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Optimized Formulation of High-Payload PLGA Nanoparticles Containing Insulin–Lauryl Sulfate Complex

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A novel poly(lactic acid-co-glycolic acid) nanoparticle loaded with insulin–lauryl sulfate complex was prepared by spontaneous emulsion solvent diffusion method. The effects of key parameters such as agitation speed, poly(vinyl alcohol) concentration, solvent composition, polymer concentration, and the volume of external aqueous phase on the properties of the nanoparticles were investigated. To enhance the drug recovery and drug content simultaneously, a response surface methodology with five-level, two-factor central composite design was employed. The weight ratio of polymer to drug and volume ratio of external aqueous phase to solvent phase were selected as controlled factors on account of their interactions found in the monofactorial investigations. The experimental datum allowed the development of quadratic models ($p < .05$) describing the inter-relationships between the dependent and independent variables. By solving the regression equation, and graphic analyzing the response surface contour and plots, the optimum values of the two factors were determined as 20/1 and 10/1. The optimized conditions led to 89.6% of drug recovery and 4.57% of drug content during nanoparticle preparation.

Keywords insulin; PLGA nanoparticles; optimization; central composite design; surface response methodology

INTRODUCTION

Because of their desirable biocompatible and biodegradable properties, colloidal carriers made of poly(lactic acid-co-glycolic acid) (PLGA) has arisen as a promising alternative for improving the transport of macromolecules such as insulin (INS) through the oral route, especially the gastrointestinal tract (Damge, Michel, Aprahamian, Couvreur, & Devissaguet, 1990). The polymeric nature of PLGA nanoparticles offers a higher stability and protects the drug from adverse external conditions when they are in contact with biological fluids (Calvo, Alonso, Vila-Jato, & Obinson, 1996; Hans & Lowman, 2002; Sakuma et al., 1997; Takeuchi, Yamomoto, &, Kawashima, 2001). Moreover, it has been shown that M-cells, which are located on the surface of Peyer's patches, are a possible pathway for transporting the nanoparticles through the epithelium of the gut (Clean et al., 1998; Jung et al., 2000).

Among the methods available for preparing nanoparticles, spontaneous emulsion solvent diffusion (SESD) method presents distinctive advantages (Murakami, Kobayashi, Takeuchi, & Kawashima, 1999; Niwa, Takeuchi, Hino, Kunou, & Kawashima, 2001), in that it enables the production of nanosacle particulates (100–300 nm) without extend energy input such as shearing, sonication, or high temperatures. Thus this method is very suitable for loading bioactive protein and peptide drugs due to their instability. The spontaneous formation of nanoparticles is governed by the so-called Marangoni effect, under which an interfacial turbulence was generated at the interface of the water miscible solvent and the aqueous phase and resulted from diffusion and surface tension variations (Quintanar-Guerrero, Allémann, Fessi, & Doelker, 1998). Nevertheless, the method available for preparing biodegradable nanoparticles is basically applicable to lipophilic drugs, which are soluble in organic phase such as ethanol or acetone. To encapsulate water-soluble drugs still remains a challenge. Because of the week affinity between hydrophilic drug substances and liposoluble polymers, the drug encapsulated always has a tendency to move from the organic phase to the outer aqueous phase, which induces drugs leaking from the precipitating matrix and leads very low drug entrapment efficiency.

In our previous studies, hydrophobic ion pairing (HIP) strategy has been employed to solve the problem (Cui, Shi, Zhang, Tao, & Kawashima, 2006; Shi, Cui, Yamamoto, &

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Kawashima 2007). The complex formation between INS and sodium lauryl sulfate (SDS) was performed in acidic solution (pH 2), in which the medium pH value was below the INS isoelectric point (pI) (pI = 5.5) and the basic amino groups of INS become completely protonated. Thus SDS can be ion-paired with positively charged basic groups of INS using its negatively charged sulfate group. As addition of SDS into INS solution, the aqueous solubility of complex was reduced, tending to aggregate with each other and forming white precipitates. After complexed with the amphiphilic surfactant, the model drug INS displayed much more hydrophobic and an increase in partition coefficient. The enhanced hydrophobicity of complex improves the affinity of INS and hydrophobic PLGA and also permits INS codissolving with the polymer in nonaqueous solvents. Thus the satisfactory drug loading efficiency was expected to achieve.

In this study, PLGA nanoparticles loaded with INS–SDS complex were prepared by SESD method. Monofactorial experiments were first employed to investgate the effects of key parameters including agitation speed, poly(vinyl alcohol) (PVA) concentration, solvent composition, polymer concentration, and volume of external aqueous phase on the properties of the nanoparticles. Then the weight ratio of polymer to drug and volume ratio of external aqueous phase to solvent phase, two important factors affecting the drug loading efficiency, were selected to find the optimum levels to maximize the drug recovery and drug content simultaneously. The multiobjective optimization process was carried out through the central composite design (CCD) and response surface methodology (RSM), which allows estimating the main and interaction effects of the investigated factors and predicting the best performance conditions with a minimum run of experiments.

MATERIALS AND METHODS

Materials

Bovine INS (29 IU/mg) was purchased from SIGMA Chemical Co., Ltd., (St. Louis, MO, USA). SDS was supplied by Nacalai Tesque Inc., (Kyoto, Japan). PLGA 7520 (75:25, average M_W = 20,000) was obtained from the Wako Pure Chemical Ind., Ltd., (Osaka, Japan). PVA-403 was supplied by Kuraray Co., Ltd., (Osaka, Japan). All other reagents were of analytical grade.

Methods

Preparation of Nanoparticles

PLGA nanoparticles were prepared according to a modified SESD method (Kawashima, Yamamoto, Takeuchi, Fujioka, & Hino, 1999). Briefly, PLGA, INS, and SDS were codissolved completely in the mixture of acetone (1 mL) and slight 0.01 M hydrochloric acid. The resultant polymer—drug solution was poured into 10 mL PVA aqueous solution (1.0%, wt/vol). The emulsified system was stirred at 400 rpm for 1 h using a propeller-type agitator with three blades (Heidon 600G, Shinto Scientific Co., Ltd., Tokyo, Japan). The entire dispersed system was then subjected to centrifugation (40,000 × *g* for 15 min; Kubota 7780, Kubota Co., Ltd., Osaka, Japan). The nanoparticles were washed two times with distilled water to remove free drug and PVA before freeze-drying. The nanoparticle yield (NY) was calculated from Equation 1.

$$\mathrm{NY}\% = \frac{\text{mass of nanoparticle recovered}}{\text{mass of (PLGA + INS + SDS) used in formulation}} \times 100. \quad (1)$$

Determination of Drug Loading Efficiency

The freeze-dried nanoparticles were dissolved in acetonitrile, to which 0.01 M hydrochloric acid was added to preferentially precipitate the polymer. The drug content in the supernatant after centrifugation (40,000 × *g* for 15 min, Kubota 7780, Kubota Co., Ltd., Japan) was measured spectrophotometrically at 214 nm by means of a high-performance liquid chromatography (HPLC) method (LC-20AD pump, SPD-20A detector, CTO-910AS column oven, Crestpak C18S column, Shimadzu Co., Ltd., Kyoto, Japan). Drug loading efficiency was expressed both as drug recovery (DR, %) and drug content (DC, %, wt/wt); represented by Equations 2 and 3, respectively.

$$\mathrm{DR}\% = \frac{\text{mass of drug in nanoparticles}}{\text{mass of drug used in formulation}} \times 100, \quad (2)$$

$$\mathrm{DC}\% = \frac{\text{mass of drug in nanoparticles}}{\text{mass of NPs recovered}} \times 100. \quad (3)$$

Particle Size Analysis

The dried nanoparticle samples were suspended in distilled water and were sonicated before measurement. The obtained homogeneous suspensions were subjected to examination. The particle size distribution, expressed as mean diameter and polydispersity index, was determined by photon correlation spectroscopy (PCS) using Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK). Each measurement was performed in triplicate.

Experimental Design

A full factorial CCD was employed to identify the optimum level of the major variables previously selected by simple factor experiment. The experimental design with the coded (x_i) and actual (X_i) levels of independent variables are shown in Table 6. When developing the regression model, the test factors were coded in terms with the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i}, i = 1, 2, 3, \ldots, k,$$

where x_i is the dimensionless value of independent variable, X_i is the real value of independent variable, X_0 is the real value of the independent variable at the center point, and ΔX_i is the step change value.

Statistical Analysis

The analysis of experimental data was carried out using an RSM. A quadratic model expressed as following equation was used to correlate the response variable (Y) to the independent variables (X).

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j, \quad (4)$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients, for intercept, linear, quadratic, and interaction terms, respectively. The optimum levels of the selected variables were obtained by solving the regression equation and also by analyzing the response surface contour and surface plots. The statistical analyses were carried out using multiple regressions and analysis of variance (ANOVA) with the program Expert Design (Version 7.0, Stat-Ease. Inc., Minneapolis, MN, USA).

RESULTS AND DISCUSSION

Various formulation parameters were first investigated, respectively, to estimate their effects on particulate characters, for example, drug recovery, drug content, particle yield, and size. Only one factor was changed in each series of experiments, all other conditions were left constant as described in the section on Preparation of Nanoparticles.

Effect of the Agitation Speed

Table 1 shows the effect of the agitation speed on the characteristics of nanoparticles. As seen in Table 1, an increase of the agitation speed resulted in an increase of the particle yield and drug recovery. When the speed reached 600 rpm, the particle yield and drug recovery, up to 82.6 and 77.3%, respectively, could be achieved. The corresponding drug content was 4.07%. In contrast, with raise of agitation speed from 200 to 400 rpm, the reduction of both particle size and polydispersity were observed. Beyond 400 rpm, this reduction was not significant. The enhanced drug payload of nanoparticles with an increase in agitation speed indicates that velocity of polymer phase separation and deposition plays an important role in the particle formation and drug loading. In our previous studies, the hydrophobicity of INS was largely improved after complexed with SDS and could be codissolved with polymer in the organic solvent as molecular complex form. During diffusion process of polymer solution into aqueous PVA solution, the complex would be redistributed between organic phase and external aqueous phase. Intensive agitation can accelerate the diffusion speed of solvent into external aqueous phase, which is favorable for particle precipitation and drug entrapment.

Effect of PVA Concentration

As shown in Table 2, an increase in the PVA concentration of the external aqueous phase led to an increase in the particle yield and drug recovery. In addition, the enhanced PVA concentration resulted in a larger particle size and a lower polydispersity index. When the PVA concentration was beyond 2%, little changes in the particle yield and drug recovery were observed besides some reduction of particle polydispersity. The changes of particle size displayed in this study differ from those of Zambaux et al. (1998) who reported a decrease with increasing PVA concentration. The difference could be due to the different nanoparticle preparation method. In our study based on the spontaneous emulsification solvent diffusion method, the particle size reduction was dependent on the speed of solvent diffusion and polymer precipitation. The increased particle size observed at high PVA concentration could be attributed to the worse dispersibility of polymer solution into aqueous phase, which was caused by the increase of viscosity of PVA solution. In contrast, the results of increased drug recovery and decreased particle polydispersity at higher PVA concentration are concordant with those of Lamprecht et al. (1999). These can be explained by the stabilizing effect of PVA on nanoparticles. At higher concentration of PVA, more surfactant molecules

TABLE 1
Effect of Agitation Speed on Nanoparticle Properties

Agitation Speed (rpm)	Particle Yield[a] (%)	Drug Recovery[a] (%)	Drug Content[a] (%)	Size[a] (nm)	PDI[a]
200	78.3 ± 2.9	56.4 ± 2.3	3.13 ± 0.15	298 ± 20	0.161 ± 0.043
400	81.7 ± 3.3	63.3 ± 2.4	3.37 ± 0.13	271 ± 16	0.125 ± 0.032
600	82.6 ± 3.6	77.3 ± 1.6	4.07 ± 0.08	271 ± 11	0.117 ± 0.028

[a]Data are shown as mean ± standard deviation (*SD*) obtained from triplicate samples.

TABLE 2
Effect of PVA Concentration on Nanoparticle Properties

PVA (%)	Particle Yield[a] (%)	Drug Recovery[a] (%)	Drug Content[a] (%)	Size[a] (nm)	PDI[a]
1	81.7 ± 3.3	63.3 ± 2.4	3.37 ± 0.13	271 ± 16	0.125 ± 0.032
2	82.6 ± 2.5	69.0 ± 1.7	3.63 ± 0.09	274 ± 12	0.090 ± 0.015
3	82.0 ± 2.8	69.5 ± 2.0	3.72 ± 0.10	287 ± 10	0.083 ± 0.016

[a]Data are shown as mean ± standard deviation (*SD*) obtained from triplicate samples.

covered the interface between solvent and external aqueous phase, protecting quasi-emulsion droplets from coalescence.

Effect of Solvent Composition

It is clearly shown in Table 3 that the addition of ethanol to unitary acetone system led to a marked particle size reduction. Moreover, an increase in the particle yield and drug recovery with an increase in the volume ratio of ethanol to acetone was also observed. The binary solvent system with ethanol percentage of 1/3 provided an excellent particle yield of 82.2%, drug recovery of 75.3% (wt/wt), relative high drug content of 3.98% (wt/wt) and smaller particle size of 230 nm. Such a result can be explained by the well-known Marangoni effect (Quintanar-Guerrero et al., 1998). In the binary solvent system of acetone and ethanol, acetone is regarded as the "good solvent" for PLGA whereas ethanol the "poor solvent." Thus when the polymer solution was dispersed into the external aqueous phase and followed by an interfacial turbulence happening, the addition of ethanol instead of single acetone made polymer easy to diffuse out of the quasi-emulsion droplets and deposit because of poorer affinity of ethanol to polymer than that of acetone. Just as the explanation mentioned aforesaid, it was due to the addition of ethanol that accelerated the diffusion speed of polymer solution into external aqueous phase and facilitated the particle formation and drug loading efficiency.

Effect of Polymer Concentration

An increase in the concentration of polymer in the organic phase led to an increase of both the particles yield and size (shown in Table 4). In the same way, when the weight ratio of polymer to drug was increased by increasing the amount of PLGA in the organic phase, an increase in drug recovery was observed, but drug content was reduced correspondingly. The results of the study were concordant with those of Görner et al. (1999) who showed that an increase in polymer concentration led to an increase in the drug payload because of the enhancement of emulsion droplets viscosity and consequently detention of drugs distributing into external aqueous phase.

TABLE 3
Effect of Ethanol Amount on Nanoparticle Properties

Ethanol/Acetone (vol/vol)	Particle Yield[a] (%)	Drug Recovery[a] (%)	Drug Content[a] (%)	Size[a] (nm)	PDI[a]
0	81.7 ± 3.3	63.3 ± 2.4	3.37 ± 0.13	271 ± 16	0.125 ± 0.032
0.3	78.3 ± 2.9	67.5 ± 1.7	3.75 ± 0.09	237 ± 9	0.057 ± 0.014
0.5	82.2 ± 4.1	75.3 ± 2.0	3.98 ± 0.11	230 ± 10	0.114 ± 0.033

[a]Data are shown as mean ± standard deviation (*SD*) obtained from triplicate samples.

TABLE 4
Effect of PLGA Concentration on Nanoparticle Properties

PLGA Concentration (mg/mL)	Particle Yield[a] (%)	Drug Recovery[a] (%)	Drug Content[a] (%)	Size[a] (nm)	PDI[a]
10	66.9 ± 4.7	52.5 ± 2.5	6.03 ± 0.28	240 ± 13	0.129 ± 0.039
20	81.7 ± 3.3	63.3 ± 2.4	3.37 ± 0.13	271 ± 16	0.125 ± 0.032
30	83 ± 3.1	69.8 ± 1.7	2.55 ± 0.06	271 ± 18	0.127 ± 0.048

[a]Data are shown as mean ± standard deviation (*SD*) obtained from triplicate samples.

TABLE 5
Effect of PVA/Solvent Volume Ratio on Nanoparticle Properties

PVA/Solvent (vol/vol)	Particle Yield[a] (%)	Drug Recovery[a] (%)	Drug Content[a] (%)	Size[a] (nm)	PDI[a]
5	85.6 ± 5.1	70.9 ± 3.1	3.93 ± 0.17	281 ± 24	0.140 ± 0.039
10	81.7 ± 3.3	63.3 ± 2.4	3.37 ± 0.13	271 ± 16	0.125 ± 0.032
20	78.3 ± 3.6	75.9 ± 1.7	4.22 ± 0.09	244 ± 11	0.184 ± 0.041

[a]Data are shown as mean ± standard deviation (*SD*) obtained from triplicate samples.

Effect of Volume of External Aqueous Phase

Table 5 shows that the volume ratio of external aqueous phase to organic phase has a diverse influence on the characters of nanoparticles. When the volume ratio was increased from 5/1 to 10/1, low particle yield of 81.7% and drug recovery of 63.3% were observed, respectively. However when the volume ratio went on increasing, both the particle yield and drug recovery were raised to 78.3 and 75.9%, respectively. Meanwhile, the particle size was decreased from 271 to 244 nm. As mentioned above, the drug loading efficiency of PLGA nanoparticles was mainly dependent on the diffusion speed of solvent phase into external aqueous phase, consequently affecting the polymer precipitation rate. Larger volume of dispersion medium provided the enough space for more oil/water interface formation, thus promoting particle precipitation as quickly as possible.

Optimization Process

It was found in the mentioned results of simple factor experiments that there existed some interaction between the polymer amounts and external aqueous phase volume. Thus an RSM with five-level, two-factor central composite design was employed because it allows the estimation of the influence of these combined factors on nanoparticulate properties at the same time. To reduce manufacturing costs and polymer toxicity, more entrapped drugs and fewer polymers used were expected. So both the drug recovery and drug content were selected as optimization targets for the highest values.

According to the results of simple factor experiment, the solvent composition within basic formulation was replaced by binary solvents of ethanol and acetone (1:2) because they provided an excellent drug recovery relative to acetone. In addition, the weight ratio of polymer to drug (PLGA/INS) and volume ratio of external aqueous phase to solvent phase (PVA/solvent) were chosen as controlled variables. For each factor, the experimental range was selected on the basis of the results of preliminary experiments. The layout of the design is shown in Table 6.

The CCD experimental plan and results are presented in Table 7. Multiple regression analysis was employed to fit the second-order polynomial equation, expressing a mathematical relationship between the responses of drug recovery (Y), drug content (Y'), and their corresponding independent variables.

TABLE 6
Independent Variables and Their Levels Investigated in the Central Composite Design

	Coded Level				
Factor	$-\sqrt{2}$	–1	0	1	$\sqrt{2}$
X_1[a]	6	10	20	30	34
X_2[b]	5	8.7	17.5	26.3	30

[a]X_1 is the weight ratio of PLGA/INS.
[b]X_2 is the volume ratio of PVA/solvent.

$$Y = 82.30 + 13.13x_1 - 13.15x_2 - 10.36x_1^2 - 8.40x_2^2 - 0.46x_1x_2, \quad (5)$$

$$Y' = 4.35 - 1.55x_1 - 0.67x_2 + 0.27x_1^2 - 0.45x_2^2 + 0.22x_1x_2, \quad (6)$$

where x_1 and x_2 represent, respectively, coded values of PLGA/INS weight ratio and of PVA/solvent volume ratio.

The statistical significance of the models was verified by F test and the ANOVA for the fitted second-order polynomial equations summarized in Table 8. Both the calculated F values of the fitted models are greater than $F_{0.05}(5,4)$, indicating that the models were significant at the probability level of $\alpha = 0.05$. In addition, the value of the determination coefficients ($R^2_{DR} = 0.9978, R^2_{DC} = 0.9938$) indicate the goodness of fit of the models. The excellent values of the correlation coefficients (R), as high as 0.9911 for drug recovery and 0.9861 for drug content, suggested a good correlation of the experimental and predicted values (as shown in Figure 1).

Figure 2 depicts the response surface and contour plots showing the effects of PLGA/INS weight ratio and PVA/solvent volume ratio on drug recovery during nanoparticle preparation. It was evident that the drug recovery steadily increased with increasing the weight ratio of PLGA/INS up to 26.5/1 (wt/wt), but decreased slightly beyond this ratio at low volume ratio of PVA/solvent. The results of the study were concordant

TABLE 7
Experimental Design Matrix With Observed Values of the Objectives Variables

Run No.	x_1	x_2	Size (nm)	PDI	Nanoparticle Recovery (%)	Drug Recovery (%)	Drug Content (%)
1	1	–1	261	0.069	89.39	88.95	3.02
2	0	$-\sqrt{2}$	240	0.055	81.30	85.46	4.57
3	0	$\sqrt{2}$	257	0.029	81.30	45.74	2.45
4	$\sqrt{2}$	0	259	0.041	90.0	80.75	2.62
5	1	1	282	0.096	83.94	63.50	2.29
6	–1	1	214	0.087	69.23	38.85	4.76
7	0	0	257	0.063	82.17	82.11	4.34
8	$-\sqrt{2}$	0	173	0.092	54.44	42.62	7.28
9	0	0	252	0.057	80.29	82.50	4.36
10	–1	–1	211	0.037	75.38	62.46	6.37

TABLE 8
Analysis of Variance (ANOVA) for the Model Regression Representing Drug Recovery and Drug Content

Source	Sum of Squares	DF	Mean Square	F Value	$F_{0.05}$
Drug recovery (%)					
Model	3,343.54	5	668.71	359.52	6.26
Residue	7.45	4	1.86		
Lack of fit	7.37	3	2.46	30.75	251.7
Pure error	0.08	1	0.08		
Total	3,350.99	9			
Drug content (%)					
Model	25.1535	5	5.0307	16.99	6.26
Residue	0.1718	4	0.0430		
Lack of fit	0.1716	3	0.0572	190.67	251.7
Pure error	0.0003	1	0.0003		
Total	25.3253	9			

with those of Lamprecht et al. (1999) who showed that an increase in weight ratio of polymer to drug, that is, increase in polymer concentration, would led to an increase in the drug payload because of the enhancement of emulsion droplets viscosity and consequently detention of drug distributing into external aqueous phase. Whereas at high PVA/solvent volume ratio, the increase in the drug recovery was insignificant as the PLGA/INS weight ratio was increased. In the case of higher volume ratio of PVA/solvent, the drug recovery of PLGA nanoparticles was mainly dependent on the diffusion speed of solvent phase into external aqueous phase. Larger volume of dispersion medium provides the enough space for more oil/water interface formation, and which promotes the particle precipitation as quick as possible. On the other hand, when the polymer amount was not enough (e.g., 10/1 for the weight ratio of PLGA/INS), the drug recovery decreased as PVA/sol-

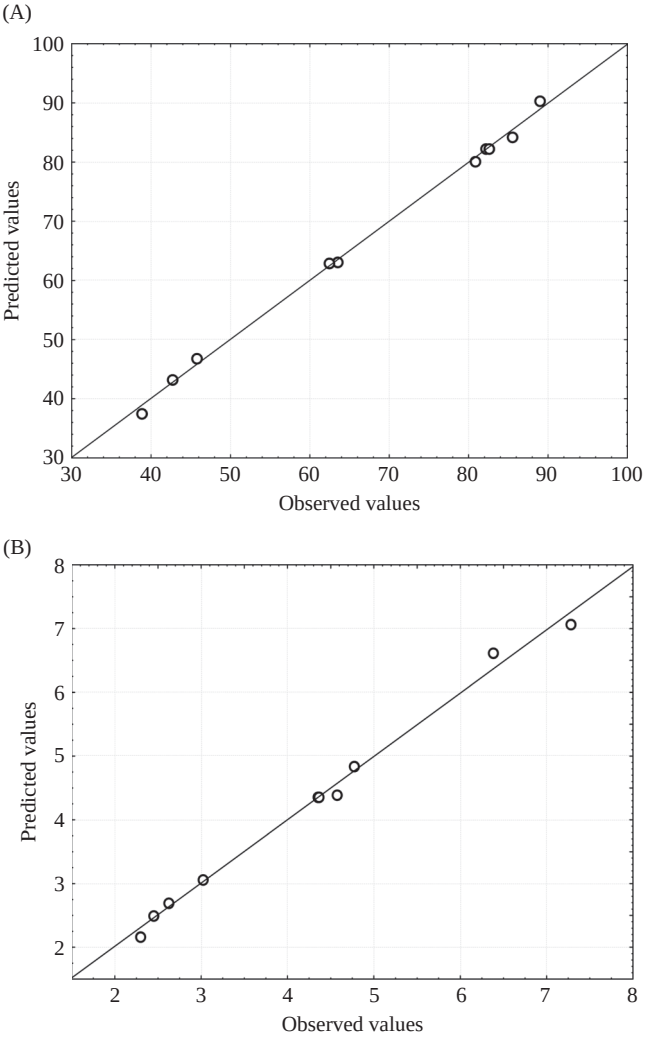

FIGURE 1. Predicted versus observed values plot for drug recovery (A) and drug content (B).

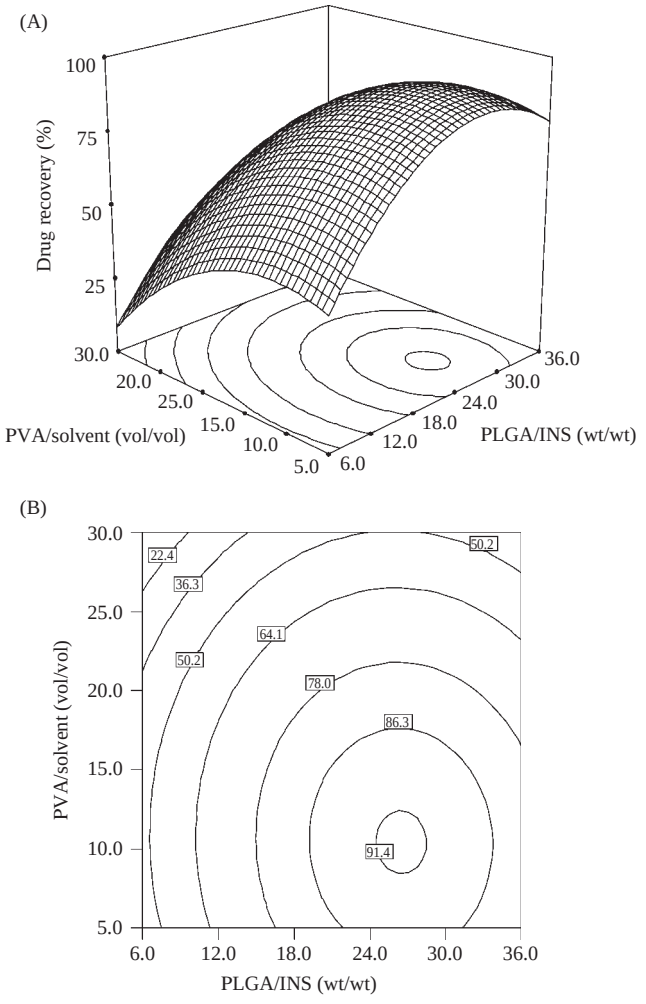

FIGURE 2. Response surface (A) and contour plot (B) of PLGA/INS weight ratio and PVA/solvent volume ratio on drug recovery.

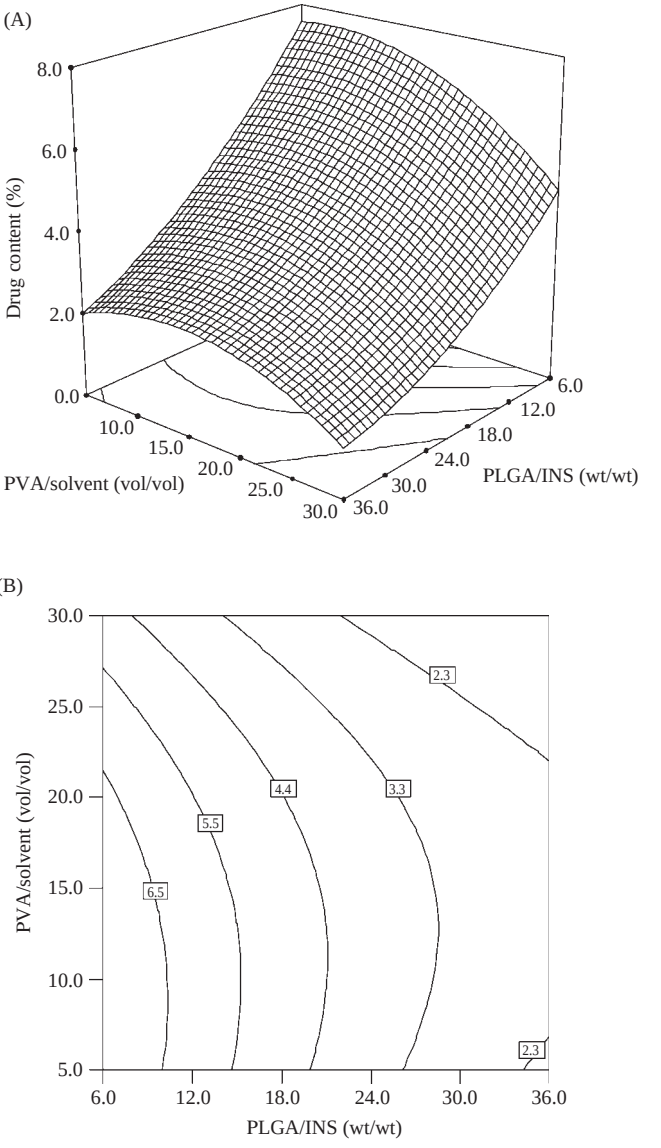

FIGURE 3. Response surface (A) and contour plot (B) of PLGA/INS weight ratio and PVA/solvent volume ratio on drug content.

vent volume ratio was increased in the range of 12/1 to 30/1 (vol/vol). The results indicated that under this ratio, the drug recovery was mainly determined by polymer concentration, and the effect of external aqueous volume was not significant.

The effects of PLGA/INS weight ratio and PVA/solvent volume ratio on drug content are shown in Figure 3. The response surface was surprisingly very different from that of drug recovery. It was found that at low weight ratio of PLGA/INS, an increase in the volume ratio of PVA/solvent led to a reduction of drug content. In contrast, when the PLGA/INS weight ratio was larger than 20/1, the changes of drug content was insignificant as increasing the PVA/solvent volume ratio. The results suggested that at low PLGA/INS weight ratio, PVA/solvent volume ratio played an important factor affecting the drug content. However, at high PLGA/INS weight ratio, the influence of PVA/solvent volume ratio on drug content was not significant.

The optimum point for nanoparticle preparation was obtained by graphic method and partial derivative resolution of Equations 5 and 6, at which the acceptable drug recovery (>80%) and highest drug content were expected to be achieved using the lowest amount of polymer. In addition, the optimum zone for nanoparticle preparation can be found readily from Figure 4 (as indicated in shadow area), which was obtained by overlapping Figures 2B and 3B. It was suggested that PLGA/INS weight ratio (18–20) and PVA/solvent volume ratio (10–15) should be employed for the practical preparation. At the recommended conditions, 85–90% of drug recovery and 4–5% of drug content were expected to be achieved.

The adequacy of the predicted models was examined by additional independent experiments at the suggested optimal preparation conditions. Triple experiments were carried out under optimum experimental conditions with PLGA/INS

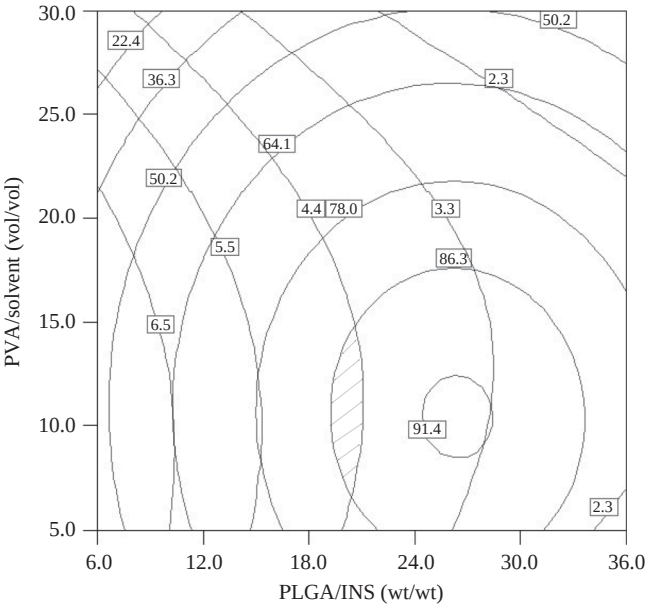

FIGURE 4. Overlapped contour plot for drug recovery and drug content.

weight ratio of 20/1 and PVA/solvent volume ratio of 10/1. Under the suggested optimum conditions, the actual average value of 89.6% of drug recovery and 4.57% of drug content were obtained. Thus, the optimized nanoparticle preparation for drug loading efficiency was successfully developed by CCD and RSM.

CONCLUSIONS

In this article, INS complexed with SDS-loaded PLGA nanoparticles was prepared by SESD method. A five-level, two-factor CCD and RSM was successfully applied to the determination of the optimum formulation conditions to improve the drug recovery and drug content during nanoparticle preparation. The research results indicated that RSM was a powerful and efficient tool for formulation optimization during the nanoparticle preparations.

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