drug content, encapsulation efficiency, mean diameter, diameter span and the cumulative percentage of the drug released in the first day after incubation (marked as  $F_{1d}$ , and it was also calculated as the initial burst). Multiple linear regression and second-order polynomial model were fitted to the data, and the resulting equations were used to produce five dimensional response graphs, by which optimal experimental conditions were selected. The results showed that all response variables were greatly dependent on three independent variables, and the optimal conditions were Span80 concentration 1.5%, poly(lactic-co-glycolic acid) concentration 17.5%, and theoretical drug content 6%. According to the optimal conditions, the drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$  of prepared microspheres were 4.37%, 72.8%, 64.1  $\mu\text{m}$ , 1.36 and 8.93%, respectively.

**Key words** nalmefene; poly(lactic-co-glycolic acid); microsphere; central composite design-response surface methodology

Nalmefene is a  $\mu$  selective opioid antagonist which is similar to naloxone in structure and pharmacology. Receptor binding studies have shown that nalmefene, like naloxone and the other opioid antagonist naltrexone, had high affinity for  $\mu$ ,  $\kappa$  and  $\delta$  receptors, furthermore, it bound more tightly with these receptors than naltrexone.<sup>1–3</sup> In animal models, nalmefene has many properties of opioid antagonist which have been demonstrated under a wide range of conditions. Studies on humans have also demonstrated opioid antagonist effects of nalmefene.<sup>4</sup> In addition to the reversal of opioid agonist effects, nalmefene provides an alternative to methadone for the treatment of opioid dependence.<sup>1,4,5</sup> Nalmefene has specific pharmacological properties which may make it more useful than other opioid antagonists as an abstinence treatment for opioid dependence.<sup>4,6</sup> The number of narcotics-abusers in China is quite staggering. According to statistics of government, heroin-abusers are estimated to be 0.791 million. Many relevant medical agencies had made continuous efforts to reduce the population of narcotic-abusers, but seldom achieved the ideal goal. Statistics showed that 98.2% subjects who entered those treatment programs had a dismal record of relapse, and only a small proportion remained clean long after the program. The disease caused by narcotics abuse remains a scourge of society. It becomes an increasingly important task for us to help addicted individuals to get rid of their dependency or at least decrease the level of dependency to make them be a functional member of society.

The relapse of addiction is often caused by the compliance and lack of retention.<sup>5,7–9</sup> A repetitive treatment act, such as asking subjects to take a pill daily, is not easy thing, even they have no doubt of taking the pill. When the narcotics-abuser has physiological and emotional needs for the abused substance, the therapeutic routine becomes more difficult. The lack of perseverance of the subject decreases the chance of success of the treatment. Therefore, it is of great impor-

tance to be able to reduce the level of involvement of the subject during medicinal treatments, particularly those treatments involving a particular regimen.

Many experts adopt sustained-release method to reduce the involvement of compliance. Various slow-release biodegradable microspheres have been developed for a variety of drugs, and some of them are commercialized. Among various biodegradable polymers, the poly(lactic-co-glycolic acid) (PLGA) is the most widely studied and used.<sup>10–12</sup> The properties of the microspheres are sensitive to many variable-ness of preparation and condition of the preparing and selecting process. In this study, we have studied the effect of formulation variables of sustained release microspheres on several response variables, and subsequently, we have utilized response surface methodology to optimize the formulation after constructing a desirable function that combines three response variables.

### Experimental

**Materials and Apparatus** PLGA ( $W_n$  20000, lactide/glycolide ratio, 75/25) was purchased from Chengdu institute of organic chemistry, Chinese Academy of Sciences; Nalmefene base was obtained from Beijing institute of pharmacology and toxicology; Paraffin liquid, acetonitrile (AN) and dichloromethane (DCM) were obtained from Beijing chemical reagents company; Span80 was obtained from Fisher Scientific (Hongkong) Ltd. All other materials or solvents were of reagent or analytical grade.

**Preparation of Microspheres** O/O emulsification/solvent evaporation method was applied to fabricate nalmefene-PLGA microspheres.<sup>10,13,14</sup> An amount of PLGA and nalmefene were added to 1 ml of solvent (DCM:AN=1:1, v/v).<sup>15</sup> After completely dissolved, it was poured into Paraffin liquid containing Span80 as emulsifier. Then the mixture was emulsified by a constant stirring at 600 rpm for 10 min by using a propeller stirrer (SXJQ-1, Zhengzhou, China) under 25 °C. Then stirred at 500 rpm under 25 °C continuing for 10 h to evaporate the organic solvent. The harden microspheres were washed three times with hexane, then rinsed with distilled water, and dried under vacuum.

**Optimization of Preparation Using Central Composite Design** Experimental Designs: Preliminary experiments indicated that the variables mostly affect the preparation of microspheres prepared by the emulsification/solvent evaporation technique were emulsifier concentration, drug load-

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ing, and polymer concentration. Other variables investigated in the preliminary study were stirring speed, volume of the outer oil phase, and the composition of the inner oil phase. Thus, a central composite experimental design with uniform precision rotatable properties was used to systematically investigate the effect of, and the interactions among the three critical formulation variables on the drug content, encapsulation efficiency, mean diameter, diameter span and the cumulative percentage of the drug released in the first day after incubation (marked as  $F_{1d}$ , and it was also calculated as the initial burst). Central composite design enables several independent variables to be investigated at the same time using a relatively small number of experiments.<sup>16–20</sup> The independent variables in our studies were Span80 concentration ( $X_1$ ), theoretical drug content ( $X_2$ ), and PLGA content in the inner phase of the emulsion ( $X_3$ ). For each factor, an experimental range was selected, based on the results of preliminary experiments and taking into consideration the feasibility of preparation of the microspheres at the extreme values. Tables 1 and 2 show the experimental design and the 20 formulations (15 distinct experiments + 5 replicates of the central point) preparation.<sup>21</sup>

**Model Fitting and Prediction:** The drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$  values were individually fitted to a multiple linear regression and a second-order polynomial model based on response surface regression, using the computer program SPSS v10.0 software (SPSS, Chicago, U.S.A.).<sup>17,21</sup> Mathematical model equations were as following:

$$\text{multiple linear regression: } Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3$$

second-order polynomial regression:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3$$

After stepwise regression, models with a higher multiple correlation coefficient were obtained. According to the fitting model, response surfaces that showed the relationships between the response variable (drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$ ) and formulation variables were generated. The resulting models were then used to predict the response variable within the experimental range, especially at the experimental conditions under which the optimized microsphere was expected to be obtained. Five batches of microspheres were prepared at the predicted optimum conditions and evaluated the responses: drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$ .

**HPLC Analysis of Nalmefene** For the drug content test and *in vitro* release studies, the drug concentrations were detected with HPLC.<sup>22,23</sup> The HPLC method for the analysis of drug content and of the drug released during the *in vitro* release test was as following. The HPLC system was an N2000 (Zhejiang Univ., China). Chromatographic conditions: column, Bondclone  $C_{18}$ , 10 mm,  $250 \times 4.60$  mm, 5 micron (Phenomenex, U.S.); mobile phase,  $KH_2PO_4$  (pH 4.0; 0.02 M)/methanol/triethylamine (67:33:0.2 v/v/v); flow rate, 1.0 ml/min; temperature, 25 °C; wavelength set, 284 nm; and injection volume, 20  $\mu$ l. The drug concentrations were determined from standard curves in the range 21.44–343.04  $\mu$ g  $\cdot$  ml<sup>-1</sup>.

**Microspheres Characterization** Morphological and Topographical Characterization: Microspheres were observed and photographed with optical microscope (OLYMPUS BX-50, Japan) and scanning electron microscopy (Hitachi S-450, Japan). Their diameters were determined with a pre-calibrated graduated eyepiece. One hundred measurements were averaged for each microsphere formation.<sup>11</sup>

**Determination of Nalmefene Content:** Ten milligrams of nalmefene microspheres were dissolved in DCM (1 ml); the drug was extracted with 5 ml of 0.001 mol  $\cdot$  l<sup>-1</sup> HCl. After vortex for 2 min and centrifugation for 10 min at 3750 rpm, 20  $\mu$ l of aqueous phase were tested by the HPLC method described in Results and Discussion. The encapsulation efficiency was expressed as the ratio of detected and added drug amount.

**Release Studies of Nalmefene Microspheres *in Vitro*:** The dialysis method was utilized for the study of the drug release *in vitro*.<sup>24</sup> In brief, about 40 mg of microspheres were weighted and added to dialysis bag with cut off molecular weight 1 kDa, 5 ml phosphate buffered saline (PBS, 0.01 M, pH 7.4, containing 0.02%  $NaN_3$ ) was then added. The dialysis bag containing microsphere suspension was kept in a beaker flasks filling 45 ml PBS as the release medium and shaken at a rate of 72 rpm at 37 °C. Four hundred microliters of medium was drawn out at the predetermined day intervals and the same volume of fresh PBS was replenished. Mediums were filtered through a 0.45  $\mu$ m filter and sampled on the HPLC column.

***In Vitro* Polymer Degradation:** *In vitro* degradation study of placebo and drug-loaded microspheres was carried out in the same PBS mediums that were used in the release experiment *in vitro*. The suspensions of 4 mg of mi-

crosheres in 10 ml of the buffers were shaken at 72 rpm and 37.0 °C. At pre-set intervals, the vials were centrifuged at 5000 rpm for 20 min. After removing the upper clear solution, the microspheres were dried under vacuum for 48 h. Mass loss was determined gravimetrically.<sup>25</sup>

## Results and Discussion

**Microspheres Characterization** The Optical microscopy revealed that all microspheres obtained from the experiment design were opaque, discrete and spherical particles with smooth surfaces. The particle size is an important property of microsphere, as it can influence the biopharmaceutical properties of the particle preparations.<sup>15</sup> The results of particle size and span analysis were given in column 4 and 5 of Table 1, The mean particle size of microspheres prepared from the formulations was  $50.0 \pm 18.5 \mu$ m, being considered suitable for intravitreal administration through a 27G needle (inner diameter 0.19 mm). Figure 2D depicted the relationship between microsphere diameter and the formulation variables. It was clear that the diameter was highly dependent on the Span80 concentration ( $p < 0.05$ ). As the Span80 concentration increased, the diameter decreased significantly. This increase corresponded to the negative coefficient of  $X_1$ . The phenomenon was attributed to smaller volume of the initial droplets in the emulsion due to greater reduction in the interfacial tension.

Column 1 and 2 in Table 2 showed the results of drug content and encapsulation efficiency. At preliminary experiments, we prepared microsphere using nalmefene hydrochlorate as model drug, but the encapsulation efficiency was very

Table 1. Experimental Design

Factor	Level				
	-a	-1	0	+1	+a
$X_1$ (%)	0.5	1.13	2	2.87	3.5
$X_2$ (%)	10	13.17	17.5	21.83	25
$X_3$ (%)	5	7.11	10	12.89	15

a=1.732.

Table 2. Microsphere Formulation Variables and Physical Properties

No.	Drug content (%)	Encapsulation efficiency (%)	Mean diameter ( $\mu$ m)	Diameter span	$F_{1d}$ (%)
1 (-a, 0, 0)	7.43	74.3	87.3	1.05	26.95
2 (-1, -1, -1)	5.58	78.5	74.3	1.24	24.38
3 (-1, +1, -1)	6.11	85.9	73.6	1.50	11.36
4 (-1, -1, +1)	7.68	59.6	68.4	0.82	39.81
5 (-1, +1, +1)	9.13	70.9	69.7	1.20	28.57
6 (0, -a, 0)	6.58	65.8	39.2	0.97	28.61
7 (0, +a, 0)	7.47	74.7	67.4	0.82	6.74
8 (0, 0, -a)	3.20	64.1	39.4	0.92	1.99
9 (0, 0, +a)	8.54	56.9	63.9	1.10	23.53
10 (+1, -1, -1)	5.21	73.3	27.0	0.50	19.09
11 (+1, +1, -1)	5.46	76.8	41.6	0.85	10.25
12 (+1, -1, +1)	6.96	54.0	28.4	1.22	28.45
13 (+1, +1, +1)	7.85	60.9	34.2	0.98	7.91
14 (+a, 0, 0)	6.48	64.8	22.9	1.01	8.80
15 (0, 0, 0)	7.05	70.5	31.5	1.44	15.89
16 (0, 0, 0)	6.95	69.5	47.7	1.25	24.64
17 (0, 0, 0)	6.94	69.4	44.6	1.15	23.68
18 (0, 0, 0)	7.12	71.2	45.4	1.20	21.71
19 (0, 0, 0)	6.94	69.4	43.8	1.40	22.72
20 (0, 0, 0)	6.87	68.7	49.6	1.28	21.66

low, then nalmefene base was tried and we got microspheres with high encapsulation efficiency. The increased encapsulation efficiency may be attributed to the hydrophobic nature of nalmefene base as well as PLGA polymer. Figure 2A described the relation between drug content and the formulation variables. It was clear that the drug content was significantly affected by the theoretical drug content ( $p < 0.001$ ). As the theoretical drug content increased, the drug content increased significantly, but the encapsulation efficiency increased initially, after reaching a maximum level, it started to decline slightly as the theoretical drug content was further increased.

The initial burst release is always attributed to the rapid release by diffusion of dissolved drug initially deposited inside the pores. The most commonly supported hypothesis to explain the burst is that some drug particles could have migrated to the surface during the drying of microspheres.<sup>26</sup> Column 5 showed the results of  $F_{1d}$ . In this work, the percentage of initial burst release for all formulations ranged between 1.99% and 28.61% of formulations respectively. Despite the fact that increasing theoretical drug content increased the mean diameter, and an increase in  $F_{1d}$  was observed, a decreased  $F_{1d}$  was found with the Span80 concentration increased. A possible explanation was that the diameter increased with the Span80 concentration decreased, on the basis of the mechanism of emulsification/solvent evaporation method, microspheres with higher diameter would have higher porosity resulting in a faster drug release.

The nalmefene release profiles of all formulations were investigated in pH 7.4 PBS. Nalmefene was released from microsphere nearly as a one-order release in two weeks, then it followed by a slow release. The cumulative amount of nalmefene released in 14 d was very important to reach therapeutic levels with the minimum dose of microspheres because if the drug release was too low the drug concentration would not be enough to reach the minimum effective concentration.

**Model Fitting** The drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$  values were individually fitted to a multiple linear regression.<sup>21</sup> The model could be described by the following equations:

$$\text{drug content (\%)} = 1.623 - 0.384X_1 + 0.0769X_2 + 0.458X_3 \quad (p < 0.001, r = 0.946)$$

$$\text{encapsulation efficiency (\%)} = 83.915 - 3.815X_1 + 0.734X_2 - 2.018X_3 \quad (p < 0.001, r = 0.835)$$

$$\text{mean diameter (\mu m)} = 67.077 - 21.911X_1 + 1.152X_2 + 0.658X_3 \quad (p < 0.001, r = 0.919)$$

$$\text{diameter span} = 1.055 - 0.105X_1 + 0.008086X_2 + 0.01092X_3 \quad (p (=0.509) > 0.05, r = 0.362)$$

$$F_{1d} (\%) = 38.718 - 5.746X_1 - 1.51X_2 + 1.903X_3 \quad (p < 0.001, r = 0.898)$$

The results of multiple linear regression showed that coefficients of multiple correlation were low and the linear correlation was not a good fitting. Then, the drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$  values were individually fitted to a second-order polynomial model.<sup>17,21</sup> The model could be described by the following equations:

$$\begin{aligned} \text{drug content (\%)} &= -0.774 + 0.498X_1 - 0.099X_2 + 1.109X_3 + 0.02337X_1^2 + 0.002167X_2^2 \\ &\quad - 0.0413X_3^2 - 0.0279X_1X_2 - 0.0487X_1X_3 + 0.01558X_2X_3 \end{aligned} \quad (p < 0.001, r = 0.988)$$

The fitting results indicated strong correlation between drug content and theoretical drug content ( $p < 0.001$ ).

$$\begin{aligned} \text{encapsulation efficiency (\%)} &= 72.291 - 1.242X_1 - 0.882X_2 + 2.784X_3 + 0.723X_1^2 + 0.04107X_2^2 \\ &\quad - 0.297X_3^2 - 0.275X_1X_2 - 0.0646X_1X_3 + 0.07292X_2X_3 \end{aligned} \quad (p < 0.01, r = 0.908)$$

The fitting results indicated strong correlation between drug encapsulation efficiency and theoretical drug content ( $p < 0.05$ ).

$$\begin{aligned} \text{mean diameter (\mu m)} &= 171.355 - 54.175X_1 - 4.974X_2 - 4.27X_3 + 4.719X_1^2 + 0.157X_2^2 \\ &\quad + 0.287X_3^2 + 0.657X_1X_2 + 0.189X_1X_3 - 0.0679X_2X_3 \end{aligned} \quad (p < 0.001, r = 0.952)$$

The fitting results indicated strong correlation between Span80 concentration and mean diameter ( $p < 0.05$ ).

$$\begin{aligned} \text{diameter span} &= -1.93 - 0.185X_1 + 0.312X_2 + 0.13X_3 - 0.0983X_1^2 - 0.00634X_2^2 \\ &\quad - 0.00966X_3^2 - 0.0176X_1X_2 + 0.07805X_1X_3 - 0.00469X_2X_3 \end{aligned} \quad (p < 0.05, r = 0.870)$$

The fitting results indicated strong correlation between PLGA concentration and diameter span ( $p < 0.05$ ), and the interaction of Span80 concentration and theoretical drug content had strong correlation with diameter span ( $p < 0.05$ ).

$$\begin{aligned} F_{1d} (\%) &= -39.804 + 11.525X_1 + 0.508X_2 + 10.995X_3 - 0.39X_1^2 - 0.0196X_2^2 \\ &\quad - 0.241X_3^2 - 0.17X_1X_2 - 1.274X_1X_3 - 0.0991X_2X_3 \end{aligned} \quad (p < 0.005, r = 0.950)$$

The fitting results indicated strong correlation between theoretical drug content and  $F_{1d}$  ( $p < 0.05$ ).

Table 3 showed the  $r$  obtained from two models. We could conclude undoubtedly that the second-order polynomial model was more suitable than the multiple linear regression in this text, so the latter one was desirable.

**Prediction** Based on the mathematical model, predicted response surface of each dependent variable (drug content, encapsulation efficiency, mean diameter, diameter span and  $F_{1d}$ ) was drawn using Origin v6.0. Software from Microcal Software, Inc. (Northampton, MA, U.S.A.).<sup>21</sup> Because response surface was three-dimension (3-D) graphics with two independent variables expressed once, one independent variable must be fixed. Viewing the coefficients of mathematical

Table 3. Comparison of the Obtained  $r$  from Two Models

	$r$	
	Multiple linear regression	Second-order polynomial model
Drug content (%)	0.946	0.988
Encapsulation efficiency (%)	0.835	0.908
Mean diameter ( $\mu\text{m}$ )	0.919	0.952
Diameter span	0.362*	0.870
$F_{1d}$ (%)	0.898	0.950

\*  $p (=0.509) > 0.05$ , no significant correlation.

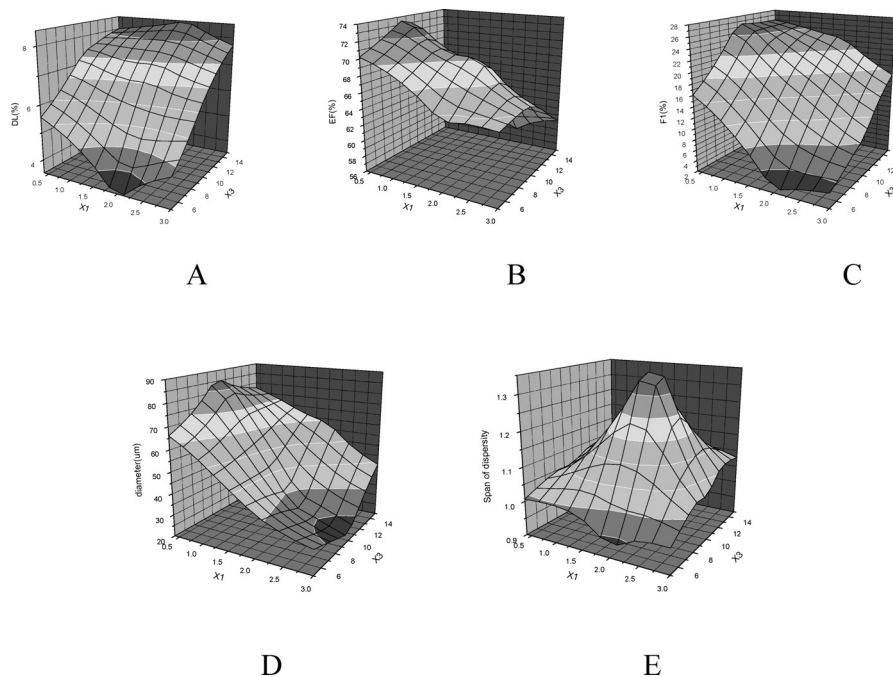


Fig. 1. Predicted Response Surfaces (A: Drug Content as a Function of Span Concentration ( $X_1$ ) and Theoretical Drug Content ( $X_3$ ); B: Drug Encapsulation Efficiency as a Function of Span Concentration ( $X_1$ ) and Theoretical Drug Content ( $X_3$ ); C:  $F_{1d}$  as a Function of Span Concentration ( $X_1$ ) and Theoretical Drug Content ( $X_3$ ); D: Mean Diameter as a Function of Span Concentration ( $X_1$ ) and Theoretical Drug Content ( $X_3$ ); E: Diameter Span as a Function of Span Concentration ( $X_1$ ) and Theoretical Drug Content ( $X_3$ )), with PLGA Concentration ( $X_2$ ) Equal to 17.5%

Table 4. Comparison of the Observed and Predicted Values of Drug Content, Encapsulation Efficiency, Mean Diameter, Diameter Span and  $F_{1d}$  of the Batch of Microspheres Prepared under Predicted Optimum Condition

Response variable	Predicted response	Observed response	Bias <sup>a)</sup> (%)
Drug content (%)	4.59	4.37	5.03
Encapsulation efficiency (%)	75.06	72.8	3.10
Mean diameter ( $\mu\text{m}$ )	58.4	64.1	-8.89
Diameter span	1.27	1.36	-6.62
$F_{1d}$ (%)	10.48	8.93	17.36

a) Bias (%)=(predicted response-observed response)/observed response.

models, we could conclude the PLGA concentration ( $X_2$ ) had less influence on the each dependent variable than Span concentration ( $X_1$ ) and theoretical drug content ( $X_3$ ). PLGA concentration ( $X_2$ ) was fixed as mid-value (17.5%), and predicted response surfaces were drawn as Fig. 1.

The sector of predicted response surfaces was read with high drug encapsulation efficiency, mini- diameter span, small  $F_{1d}$  and suitable mean diameter, and here was Span80 concentration ( $X_1$ )=1.5%, PLGA concentration ( $X_2$ )=17.5% and theoretical drug content ( $X_3$ )=6%, in other word, the optimized formulation of microspheres is  $X_1$ =1.5%,  $X_2$ =17.5%,  $X_3$ =6%.

**Optimized Microspheres** Five batches of optimized microspheres were prepared under the optimized formulation and were pooled together for testing. The responses' values were calculated and compared to the corresponding predicted values. The results are given in Table 4.

Figures 2 and 3 showed the microspheres morphology prepared under predicted optimum condition.

To understand the characteristics of drug release from optimized microspheres, *in vitro* drug release and polymer

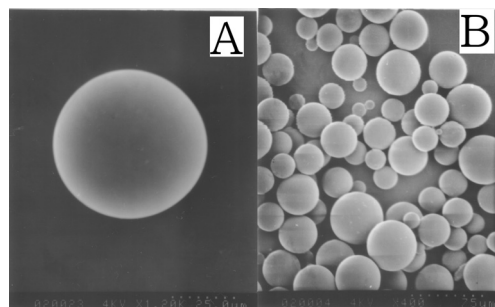


Fig. 2. Scanning Electron Microscopy Photograph of Nalmefene Microspheres (A:  $\times 1200$ , B:  $\times 400$ )

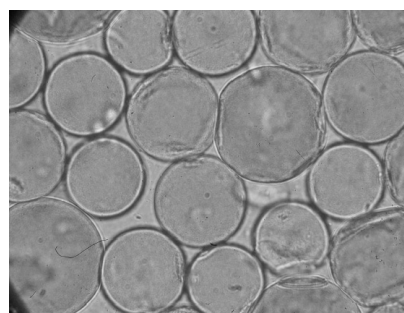


Fig. 3. Optical Micrograph of Nalmefene Microspheres ( $\times 600$ )

degradation experiments were carried out. The profile of drug release of nalmefene microsphere was illustrated in Fig. 3. The accumulated amount of drug released in 3 weeks was about 76.0% and in 4 weeks was 83.5%, the  $T_{1/2}$  was about 12 d. The burst release was 8.0%. Higuchi equation was  $\ln(100-Q)=78.37-17.01t$  ( $r=0.988$ ). It was obvious that

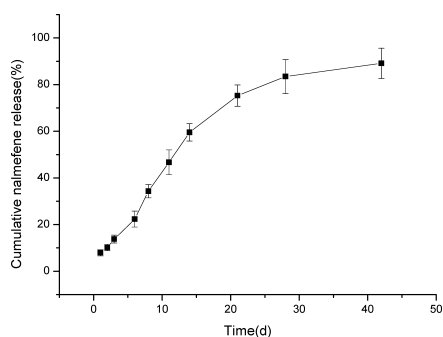


Fig. 4. *In Vitro* Drug Release Profile of Nalmefene from Microspheres ( $n=5$ )

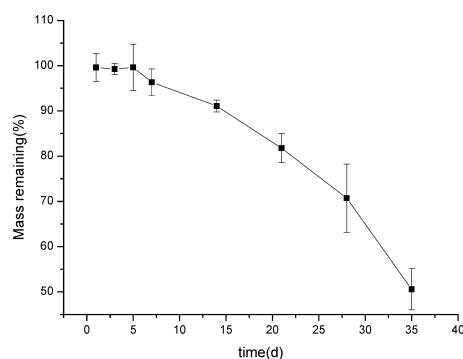


Fig. 5. Mass Loss of Biodegradable Microspheres in PBS *in Vitro* ( $n=5$ )

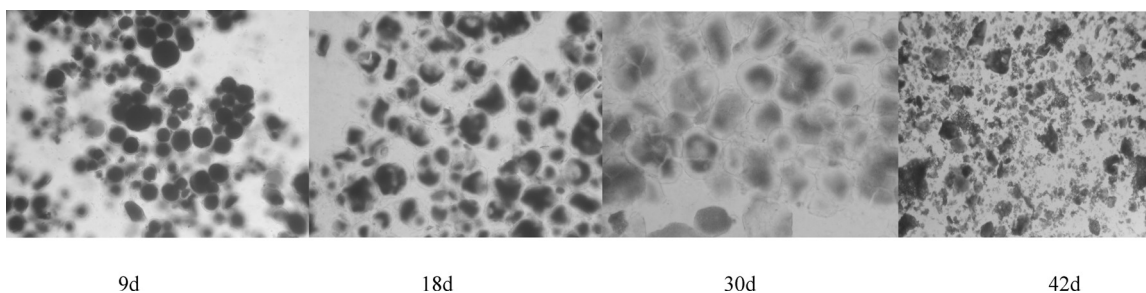


Fig. 6. Microscope Pictures of Nalmefene Microspheres during Incubation in 50 ml of 0.05 M Phosphate Buffer (pH 7.4)

nalmefene was released from the microspheres in a steady and sustained fashion.

Statistical analysis of drug release results were performed with Microsoft Excel (Microsoft, Washington, U.S.A.).

Figure 5 showed the mass loss profiles of microspheres during 5 weeks of degradation in PBS at 37 °C. Initially, the weight remained relatively constant during the first 7 d. Then, the PLGA microspheres weight started to decrease rapidly, losing 50% in 35 d. The microscope pictures of nalmefene microspheres during incubation in PBS also described the degradation. From Fig. 6 we could conclude that the microspheres had spherical surfaces initially. Then, the microspheres became more irregularly shaped after 18 and 30 d of degradation. After 42 d incubation, the microspheres' matrix collapsed completely.

## Conclusion

A nalmefene-loaded sustained-release microsphere formulation was optimized using response surface methodology by fitting a second-order model to the response data. The model was found to be satisfactory for describing the relationships between formulation variables and individual response variables. The optimization method enabled us to predict the values of response variables within the experimental range with good agreement between the predicted and experimental values. The optimized microspheres were able to provide a long-term release of nalmefene.

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