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Application of Plackett-Burman Design and Box-Behnken Design to Achieve Process Optimization for Geniposide Submicron Emulsion

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The objective of this study was to prepare and characterize geniposide submicron emulsion (GP-SME) loaded the geniposide phytosomes (GP-PS) geniposide and optimize the process variables. The physicochemical properties of GP-PS obtained were investigated by means of differential scanning calorimetry. A screening experiment with Plackett-Burman design and response surface methodology with Box-Behnken design was used to optimize the process parameters of GP-SME. The optimum process conditions were finally obtained by using a desirability function. The differential scanning calorimetry studies of GP-PS demonstrated that GP and phospholipids in the GP-PS were combined by noncovalent bond, not forming a new compound. A Plackett-Burman design was initially employed and it was found that stirring velocity, homogenization pressure and homogenization cycles were the most important variables that affected the particles size, polydispersity index, and entrapment efficiency of GP-SME. Results showed that the optimum stirring velocity, homogenization pressure, and cycles were 16000 rpm, 50 Mpa, and 10 cycles, respectively. The mean diameter, polydispersity index, and entrapment efficiency of GP-SME were 258.2 nm, 0.243, 72.56%, respectively.

Keywords Box-Behnken design, geniposide submicron emulsion loaded geniposide phytosomes, Plackett-Burman design, process optimization

INTRODUCTION

Geniposide (GP, Figure 1) is one of the major iridoid glycosides in the fruit of *gardenia jasminoides* Ellis (Rubiaceae).[1] GP has been proved to distinctly treat hepatic and inflammatory conditions,[2–4] which has been widely used as a herbal medicine in the treatment of liver and gall bladder disorders.[5] The hepato-protective effect of GP has been reported, which may facilitate the conjugation and biliary extraction of naphthylisothiocyanate and/or its toxic metabolites.[6] However, the slight liposolubility of GP resulted in the poor permeation across the intestinal epithelial cells and minor the gastrointestinal (GI) tract absorption in rats.

Submiron emulsion (SME) is a potentially interesting drug delivery system.[7] It can enhance drug activity and increase drug bioavailability.[8] GP was a drug easily soluble in water and poorly soluble in oil.[9] The GP emulsion was not possibly prepared by applying the simple production process as used for emulsion loaded with diazepam, etomidate or propofol (i.e., dissolving the drug in the oil and preparing the emulsion), otherwise, GP would be very difficultly incorporated into oil phase of emulsion. Various approaches have been investigated to improve the solubility in the oil of biologically active constituents of synthetic and natural origin. Apart from other methods used for modifying the solubility in the oil, the complexes with phospholipids (PLs) has been demonstrated to show improvement in distribution in the oil phase of the active constituents.[9,10] Therefore, to develop the drugs as lipid complexes (also termed phytosomes, PS) might be a potential approach to improve the liposolubility of drug.[10,11] In this article, a new kind of geniposide submicron emulsion (GP-SME) loaded with PS was studied by means of novel complexes-homogenization method.

However, there are many process factors which affect the physicochemical properties of GP-SME, such as particle size, particle distribution and entrapment efficiency. Some of these are stirring velocity, emulsification time,

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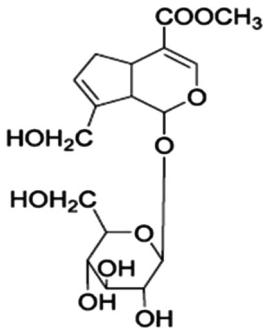

FIG. 1. The chemical structure of geniposide.

emulsification temperature, homogenization pressure, and homogenization circles. How to determine which actual valued of these variables result in a response value that is near to the optimum. The conventional approach requires that only one variable be changed at a time to determine its effect, which was not feasible to independently study each factor for its effect on particle size. Moreover, such experiments would not account for the interactions between the factors. An efficient way to screen for the important factors among a large number of variables was the use of the Plackett-Burman design. The Plackett-Burman design is a two-level multifactor design based on the rationale known as balanced incomplete blocks.[12] The key is forming various combinations of the factors with varying amounts. With the help of this design, up to N-1 factors can be studied in N assemblies. The results obtained with the Plackett-Burman design indicated limitations with respect to process factors.

After finding the critical factors, the next step was to optimize the actual values of these process factors. A number of optimization techniques could achieve this purpose, which have been described elsewhere.[13] The Box-Behnken design was originally introduced by Box and Wilson.[14] In this study, the Box-Behnken design was used. Since three process parameters limited the particles size, particle distribution, and entrapment efficiency of SME. The Box-Behnken design for three factors was used. This design proved to be crucial in achieving process optimization of GP-SME.

The main objectives of this study were:

1. to prepare GP-PS by a simple method and evaluate GP-PS for physicochemical characterization by means of differential scanning calorimetry (DSC);
2. to investigate systemically the preparation procedure optimization of the GP-SME by means of Plackett-Burman design; to evaluate process factors which affect the physicochemical properties of GP-SME, such as stirring velocity, homogenization pressure, homogenization circles, etc.;
3. to get the acceptable GP-SME, the Box-Behnken design approach was used for optimization of process variables on the mean diameter (MD), polydispersity (PDI), and entrapment efficiency of GP-SME.

MATERIALS AND METHODS

Materials

GP was purchased from Shuang-zi-ye Ltd. (Sichuan, China), purity 97%. Purified soybean oil (LCT) for parenteral use (Tieling BeiYa Pharmaceutical Co., Tieling, China); soybean lecithin and medium chain triglyceride (MCT; Lipoid GmbH, Ludwigshafen, Germany). All other chemicals and reagents were of analytical and used as received.

Preparation of the GP-SME

GP-SME was prepared as follows.

Preparation GP-PS

The phospholipids and GP at a ratio 1:3 were placed in a 100 ml round-bottom flask and dissolved in tetrahydrofuran (30 ml per mg GP). The tetrahydrofuran (60 ml) was used as reaction medium. The reaction temperature of the complexes was controlled using water bath (HH-6, Guohua Apparatus Center, China) and was maintained at the specified temperature for a suitable drug concentration. After then the tetrahydrofuran was evaporated off under vacuum at 40°C for 6 hours, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh.

Preparation GP-SME

The preparation of the GP-SME involved four steps, as follows.

(a) *Preparation of the lipid phase*: The GP-PS and α-tocopherol were dissolved in oil phase (MCT and soybean oil with a proper ratio) at predetermined temperature, in which some of the soybean lecithin had already been uniformly dissolved.
(b) *Preparation of the water phase*: The water and glycerol were mixed at predetermined temperature in a water bath.
(c) *Preparation of the coarse emulsion*: The water phase was stirred at a predetermined temperature by high-speed stirrer (FA25, Fluka, Germany). Stirring conditions were typically 10000–16000 rpm of 2–10 cycles at 60°C. The lipid phase was slowly injected into the lipid phase to obtain coarse emulsion.
(d) *Homogenization*: A fine emulsion was prepared by passing the coarse emulsion through a high-pressure homogenizer (AH1100D, ATS Engineering Inc., Canada). Homogenization conditions were typically 20–80 Mpa

of 2 to 10 cycles at 25°C.Afterward, the pH was adjusted to 6–7 with 0.1 N sodium hydroxide solutions.

Differential Scanning Calorimetry

The samples sealed in the aluminum crimp cell were heated at the speed of $20°C \cdot ml^{-1}$ from 0 to 200°C in the atmosphere of nitrogen (Q100, Perkin-Elmer, USA). Peak transition onset temperature was determined by means of an analyzer. The peak transition onset temperatures of phospholipids, pure geniposide, the mixture of phospholipids, and geniposide, and the GP-PS were compared.

Particle Size

The mean diameter (MD) and size distribution were performed using Zetasizer Narcos (Malvern, UK).The obtained distribution was a volume distribution, the width of the particle size distribution was expressed as PDI.

Entrapment Efficiency (EE%) of GP-SME

The content of GP in SME was determined as follows. Approximately 1 ml of SME was dissolved in 50 ml of methanol, and a 20 μl aliquot of the resulting solution was injected into a HPLC system. Kromasil 100-5C_{18} column (250 × 4.6 mm, 5 μm), was kept at 25°C. The mobile phase was a mixture of acetonitrile: H_2O (15: 85, v/v). The flow rate was 1.0 ml/min. Effluent was monitored at 238 nm.

The emulsions with GP were centrifuged at 18,000 × g for 30 minutes (4°C) in a Sigma ultracentrifuge (SIGMA3-18 K, German) in order to separate the incorporated drug and the nonincorporated drug. The GP was analyzed by HPLC for the unincorporated drug concentration to determine the entrapment percentage.

The concentrations of GP in the emulsion (n_1) and free drug in the aqueous (the unincorporated drug) (n_2) were assayed by HPLC after dilution with methanol. EE% could be achieved by the following equation: $EE\% = (n_1 - n_2)/n_1 \times 100\%$.

Plackett-Burman Design

The procedure for designing various assemblies in the Plackett-Burman design is given elsewhere.[15] Another important part of the Plackett-Burman screening design was the choice of dummies. A dummy is a component whose level does not change in the design. Factors known to have no effect can be chosen as dummies. Or any factor not chosen as a variable can be included as a dummy. The dummies are used to obtain the estimate of error and normally three dummy variables will provide an adequate estimate of error.[12] In the experiments performed, emulsification temperature (60°C), oil phase ratio (10%) and phospholipids types (soybean lecithin) served as dummies.

In this study, the lower levels and higher levels of the factors were chosen, respectively (Table 1). Table 2 shows the Plackett-Burman design used in this study. Noting that −1 and +1 in a given assembly designate the lower and higher levels of the corresponding factors, respectively, caution must be exercised while setting the level differential, as a small differential may not show any effect, and a large differentia for a sensitive factor can mask other factors.[16]

These experiments were designed and analyzed by Design-Expert 7.1.3. Experiments were performed in randomized order according to the run number that was arranged by the software. The response values were the mean of three duplicate measurements. Analysis of variance (ANOVA) was used to estimate the significance of main effects and interactions. Factors with a negligible effect on the response at a significance level of 95% were screened out. The remaining factors that affected the response were optimized further.

Experimental Design of Box-Behnken Design

Stirring velocity, homogenization pressure, and homogenization cycles were left to be optimized after the Plackett-Burman design. To reduce the number of trials

TABLE 1
Process factors and their two levels used in the Plackett-Burman design

Variable no.	Factors	Unit	Lower level (−1)	Higher level (1)
A	Stirring velocity	rpm	10000	16000
B (Fixed)	Emusification temperature	°C	60	60
C	Sitrring cycles	cycle	2	8
D	Homogenization pressure	Mpa	10	80
E	Homogenization cycles	cycle	2	10
F (Fixed)	Phospholipids types	type	Soybean lecithin	Soybean lecithin
G	Synperonic F68	%	0	2
H (Fixed)	Oil phase ratio	%	10	10
I	MCT ratio	%	0	2

TABLE 2
Plackett-Burman design and the corresponding response measurements

Standard no.	Run	Process factors											Response values		
		A	B[a]	C	D	E	F[a]	G	H[a]	I	J[a]	K[a]	MD (nm)	PDI	EE%
1	11	−1	−1	1	−1	1	1	−1	1	1	1	−1	196.5 ± 13.5	0.261 ± 0.067	66.16 ± 1.32
2	7	−1	1	1	1	−1	1	−1	1	−1	1	1	330.7 ± 21.3	0.556 ± 0.032	63.35 ± 1.43
3	8	−1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1	552.5 ± 16.8	0.386 ± 0.055	74.44 ± 0.76
4	3	−1	1	−1	1	1	1	1	1	1	−1	−1	161.8 ± 20.3	0.322 ± 0.013	52.81 ± 0.94
5	4	1	−1	1	1	1	−1	−1	−1	1	−1	1	271.6 ± 13.8	0.268 ± 0.042	56.87 ± 1.03
6	1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	212.4 ± 14.2	0.256 ± 0.032	55.34 ± 1.38
7	10	−1	1	1	−1	1	−1	1	−1	−1	−1	1	323.8 ± 13.6	0.271 ± 0.061	65.37 ± 1.64
8	9	−1	−1	−1	1	−1	−1	1	−1	1	1	1	330.5 ± 18.4	0.672 ± 0.015	67.74 ± 1.16
9	6	1	−1	1	1	−1	−1	1	1	−1	−1	−1	452.6 ± 8.6	0.494 ± 0.026	70.73 ± 1.35
10	2	1	−1	−1	−1	1	1	1	1	−1	1	1	407.8 ± 16.3	0.212 ± 0.034	72.27 ± 1.68
11	1	1	1	1	−1	−1	1	1	−1	1	1	−1	703.2 ± 14.3	0.323 ± 0.041	76.43 ± 1.53
12	2	1	1	−1	−1	−1	−1	−1	1	1	−1	1	654.5 ± 16.1	0.376 ± 0.053	75.24 ± 1.29

[a]Represents a dummy variable; −1 and +1 represent the low and high levels, respectively.

and attain the highest amount of information on product properties, the screening was planned applying a Box-Behnken design.

According to the principal of Box-Behnken design, the stirring velocity (X_1, rpm), homogenization pressure (X_2, Mpa), and homogenization cycles (X_3, cycle) defined as independent values were evaluated on three response values (Table 3), and the mean diameter (Y_1, nm), PDI (Y_2), and entrapment efficiency (Y_3) were defined as response values in the mathematical modeling, respectively. Each of the 20 formulations of a trial was produced three times in order to estimate the precision of stirring velocity, homogenization press and homogenization cycles (see Table 4).

ANOVA Analysis of Model

A statistical model incorporating interactive and polynomial terms was used to evaluate the response employing the formula

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

where Y was the dependent variable, b_0 was the arithmetic mean response of the 20 runs, and b_i was the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represented the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3, and X_1X_3) showed how the response changes when three factors were simultaneously changed. The polynomial terms (X_1^2, X_2^2, and X_3^2) were included to investigate nonlinearity.

In order to ensure a good model, test for significance of the regression model, test for significance on individual model coefficients and test for lack-of-fit need to be performed. ANOVA was commonly used to summarize the

TABLE 3
Factors and levels for the BBD

Variable no.	Factors	Unit	Lower level (−1)	Higher level (1)
X_1	Stirring velocity	rpm	10000	16000
X_2	Homogenization pressure	Mpa	10	80
X_3	Homogenization cycles	cycle	2	10
Fixed	Emulsification temperature	°C	60	
Fixed	Stirring cycles	cycle	2	
Fixed	Phospholipids types	type	Soybean lecithin	
Fixed	Oil phase ratio	%	10	
Fixed	MCT ratio	%	2	

TABLE 4
Experimental design table (columns 1–5) with experimentally determined values of different dependent variables (columns 6–8). Data expressed by mean ± S.D. ($n = 3$)

Standard no.	Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
15	1	13000	45	6	359.7 ± 13.1	0.279 ± 0.017	74.58 ± 2.51
16	2	13000	45	6	359.7 ± 13.1	0.279 ± 0.017	74.58 ± 2.51
7	3	10000	45	10	447.2 ± 11.3	0.189 ± 0.016	71.14 ± 1.31
8	4	16000	45	10	312.5 ± 13.1	0.254 ± 0.034	74.06 ± 1.42
9	5	13000	10	2	531.1 ± 22.6	0.563 ± 0.023	72.57 ± 1.33
12	6	13000	80	10	213.5 ± 12.3	0.357 ± 0.018	58.78 ± 2.06
14	7	13000	45	6	367.4 ± 23.7	0.279 ± 0.017	74.58 ± 0.81
5	8	10000	45	2	421.3 ± 12.3	0.652 ± 0.016	72.28 ± 1.39
3	9	10000	80	6	336.2 ± 15.1	0.483 ± 0.024	63.32 ± 1.42
11	10	13000	10	10	496.1 ± 23.8	0.218 ± 0.032	72.23 ± 1.28
10	11	13000	80	2	254.8 ± 24.1	0.808 ± 0.022	67.12 ± 1.26
1	12	10000	10	6	578.7 ± 16.1	0.313 ± 0.031	71.19 ± 0.19
6	13	16000	45	2	478.9 ± 14.6	0.542 ± 0.025	78.78 ± 1.34
13	14	13000	45	6	356.6 ± 23.2	0.279 ± 0.017	76.78 ± 2.29
2	15	16000	10	6	524.5 ± 14.2	0.266 ± 0.023	74.58 ± 3.51
17	16	13000	45	6	342.3 ± 22.3	0.279 ± 0.017	74.58 ± 1.51
4	17	16000	80	6	182.6 ± 31.2	0.456 ± 0.025	62.89 ± 1.16

tests performed. The fitted model is considered adequate if the model is significant and the lack-of-fit is not significant.

Optimization

The search for the experimental conditions that optimize the four responses simultaneously requires the use of the desirability function approach. In this work, the Derringer desirability function was used with Design-Expert 7.1.3. Each response can be assigned an importance relative to the other responses. Importance (r_i) varies from the least important (+) a value of 1, to the most important (+++++) a value of 5. If varying degrees of importance are assigned to the different responses, the objective function is given as follows:

$$D = (d_1 r_1 \times d_2 r_2 \times \cdots d_n r_n) 1 \Big/ \sum r_i = \left(\prod_{i=1}^{n} d_i^{r_i} \right)^{1 \Big/ \sum r_i},$$

where d_i is the partial desirability function of each response obtained from the transformation of the individual response of each experiment, n is the number of responses in the measure and r_i reflects the importance of each response. If all the importance values are the same, the simultaneous objective function reduces to the normal form for desirability. Taking into account all of the requirements for each response, we can choose the process variable conditions that maximize D. One can see that a high value of D is obtained only if all individual d_i are high.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry of GP-PS

Figure 2 shows the DSC curves of phospholipids, GP physical mixture and phytosomes. DSC of phytosomes showed the endothermal peaks of GP and phospholipids are disappeared and the phase transition temperature is lower than the phase transition temperature of phospholipids, it was considered that GP and phospholipids should have some interaction, such as the combination of hydrogen bonds or van der Waals force. After the combination of GP and the phospholipids molecule polarity parts, the carbon-hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts,

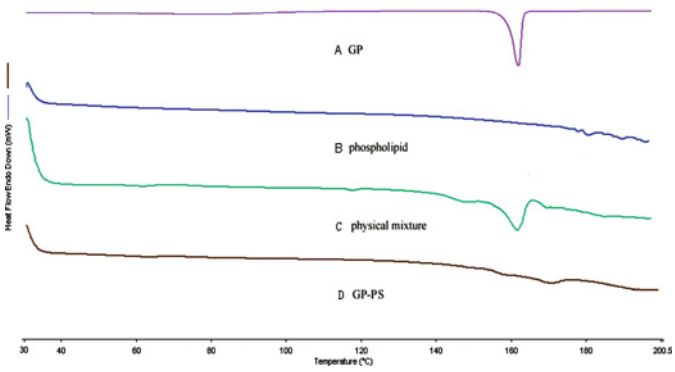

FIG. 2. DSC thermograms of GP (A), phospholipids (B), physical mixture (C), and GP-PS (D). (Figure available in color online.)

TABLE 5
Regression coefficients and their significance level

	Y1		Y2		Y3	
Source	Coefficient	*p* value	Coefficient	*p* value	Coefficient	*p* value
Constant	383.34	0.0035*	0.37	0.0035*	86.40	0.0058*
A	67.42	0.009*	−0.036	0.0288*	1.41	0.1451
B[a]	4.08	0.4684	0.0034	0.8946	−2.46	0.1521
C	−4.08	00812	0.0056	0.6867	0.086	0.9207
D	−88.87	0.00285*	0.052	0.0070*	−5.28	0.0014*
E	−12.22	0.0007*	−0.14	0.004*	−4.93	0.0018*
F[a]	−61.02	0.0430	−0.025	0.6424	0.42	0.6989
G	12.08	0.4916	0.005	0.2711	1.16	0.2165
H[a]	−37.75	0.1169	5.000E-003	0.1562	0.36	0.4532
I	2.92	0.8649	0.003	0.8550	0.52	0.5507
J[a]	23.94	0.2379	−0.08	0.3218	−0.59	0.7135
K[a]	24.90	0.2464	−0.22	0.0846	106.98	0.6275

[a]Represents a dummy variable.

TABLE 6
ANOVA for response surface quadratic model of Y_1

Source	Sum of squares	df	Mean square	*f* Value	*p* value	
Model	137.99	6	23.00	71.13	<0.0001	Significant
X_1	8.25	1	8.25	25.52	<0.0005	
X_2	111.71	1	111.71	345.51	<0.0001	
X_3	4.05	1	4.05	12.52	0.0054	
X_1 X_2	3.40	1	3.40	10.52	0.0088	
X_1 X_3	5.82	1	5.82	17.99	0.0017	
X_1^2	4.76	1	4.76	14.73	0.0033	
Residual	3.23	10	0.32			
Lack of fit	2.97	6	0.49	7.42	0.0564	Not significant
Pure error	0.27	4	0.067			
Cor total	141.22	16				

TABLE 7
ANOVA for response surface quadratic model of Y2

Source	Sum of squares	df	Mean square	*f* Value	*p* value	
Model	0.59	7	0.084	71.13	<0.0001	Significant
X_1	0.004	1	0.004	25.52	0.0129	
X_2	0.083	1	0.083	345.51	<0.0001	
X_3	0.35	1	0.35	12.52	<0.0001	
X_1 X_2	0.014	1	0.014	10.52	0.003	
X_2 X_3	0.0077	1	0.0077	17.99	0.002	
X_2^2	0.038	1	0.038	14.73	<0.0001	
X_3^2	0.082	1	0.082		0.0033	
Residual	0.0038	9	0.00042			
Lack of fit	0.0026	5	0.00051	1.72	0.310	Not significant
Pure error	0.0012	4	0.00029			
Cor total	0.59	16				

TABLE 8
ANOVA for response surface quadratic model of Y3

Source	Sum of squares	df	Mean square	*f* Value	*p* value	
Model	45.58	5	91.12	45.57	<0.0001	Significant
X_1	19.75	1	19.75	9.88	0.0094	
X_2	185.96	1	185.96	93.00	<0.0001	
X_3	26.14	1	26.14	13.07	0.0041	
X_2 X_3	16.00	1	16.00	8.00	0.0164	
X_2^2	207.74	1	207.74	103.89	<0.0001	
Residual	21.99	11	2.00			
Lack of fit	18.44	7	2.63	2.96	0.1554	Not significant
Pure error	3.56	4	0.89			
Cor total	477.57	16				

which made the sequence decrease between phospholipids aliphatic hydrocarbon chains, made the second endothermal peak of phospholipids disappear and depressed the phase transition temperature.

Analysis for the Plackett-Burman Design: Critical Factors for Response Values of GP-SME

Twelve experiments were carried out according to the conditions fixed by the experimental design shown in Table 2. ANOVA analysis of the results provided the weights of the experimental factors for all of the response. Test for significance of regression coefficients was shown as follows (Table 5).

From these results, it demonstrates that the particle size, PDI, entrapment efficiency behaved differently when the process variables were changed. The stirring velocity exhibited significant ($p < 0.01$) effect on these three response values. On the other hand, the homogenization pressure and stirring cycles showed a significantly ($p < 0.01$) effect on these three response values, the homogenization cycles level showed positive significant ($p < 0.01$) on the particle size. The stirring cycles, synperonic F68 and MCT Ratio showed no significant ($p > 0.05$) on the particle size, PDI, and entrapment efficiency. So the stirring velocity, homogenization pressure and homogenization cycles were selected for optimization in the next experimental design, where the levels of stirring cycles, synperonic F68 and MCT ratio were fixed, respectively.

Analysis for the Box-Behnken Design: Optimum Factors for Response Values of GP-SME

Table 4 shows the experimental conditions of the BBD along with the corresponding values observed for the three responses studied. Experimental data was fitted to the quadratic model by ANOVA. The ANOVA for the three responses is shown in Tables 6–8. The analysis showed that all of the four models were significant at 95% confidence and the lack of fit was not significant. It showed that the fitted three models were considered adequate. The calculated coefficients of all factors and the fitted models in terms of actual factors are shown in Table 9. Coefficient of determination (R^2) for the particle size, PDI, and entrapment efficiency was 0.963, 0.989, and 0.933, respectively. Surface response graphs, obtained using the fitted model, are presented in Figures 2–9.

The results corresponding to the three responses are discussed below.

The Particle Size. The three-dimensional plot of interaction stirring velocity × homogenization pressure (Figure 3) indicates that the particle size was achieved with the stirring velocity between 10000 rpm and 16000 rpm. It was increased as the power increased from 10 to 80 Mpa. Homogenization cycles significantly affected the particle size (Figure 4). As can be seen from Figure 3 and 4, the particle size decreased as the homogenization pressure and homogenization cycles increased from 580 to 180 nm.

TABLE 9
Results of regression analysis

Response	b_0	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}	R^2
Y_1	36.14	2.41E-3	7.43E-3	1.13	1.18E-7	—[a]	—[a]	8.78E-6	1.05E-4	—[a]	0.963
Y_2	1.32	3.68E-5	2.18E-3	0.21	—[a]	7.75E-5	8.73E-3	—[a]	4.89E-6	3.14E-4	0.989
Y_3	61.52	5.24E-4	0.46	0.19	—[a]	5.72E-3	—[a]	—[a]	—[a]	0.01	0.933

[a]—indicates the term was omitted in reduced model.

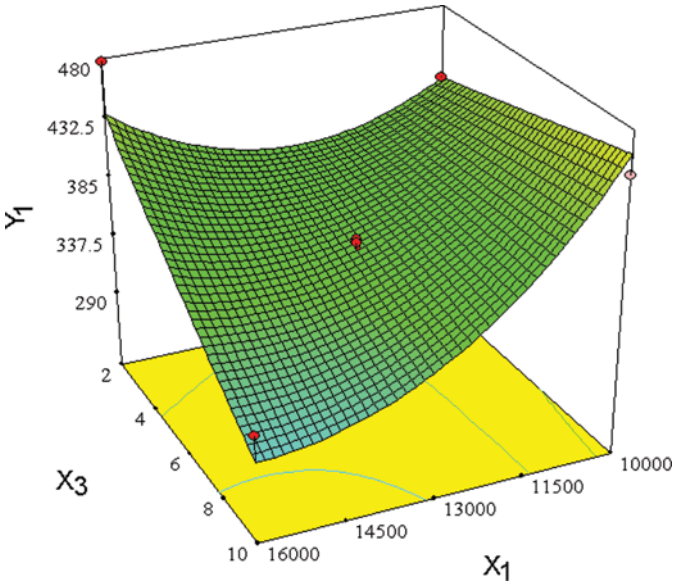

FIG. 3. Fitted surface for the particle size as a function of stirring velocity (X_1) and homogenization cycles (X_3) (homogenization pressure = 45 Mpa). (Figure available in color online.)

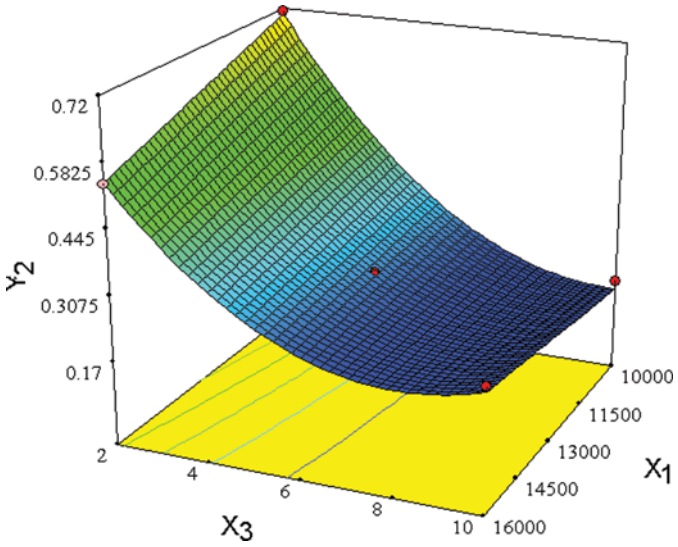

FIG. 5. Fitted surface for the PDI as a function of stirring velocity (X_1) and homogenization cycles (X_3) (homogenization pressure = 45 Mpa). (Figure available in color online.)

Polydispersity Index. Figure 5 indicates homogenization cycles affected the polydispersity index more significantly than stirring velocity. The optimum cycles were located between 6 and 10 cycles. Homogenization pressure had significant effect on the polydispersity and the minimize polydispersity index was achieved at 10 cycles and 45 Mpa (Figure 6). The optimum homogenization pressure is located between 30 and 50 Mpa.

Entrapment Efficiency. Figure 7 indicates homogenization pressure homogenization cycles significantly affected the entrapment efficiency than stirring velocity. The optimum cycles were located between 2 and 8 cycles. The optimum homogenization pressure is located between 30 and 60 Mpa.

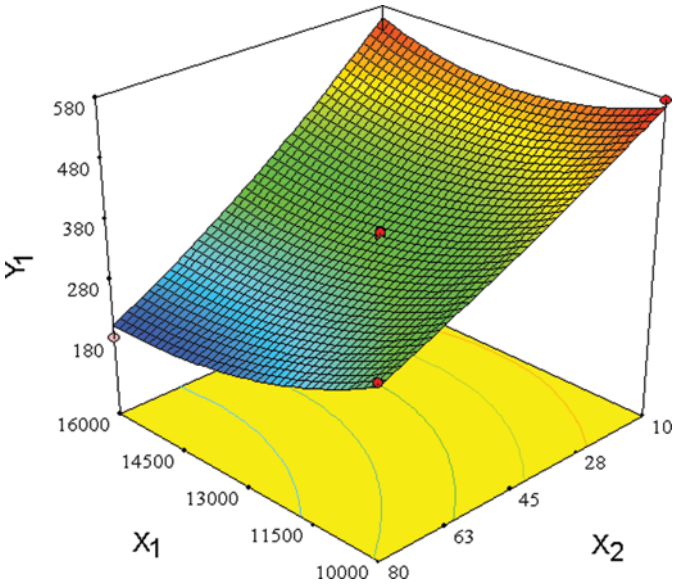

FIG. 4. Fitted surface for the particle size as a function of stirring velocity (X_1) and homogenization pressure (X_2) (homogenization cycles = 6). (Figure available in color online.)

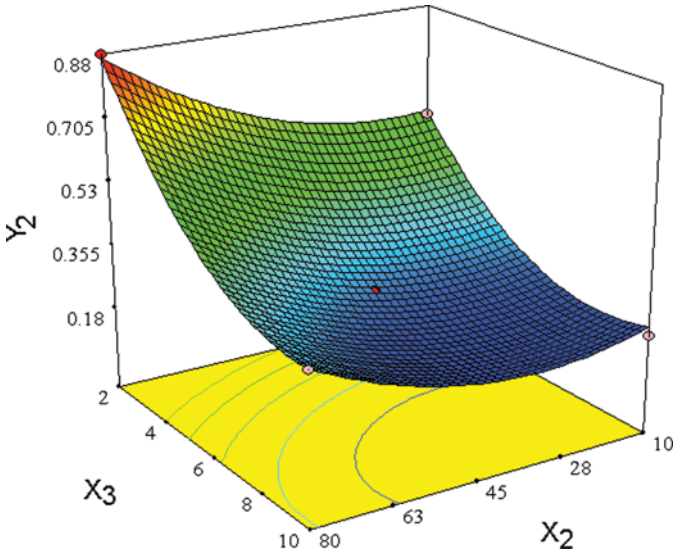

FIG. 6. Fitted surface for the PDI as a function of homogenization pressure (X_2) and homogenization cycles (X_3) (stirring velocity = 13000 rpm). (Figure available in color online.)

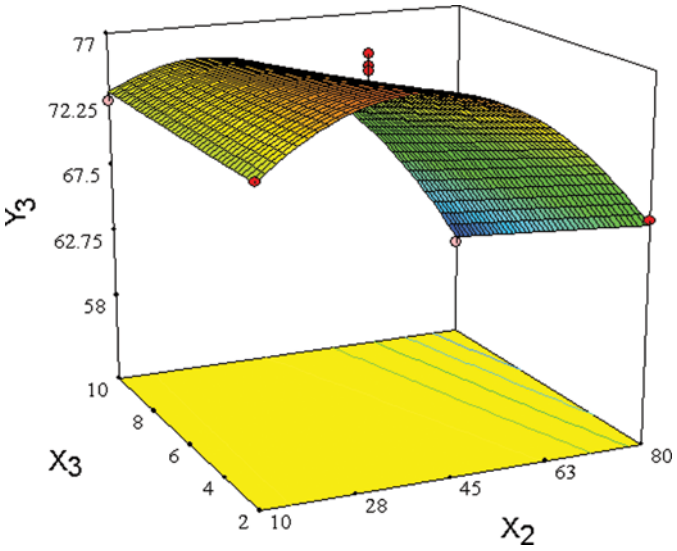

FIG. 7. Fitted surface for the entrapment efficiency (%) as a function of homogenization pressure (X_2) and homogenization cycles (X_3) (stirring velocity = 13000 rpm). (Figure available in color online.)

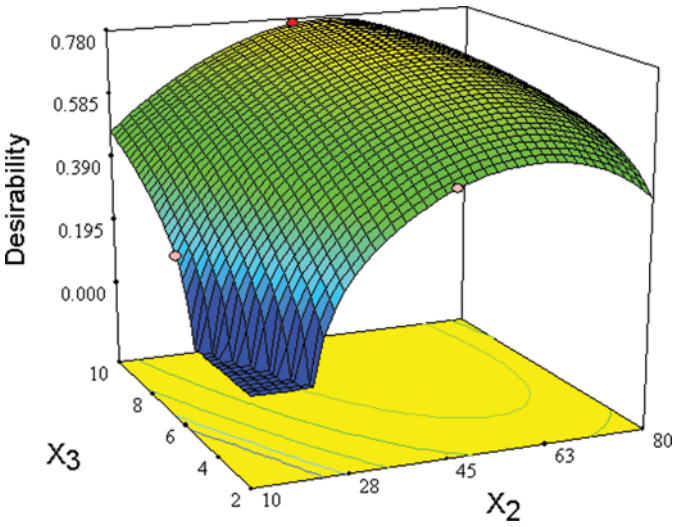

FIG. 8. Fitted surface for the desirability as a function of homogenization pressure (X_2) and homogenization cycles (X_3) (stirring velocity = 16000 rpm). (Figure available in color online.)

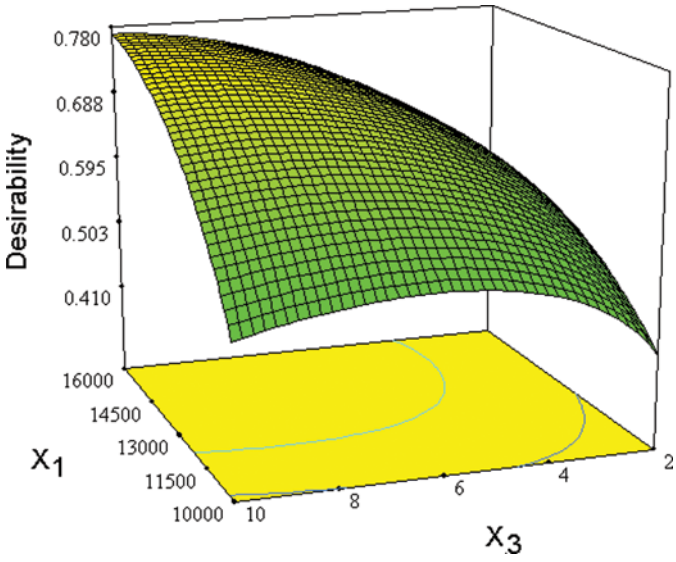

FIG. 9. Fitted surface for the desirability as a function of stirring velocity (X_1) and homogenization cycles (X_3) (homogenization pressure = 50 Mpa). (Figure available in color online.)

Desirability Optimization

The aim of optimization was to find the conditions that give the maximum entrapment efficiency, minimum particles size, and PDI. Desirability function approach was used to achieve this goal.

Constraints for this optimization which were set in the software can be seen from Table 10. Surface response graphs are presented in Figures 8–10. The desirability was 0.77. The software predicted the optimum stirring velocity, homogenization pressure and cycles was 16000 rpm, 50 Mpa, and 10 cycles, respectively. The optimum process parameter of GP-SME (100 ml) were: (a) The GP-PS 1.5% and α-tocopherol 0.02% were dissolved in 10% oil phase (MCT and soybean oil with a proper ratio 1:4) at 60°C, in which some of the soybean lecithin had already been uniformly dissolved to obtain the lipid phase; (b) the water and glycerol 2.5% were mixed at 60°C in a water bath to obtain the water phase; (c) the water phase was stirred at stirring velocity 16000 rpm for 2 cycles at

TABLE 10
Constraints of factors and responses for optimization

Name	Goal	Lower limit	Upper limit	Importance
Stirring velocity (rpm)	Is in a range	10000	16000	3
Homogenization pressure (Mpa)	Is in a range	200	600	3
Homogenization cycles	Is in a range	2	10	3
Mean diameter (nm)	Minimize	182	578	5
Polydispersity index	Minimize	0.189	0.878	3
Entrapment efficiency (%)	Maximize	58.78	78.78	4

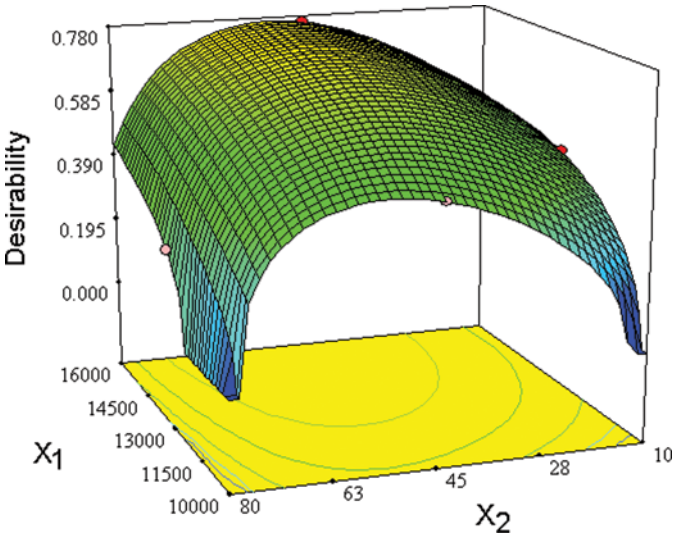

FIG. 10. Fitted surface for the desirability as a function of stirring velocity (X_1) and homogenization pressure (X_2) (homogenization cycles = 10). (Figure available in color online.)

60°C by high-speed stirrer, and lipid phase was injected into the lipid phase to obtain coarse emulsion; (d) a fine emulsion was prepared by passing the coarse emulsion through a high-pressure homogenizer. Homogenization conditions were typically 50 Mpa and 10 cycles. Afterwards, the pH was adjusted to 6–7 with 0.1 N sodium hydroxide solutions. The software predicted the particle size, PDI, and entrapment efficiency of GP-SME was 267.2 nm, 0.254, 73.48%, respectively.

Verification

In order to evaluate the optimization capability of the models generated according to the results of the Box-Behnken design, the GP-SME were prepared using the optimal process variable settings that X_1, X_2, and X_3 were equal to 16000 rpm, 50 Mpa, and 10 cycles, respectively. The particle size, PDI, and entrapment efficiency of GP-SME obtained with predicted models were shown in Table 11. The results showed good agreement on preparation properties with theoretical predictions.

TABLE 11
Model-predicted and observed values of particles size, PDI and entrapment efficiency of GP-SME prepared according to the optimal experimental conditions ($X_1 = 16000$, $X_2 = 50$, $X_3 = 10$) ($n = 3$)

Dependent variable	Predicted	Observed	Bias*/ %
Mean diameter (Y_1)	267.2 nm	258.4 nm	3.41
Polydispersity index (Y_2)	0.254	0.243	4.52
Entrapment efficiency (Y_3,)	73.48%	72.56%	1.26

*Bias was calculated according to equation: Bias/% = (predicted value-observed value)/predicted value × 100%.

CONCLUSION

In this study, GP-SME-PS was successfully prepared by the novel complex-homogenization technology, which was a technology basically combining drug phospholipids complex with the homogenization emulsification technology. The statistical analysis of plackett-burman design, Box-Behnken design and desirability function enabled us to screen several significant process factors and find out the optimum process conditions for GP-SME prescription in a quick and economical way. The optimum stirring velocity, homogenization pressure and cycles was 16000 rpm, 50 Mpa, and 10 cycles, respectively. The entrapment efficiency of emulsion was very markedly increased, and the particle size and the distribution range of particle were completely acceptable. The experimental results were in good agreement with the predicted values.

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