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Optimization of Polylactic-Co-Glycolic Acid Nanoparticles Containing Itraconazole Using 2^3 Factorial Design

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ABSTRACT

This study investigated the utility of a 2^3 factorial design and optimization process for polylactic-co-glycolic acid (PLGA) nanoparticles containing itraconazole with 5 replicates at the center of the design. Nanoparticles were prepared by solvent displacement technique with PLGA X_1 (10, 100 mg/mL), benzyl benzoate X_2 (5, 20 µg/mL), and itraconazole X_3 (200, 1800 µg/mL). Particle size (Y_1), the amount of itraconazole entrapped in the nanoparticles (Y_2), and encapsulation efficiency (Y_3) were used as responses. A validated statistical model having significant coefficient figures ($P < .001$) for the particle size (Y_1), the amount of itraconazole entrapped in the nanoparticles (Y_2), and encapsulation efficiency (Y_3) as function of the PLGA (X_1), benzyl benzoate (X_2), and itraconazole (X_3) were developed: $Y_1 = 373.75 + 66.54X_1 + 52.09X_2 + 105.06X_3 - 4.73X_1X_2 + 46.30X_1X_3$; $Y_2 = 472.93 + 73.45X_1 + 169.06X_2 + 333.03X_3 + 62.40X_1X_3 + 141.49X_2X_3$; $Y_3 = 57.36 + 6.53X_1 + 15.52X_2 - 12.59X_3 + 1.01X_1X_3 + 1.73X_2X_3$. X_1, X_2, and X_3 had a significant effect ($P < .001$) on Y_1, Y_2, and Y_3. The particle size, the amount of itraconazole entrapped in the nanoparticles, and the encapsulation efficiency of the 4 formulas were in agreement with the predictions obtained from the models ($P < .05$). An overlay plot for the 3 responses shows the boundary in which Y_1 shows the boundary in which a number of combinations of concentration of PLGA, benzyl benzoate, and itraconazole will result in a satisfactory process. Using the desirability approach with the same constraints, the solution composition having the highest overall desirability (D = 0.769) was 10 mg/mL of PLGA, 16.94 µg/mL of benzyl benzoate, and 1001.01 µg/mL of itraconazole. This approach allowed the selection of the optimum formulation ingredients for PLGA nanoparticles containing itraconazole of 500 µg/mL.

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INTRODUCTION

Nanoparticulate delivery systems, such as those based on poly(lactic-co-glycolic acid) (PLGA) polymers, have been studied extensively for many years. PLGA polymers have the advantage of being well characterized and already commercially used for microparticulate drug delivery systems.[1] PLGA polymers are biocompatible, biodegradable, and bioresorbable.[2] PLGA nanoparticles can be formed by interfacial deposition following solvent displacement technique.[3] Several compounds such as indomethacin[4] and muramyl dipeptide MDB-B30[5] have been incorporated in this technique.

The interfacial deposition following solvent displacement technique[3] differs from nanosphere preparation method by introduction of an oily component into the polymer-organic solution. First, the polymer, phospholipid mixture, and benzyl benzoate are dissolved in acetone. Then, the organic solution is poured into an aqueous phase containing a surfactant (eg, poloxamer) under moderate stirring. Acetone diffuses immediately into the aqueous phase, inducing the deposition and precipitation of the polymer around the oily droplets.[6]

Itraconazole is weakly basic (pKa = 3.7) and highly hydrophobic (octanol/water partition coefficient at pH = 8.1, log P = 5.66).[7] It is insoluble in water, so the first route of administration is oral. Large interindividual as well as intraindividual variations of its oral bioavailability have been reported.[8] The development of an injectable dosage form would be extremely useful to overcome this drawback and to offer a dosage form that would allow the necessary therapeutic dose to be achieved. This implies a preparation containing not less than 0.500 mg/mL of itraconazole.[7] Chasteigner et al[7] studied the association of itraconazole with colloidal drug carriers, except PLGA.

Table 1. Level of the Investigated Variables*

	Independent Variables		
Code Unit	**PLGA (X_1) (mg/mL)**	**Benzyl Benzoate (X_2) (µg/mL)**	**Itraconazole (X_3) (µg/mL)**
–1	10	5	200
0	55	12.5	1000
1	100	20	1800

*PLGA indicates polylactic-co-glycolic acid.

The application of an optimization technique consisting of statistical design to pharmaceutical formulation development would provide an efficient and economical method to acquire the necessary information to understand the relationship between controllable (independent) variables and performance or quality (dependent) variables or responses.[9] In addition, the optimization process provides a method to develop an empirical model equation to characterize the response as a function of the different independent variables.

The technique of optimization is well reported in the literature for the development of tablet formulations,[10-12] microcapsules,[13,14] fluid bed spray coating,[15] hydrocolloid dressing,[16] and suspension.[17] The purpose of this study was to investigate the utility of a 2^3 factorial design and optimization process to develop and improve formulation of PLGA nanoparticles containing itraconazole. The optimization process was used to generate a model equation that provides a means of evaluating changes in response due to changes in the independent variable levels.

MATERIALS AND METHODS

Materials

Poly(lactic-co-glycolic acid) with a monomer ratio of 50:50 was obtained from Boehringer Ingelheim (Ingelheim, Germany). Itraconazole was purchased from Tricon Enterprises (batch no. IT008090) (Mumbai, India). Benzyl benzoate was obtained from Sigma (St Louis, MO). Poloxamer F68 was purchased from BASF (Montreal, Canada). Acetone (high performance liquid chromatography [HPLC] grade) was obtained from Mallinckrodt Baker (Paris, France). Acetonitrile and methanol (both HPLC grade) were purchased from BDH (Dorset, Poole Dorset, UK).

Preparation of Nanoparticles

PLGA nanoparticles containing itraconazole (PGNI) were prepared according to the method already described.[3] Briefly, the accurate content of PLGA, benzyl benzoate, and itraconazole were dissolved in 10 mL acetone. The organic phase was added at a constant flow rate (0.3 mL/min) under mechanical stirring at 750 rpm to 25 mL of an aqueous phase containing 0.25% of nonionic surfactant (pluronic F68). The resulting mixture turned milky instantaneously because of the formation of nanoparticles by interfacial polymer deposition. Acetone was removed by evaporation under vacuum, and the final volume was adjusted to 10 mL.

Experimental Design

A 2^3 full factorial design with 2 replicates was used in this study. The 3 independent variables investigated were the concentrations of PLGA (X_1), benzyl benzoate (X_2), and itraconazole (X_3). The level of the 3 independent variables is shown in Table 1, and the design is presented in Table 2. Five replicates at the center of the design were investigated to allow for an independent estimation of the experimental error and to check the linearity of the factor effects.[18]

The effect of the previously mentioned variables was investigated on the following responses: the particle size and the encapsulation efficiency.

Determination of Particle Size

The particle size of PGNI was determined by photon correlation spectroscopy (PCS) using an Autosizer IIC (Malvern 4700 laser spectrometer, Malvern Instruments, Malvern, UK) with a He-Ne laser. The signals were processed by Malvern 7032-N multibit correlator (Malvern Instruments). For particle size analysis, PGNI were dispersed in ultrapure water filtrated through a 0.22-µm nylon membrane for minimizing dust. Measurements were carried out at 30°C using a 632.8 nm laser at an angle of 90°.

Determination of Encapsulation Efficiency

The amount of itraconazole entrapped in the nanoparticles (ITRAe) was assayed by HPLC in the suspension after filtration through a sintered glass filter (porosity 4, mesh size 5-15 µm). This filtration step retains the unassociated itraconazole that precipitates in the aqueous phase just after preparation because of its insolubility. The method was modified from the method described by Law et al[19] using

Table 2. Design Points for the Investigated Variables

		Level of Variables		
Formula	**Random No.**	X_1 **(mg/mL)**	X_2 **(µg/mL)**	X_3 **(µg/mL)**
1a	1	10	5	200
4a	2	10	20	1800
4b	3	10	20	1800
6a	4	100	5	1800
3a	5	10	20	200
5a	6	100	5	200
2a	7	10	5	1800
3b	8	10	20	200
1b	9	10	5	200
2b	10	10	5	1800
8a	11	100	20	1800
8b	12	100	20	1800
7a	13	100	20	200
7b	14	100	20	200
6b	15	100	5	1800
9a	16	55	12.5	1000
9b	17	55	12.5	1000
9c	18	55	12.5	1000
9d	19	55	12.5	1000
9e	20	55	12.5	1000
5b	21	100	5	200

reverse phase (RP)-HPLC equipped with UV detector (263 nm). The chromatographic analysis was performed on Waters systems (Waters Corp, Milford, MA), using RP Lichrospher 60 RP-select B, 5 µm; 4.6 × 120 mm. After appropriate dilution of the sample in methanol, 0.5 mL of internal standard solution was added. The samples were eluted with acetonitrile:water:diethylamine (50:50:0.05), at a constant flow rate of 1.1 mL/min. Ketoconazole was used as an internal standard. The retention time of ketoconazole and itraconazole were 3.1 and 6 minutes, respectively. Encapsulation efficiency was expressed as the percentage of drug versus the amount of drug in organic phase.

Response Surface Analysis

Response surface methodology is a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response.[20]

A linear regression model equation was employed for fitting the response surface in the following form:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{123}X_1X_2X_3 \quad (1)$$

where Y is the measured response of the particle size (nm), the amount of itraconazole entrapped in the nanoparticles (µg/mL) (ITRAe), and encapsulation efficiency (ITRAe [%]), B_0, intercept term, B_i and B_{ij} are, respectively, the measures of the effects of variables X_i and X_iX_j. The variable X_iX_j represents the first order interactions between X_i and X_j ($i < j$).

Data were analyzed using analysis of variance (ANOVA), and regression coefficients were calculated.

The error mean square was calculated according to the following equation:

$$MS_E = \frac{SS_E}{n_c - 1} \quad (2)$$

$$MS_E = \frac{\sum\{Y_i - \overline{Y}\}^2}{\frac{CenterPoints}{n_c - 1}} \quad (3)$$

where Y_i is the response of the ith replicates at the center of the design, $\overline{Y}$ is the mean, and n_c is the number of the replicates.

The curvature sum of squares was calculated according to the following equation:

$$SS_{curvature} = \frac{n_f n_c \{\overline{Y}_f - \overline{Y}_c\}^2}{n_f n_c} \quad (4)$$

Table 3. Observed Responses and Predicted Values*

Run No.	Size (nm)				Encapsulation Efficiency ITRAe (μg/mL)			
	Observed Response	Predicted Value	*P* value	Residual Value	Observed Response	Predicted Value	*P* value	Residual value
1	193.90	191.64	<.05	2.26	98.28	101.29	<.05	–3.01
2	425.60	422.79	<.05	2.81	976.30	980.66	<.05	–4.36
3	420.60	422.79	<.05	–2.19	983.34	980.66	<.05	2.68
4	539.60	544.29	<.05	–4.69	628.87	631.26	<.05	–2.39
5	304.30	305.26	<.05	–0.96	159.97	156.43	<.05	3.54
6	249.60	241.56	<.05	8.04	123.97	123.38	<.05	0.59
7	306.90	309.16	<.05	–2.26	355.60	359.56	<.05	–3.96
8	305.60	305.26	<.05	0.34	154.22	156.43	<.05	–2.21
9	190.00	191.64	<.05	–1.64	102.96	101.29	<.05	1.67
10	310.80	309.16	<.05	1.64	365.20	359.56	<.05	5.64
11	639.50	639.01	<.05	0.49	1256.80	1252.36	<.05	4.44
12	643.90	639.01	<.05	4.89	1249.60	1252.36	<.05	–2.76
13	337.60	336.29	<.05	1.31	176.40	178.52	<.05	–2.12
14	329.60	336.29	<.05	–6.69	179.30	178.52	<.05	–2.12
15	543.60	544.29	<.05	–0.69	631.97	631.26	<.05	0.71
16	461.20	462.92	<.05	–1.72	701.30	705.00	<.05	–3.70
17	458.30	462.92	<.05	–4.62	712.60	705.00	<.05	7.60
18	460.50	462.92	<.05	–2.42	706.20	705.00	<.05	1.20
19	467.70	462.92	<.05	4.78	699.30	705.00	<.05	–5.70
20	466.90	462.92	<.05	3.98	705.60	705.00	<.05	0.60
21	238.90	241.56	<.05	–2.66	124.12	123.38	<.05	0.74

*ITRAe indicates the amount of itraconazole entrapped in the nanoparticles. Predicted values predicted through the model Equations (9) and (10).

where n_f and n_c are the number of the factorial experiments and the experiments at the center, respectively; $\overline{Y}f$ and $\overline{Y}c$ are the means of the corresponding observations.

$SS_{curvature}$ with 1 degree of freedom was compared with the error mean square to check any curvature in the response surface as a function of different factors effect.

A relatively straightforward approach to optimizing several factor responses that works well when there are only a few process variables is to overlay the contour plots for each response. Another useful approach to optimization of multiple responses is to use the simultaneous optimization technique popularized by Derringer and Suich.[18] Their procedure makes use of desirability functions. The general approach is to first convert each response y_i into an individual desirability function d_i that varies over the range $0 \le d_i \le 1$, where if the response y_i is at its goal or target, then $d_i = 1$, and if the response is outside an acceptable region, $d_i = 0$. Then, the design variables are chosen to maximize the overall desirability as follows:

$$D = (d_1 * d_2 * \dots \dots * d_m)^{1/m} \quad (5)$$

where there are m responses.[18]

RESULTS AND DISCUSSION

The effects of the different combinations of concentration of PLGA (X_1), benzyl benzoate (X_2), and itraconazole (X_3), illustrated in Table 2, on the particle size (nm) and the amount of itraconazole entrapped in the nanoparticles (μg/mL) (ITRAe) of PGNI are shown in Table 3 as observed response.

Analysis of data was carried out using ANOVA, and the individual parameter was evaluated with the F test. Results of the response surface analysis are summarized in Table 4. The application of response surface methodology[20] yielded the following regression equations, which suggest an empirical relationship between the values of responses and the independent variables in coded unit:

$$\begin{aligned} Y_1 = {} & 373.75\ (P < .0001) + 66.54\ (P < .0001)\ X_1 \\ & + 52.09\ (P < .0001)\ X_2 + 105.06\ (P < .0001)\ X_3 \\ & - 4.73\ (P = .0006)\ X_1X_2 + 46.30\ (P < .0001)\ X_1X_3 \\ & + 1.50\ (P = .1716)\ X_2X_3 + 1.19\ (P = .2721)\ X_1X_2X_3 \end{aligned} \quad (6)$$

$$\begin{aligned} Y_2 = {} & 472.93\ (P < .0001) + 73.45\ (P < .0001)\ X_1 \\ & + 169.06\ (P < .0001)\ X_2 + 333.03\ (P < .0001)\ X_3 \\ & + 0.086\ (P = .9397)\ X_1X_2 + 62.40\ (P < .0001)\ X_1X_3 \\ & + 141.49\ (P < .0001)\ X_2X_3 + 0.75\ (P = .5121)\ X_1X_2X_3 \end{aligned} \quad (7)$$

Table 4. Response Surface Data*

Parameters	Size (nm)		Encapsulation Efficiency			
			ITRAe (µg/mL)		ITRAe (%)	
		P Value		*P* Value		*P* Value
Model	F = 3108.78	<.0001	F = 19 369.93	<.0001	F = 1314.44	<.0001
B_0	373.75	<.0001	472.93	<.0001	57.36	<.0001
B_1	66.54	<.0001	73.45	<.0001	6.53	<.0001
B_2	52.09	<.0001	169.06	<.0001	15.52	<.0001
B_3	105.06	<.0001	333.03	<.0001	–12.59	<.0001
B_{12}	–4.73	.0006	0.086	.9397	–0.14	.5261
B_{13}	46.30	<.0001	62.40	<.0001	1.01	.0006
B_{23}	1.50	.1716	141.49	<.0001	1.73	<.0001
B_{123}	1.19	.2721	0.75	.5121	0.19	.4051
Error MS	17.03		19.92		0.78	
Curve MS	30 290.62	<.0001	12 052.00	<.0001	657.38	<.0001
$F_{curvature}$	1778.94		10 300.13		847.52	
R^2	0.9994		0.9999		0.9987	
Adjusted R^2	0.9990		0.9999		0.9979	
CV	1.04		0.84		1.46	

*CV indicates coefficient of variation; F, F Value; ITRAe, the amount of itraconazole entrapped in the nanoparticles; MS, mean square; and R^2, determination coefficient.

$$Y_3 = 57.36\ (P < .0001) + 6.53\ (P < .0001)\ X_1 + 15.52\ (P < .0001)\ X_2 - 12.59\ (P < .0001)\ X_3 - 0.14\ (P = .5261)\ X_1X_2 + 1.01\ (P = .0006)\ X_1X_3 + 1.73\ (P < .0001)\ X_2X_3 + 0.19\ (P = .4051)\ X_1X_2X_3 \quad (8)$$

$$Y_1 = 373.75 + 66.54X_1 + 52.09X_2 + 105.06X_3 - 4.73X_1X_2 + 46.30X_1X_3 \quad (9)$$

$P < .001$, $R^2 = 0.9992$, Adjusted $R^2 = 0.9989$, coefficient of variation (CV) = 1.10

$$Y_2 = 472.93 + 73.45X_1 + 169.06X_2 + 333.03X_3 + 62.40X_1X_3 + 141.49X_2X_3 \quad (10)$$

$P < .001$, $R^2 = 0.9999$, Adjusted $R^2 = 0.9999$, CV = 0.80

$$Y_3 = 57.36 + 6.53X_1 + 15.52X_2 - 12.59X_3 + 1.01X_1X_3 + 1.73X_2X_3 \quad (11)$$

$P < .001$, $R^2 = 0.9986$, Adjusted $R^2 = 0.9981$, CV = 1.41

Equations 6-8 represent full model, while Equations 9-11 represent reduced model having significant coefficient figures ($P < .001$).

Where Y_1, Y_2, and Y_3 are the responses: the particle size (nm), the amount of itraconazole entrapped in the nanoparticles (ITRAe) (µg/mL), and encapsulation efficiency (ITRAe [%]), respectively. X_1, X_2, and X_3 are the code values of the independent variables (PLGA, benzyl benzoate, and itraconazole).

The predicted values were calculated by using the mathematical model from Equations 9 and 10, tabulated in Table 3.

On the particle size, the Fisher F test with a very low probability value (P_{model} > F less than 0.0001) (Table 4) demonstrated a very high significance for the regression model.[19] The goodness of fit of the model was checked by the adjusted determination coefficient (adjusted R^2). The determination coefficient (R^2) is a measure of the amount of reduction in the variability of Y obtained by using the regressor variables X_1, X_2, and X_3. However, a large value of R^2 does not necessarily imply that the regression model is a good one. Adding a variable to the model will always increase R^2, regardless of whether the additional variable is significant or not. The adjusted R^2 statistic will not always increase as variables are added to the model. If unnecessary variables are added, the value of adjusted R^2 will often decrease.[18] The results show that the value of the determination coefficient (R^2= 0.9992) was as high as the value of the adjusted determination coefficient (adjusted R^2= 0.9989), which indicated a high significance of the model.[20] A higher value of the correlation coefficient (R = 0.9996) signified an excellent correlation between the independent variables.[21] At the same time, a relatively low value of the coefficient of variation (CV = 1.10) indicated improved precision and reliability of the conducted experiments.[22]

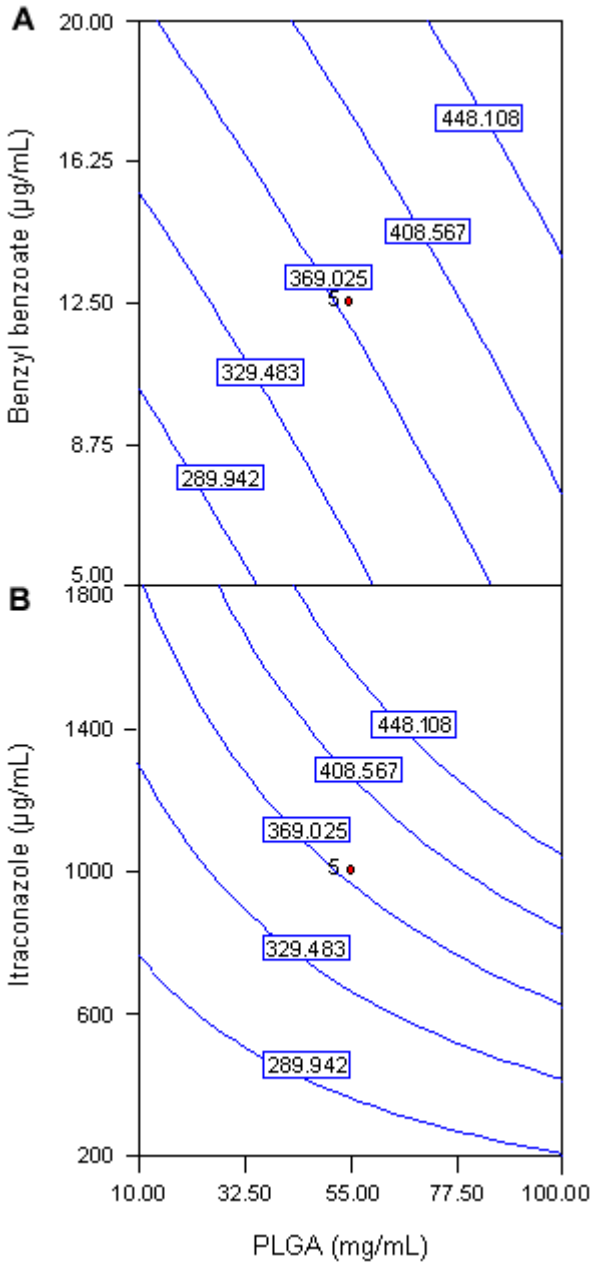

Figure 1. Effect of different variables on the particle size of nanoparticles.

It is obvious that the concentration of PLGA, benzyl benzoate, and itraconazole had significant effect on the particle size of the nanoparticles. Figures 1A and 1B show the increasing effect of PLGA, itraconazole, and benzyl benzoate. A significant synergistic interaction between PLGA and itraconazole at $P < .001$ was observed. This interaction is reflected by the pattern of the lines of Figure 1B. A significant ($P < .001$) antagonistic interaction was observed between PLGA and benzyl benzoate. This interaction is reflected by the pattern of the lines of Figure 1A. The dot at the center of the contour plot defined as the design points was 5 center points. The linearity over the region of exploration was tested by the F curve. Although the F curvature for the response surface was significant at $P < .001$, the developed model equation was a good prediction of the particle size for the 4 formulas presented in Table 5.

On the amount of itraconazole entrapped in the nanoparticles (ITRAe), the Fisher F test with a very low probability value (P_{model} > F less than 0.0001) (Table 4) demonstrated a very high significance for the regression model.[20] The value of the determination coefficient ($R^2 = 0.9999$) was as high as the value of the adjusted determination coefficient (Adjusted $R^2 = 0.9999$), which indicated a high significance of the model.[20] A relatively low value of the coefficient of variation (CV = 0.80) indicated improved precision and reliability of the conducted experiments.[22]

It is obvious that the concentration of PLGA, benzyl benzoate, and itraconazole also had significant effect on the amount of itraconazole entrapped in the nanoparticles (ITRAe). Figures 2A and 2B show the increasing effect of [PLGA], itraconazole, and benzyl benzoate, respectively. The increasing effect shown with benzyl benzoate ($P < .001$) was due to itraconazole solubility in benzyl benzoate. The greater the concentration of benzyl benzoate in organic phase, the higher becomes itraconazole dissolved in benzyl benzoate. A significant synergistic interaction between PLGA and itraconazole at $P < .001$ was observed. This interaction is reflected by the pattern of the lines of Figure 2A. A significant ($P < .001$) synergistic interaction also was observed between benzyl benzoate and itraconazole. This interaction is reflected by the pattern of the lines of Figure 2B. The linearity over the region of exploration was tested by the F curve. Although the F curvature for the response surface was significant at $P < .001$, the developed model equation was a good prediction of the amount of itraconazole entrapped in the nanoparticles for the 4 formulas presented in Table 5.

Figure 3A shows an overlay plot for the 3 responses: $300 \le Y_1$ (the particle size) ≤ 400; $450 \le Y_2$ (ITRAe) ≤ 550; and $60 \le Y_3$ (ITRAe [%]) ≤ 70. The boundary shown in Figure 3A indicates that there are a number of combinations of concentration of PLGA, benzyl benzoate, and itraconazole that will result in a satisfactory process. Figure 3B shows an overlay plot for the 3 responses: $200 \le Y_1$ (the particle size) ≤ 300; $450 \le Y_2$ (ITRAe) ≤ 550; and $60 \le Y_3$ (ITRAe [%]) ≤ 70. Figure 3B shows that there is no boundary that must be met by the process.

Using the desirability approach, the target of ITRAe was chosen to be 500 µg/mL, while the lower limit was equal to 450 µg/mL, and the upper limit was 550 µg/mL. The particle was set equal at a minimum between 300 and 400 nm. Finally, the ITRAe (%) ranged from 60% to 70%. Four solutions were found. The solution having the highest overall desirability ($D = 0.769$) was composed of 10 mg/mL of

Table 5. Comparison of the Experimental and Predicted Nanoparticle Characteristics*

Formula	Composition			Size (nm)			Encapsulation Efficiency ITRAe (µg/mL)		
	X_1	X_2	X_3	Exp	Pred	*P* value	Exp	Pred	*P* value
1	55	20	1000	424.51	425.84	<.05	650.62	641.99	<.05
2	10	12.5	1000	305.03	307.21	<.05	408.91	399.48	<.05
3	100	12.5	200	289.63	288.93	<.05	151.36	150.95	<.05
4	10	16.94	1001.01	336.87	340.91	<.05	506.58	499.99	<.05

*Exp indicates experimentally determined; ITRAe, the amount of itraconazole entrapped in the nanoparticles; and Pred, predicted through the model Equations (9) and (10).

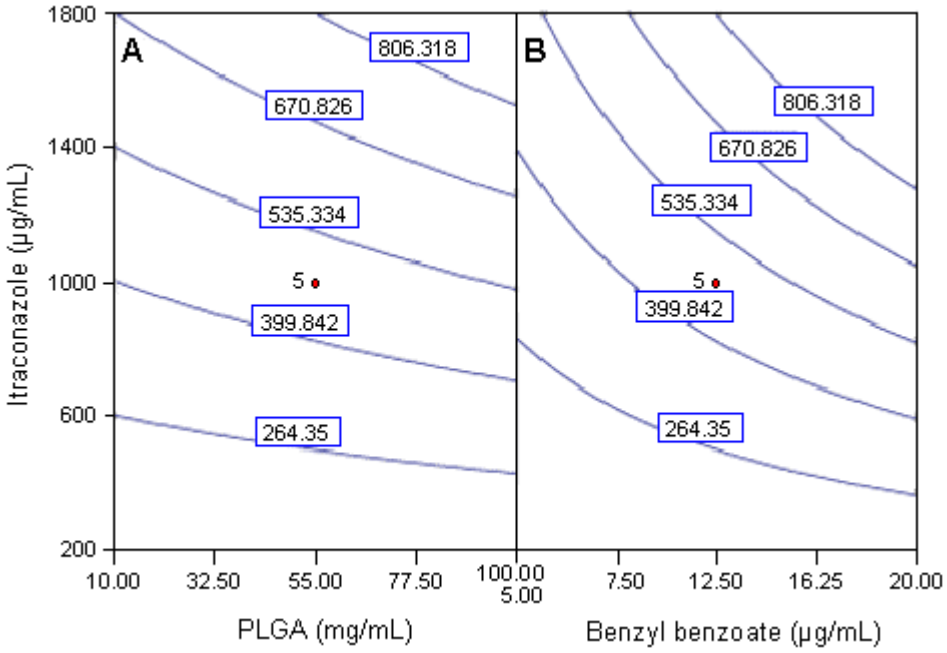

Figure 2. Effect of different variables on ITRAe.

PLGA, 16.94 µg/mL of benzyl benzoate, and 1001.01 µg/mL of itraconazole. The desirability function response surface and contour plot are shown in Figures 4A and 4B, respectively. Verification of the predicted value was made by using nanoparticles prepared using the optimized conditions (Formula 4 in Table 5). The particle size and amount of itraconazole entrapped in nanoparticles are in the 95% prediction interval. These results therefore corroborate the predicted values, and the effectiveness of the model.

CONCLUSION

PLGA nanoparticles containing itraconazole were successfully prepared by using solvent displacement technique. The formulation study using 2^3 factorial design and response surface methodology allowed one to obtain the optimum formulation. The concentration of PLGA, benzyl benzoate, and itraconazole had significant effect on the particle size of the nanoparticles , and the amount of itraconazole entrapped in the nanoparticles (ITRAe).The PLGA nanoparticles containing itraconazole prepared by 10 µg/mL of *PLGA*, 16.94 µg/mL of benzyl benzoate and 1001.01 mg/mL of itraconazole were the optimum formulation. Observed responses were in close agreement with the predicted values of the optimized formulation, thereby demonstrating the feasibility of the optimization procedure in developing nanoparticle formulations.

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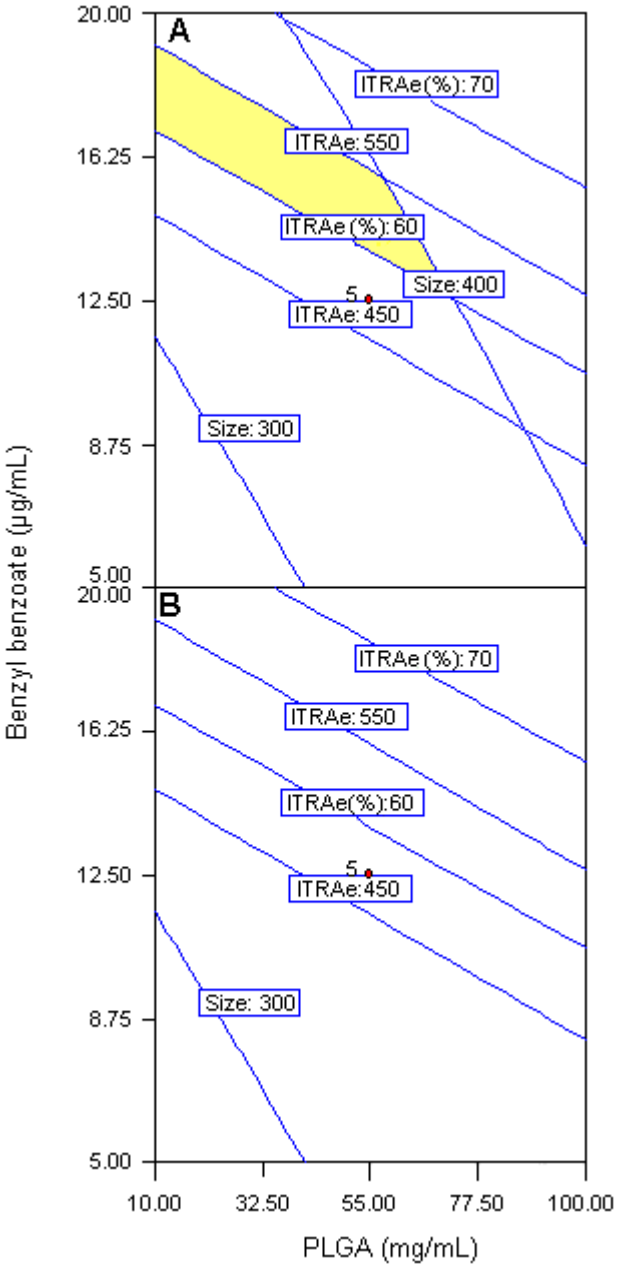

Figure 3. The overlay plots for the particle size, ITRAe, and ITRAe (%).

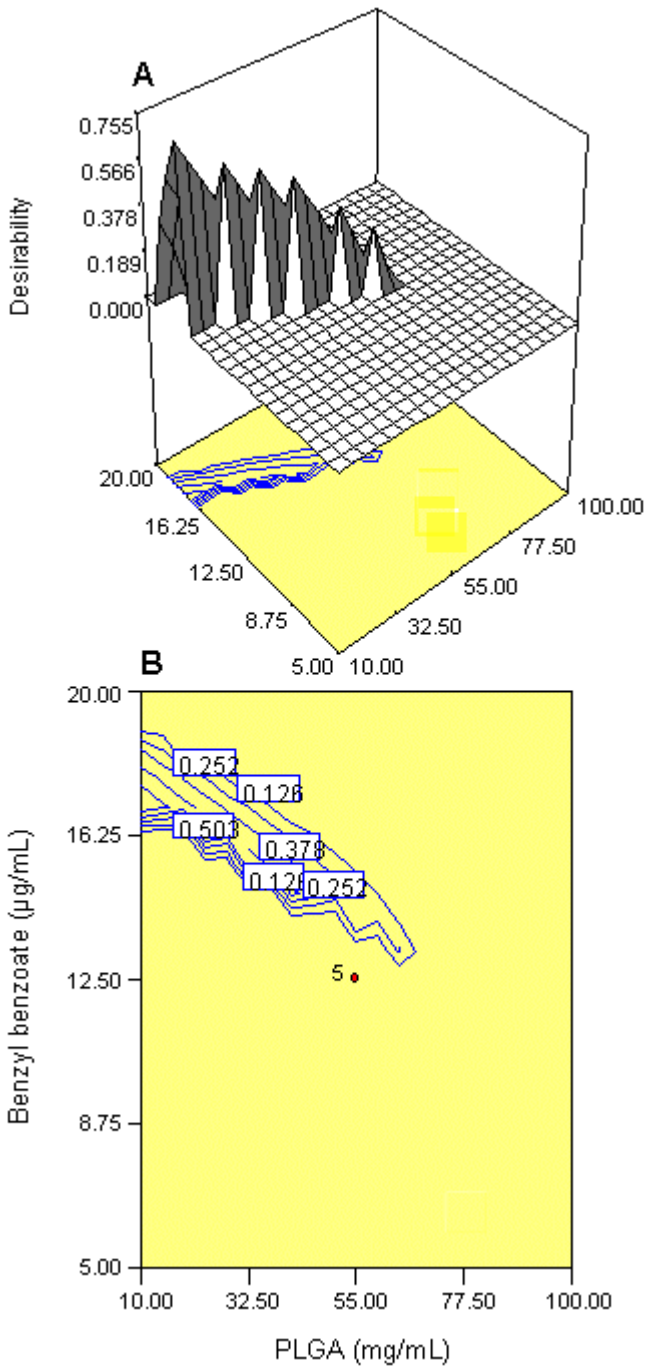

Figure 4. Desirability function response surface and contour plot.

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