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Effect of Different Formulation Variables on Some Particle Characteristics of Poly (DL-lactide-co-glycolide) Nanoparticles

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(Received July 23, 2007; Accepted December 26, 2007)

This study investigates the effect of some formulation variables on particulate characteristics of poly (DL-lactide-co-glycolide) (PLGA) copolymer nanoparticles by applying 2^3 factorial design and response surface methodology (RSM). Nanoparticles were prepared by solvent displacement technique. Initially, appropriate formulation factors for elaboration of polymeric particles were selected by screening. A 2^3 full factorial design was employed to evaluate the influence of three formulation variables, polymer concentration (X_1), dispersant concentration (X_2) and phase volume ratio (X_3) on the percentage of total particles at submicron range (Y_1), mean diameter (Y_2) and specific surface area (Y_3) as particle characteristics. The results showed that all the three variables had significant influence on mean diameter of particles and amount of particles at submicron range. Simultaneous change of polymer concentration and dispersant concentration had significant effect on specific surface area of particles. Span value as an index of polydispersity indicated uniformity in particle size distribution.

Key words——nanoparticles; poly (DL-lactide-co-glycolide); factorial design; response surface methodology

INTRODUCTION

Drug delivery systems consisting of biodegradable polymers have become very popular during last few decades due to the possibility of their administration by parenteral routes.[1] Because of their biodegradability and biocompatibility, poly lactic acids and its copolymers with glycolic acid are widely employed for the preparation of sustained release preparations.[2–8]

The colloidal suspension of polymeric nanoparticles is usually prepared by using some sophisticated devices like ultra-sonicator[9] and high pressure homogenizer.[10] In present study solvent displacement technique[11] has been chosen to prepare nanoparticles as a conventional laboratory technique. In this technique a water miscible liquid is used to dissolve the internal phase. Although the solvent displacement technique is conceptually simple, many formulation variables can influence the final product. The formulation variables include principally the emulsification procedure, the ratio and nature of both phases, concentration of polymer, concentration of surfactant, stirring speed and rate of evaporation of organic phase *etc*. The application of an experimental design to pharmaceutical formulation development would provide an efficient and economic method to acquire the necessary information to understand the relationship between independent variables and dependent variables or responses.[12] The statistical optimization technique has been studied in formulation development including tablet, microencapsulated drug delivery system and nanoparticles by different researchers.[13–18] In addition, this process provides a method to develop an empirical model equation to characterize the response as a function of different independent variables. Nanoparticles were successfully prepared at a stirring rate of 750 rpm by mechanical stirring.[19] Influence of organic solvent evaporation rate on particle size distribution was studied by Mainardes and Evangelista.[20] However there are few reports that comprehensively describe the effects of combinations of formulation variables on the micromeritic properties of particles prepared by solvent displacement technique.[21] The present study describes the effect of formulation variables including concentration of polymer in internal phase, concentration of dispersant in external phase and the phase volume ratio on the percentage of total particles at submicron range, mean diameter and specific surface area of the particles by applying a 2^3 full factorial design and response surface methodology (RSM).

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EXPERIMENTAL MATERIALS

Poly (DL-lactide-co-glycolide) with a monomer ratio of 50 : 50 was a kind gift from Sun Pharma Advanced Research Centre (SPARC), Vadodara, India. The dispersant Polysorbate 20 was purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Acetone was supplied by Merck India and used without further purification. A Mastersizer (Malvern laser diffraction particle size analyzer, Malvern Instruments, U.K.) was employed to determine volumetric particle size distribution.

Preparation of Nanoparticulate Suspension The preparation of nanoparticles was based on solvent displacement process. The required amount of polymer was dissolved in acetone. The organic phase was added to the aqueous phase containing dispersant at a constant flow rate (0.3 ml/m) under mechanical stirring at 800 rpm. Acetone was removed at room temperature with constant stirring at 800 rpm for 5 h.

Experimental Design A 2^3 full factorial design with two replicates was used in this study. The three independent variables investigated were concentration of PLGA (X_1), concentration of dispersant (X_2) and phase volume ratio (X_3). The level of three independent variables is shown in Table 1 and the design is presented in Table 2.

The effect of previously mentioned variables was investigated on the following responses: the percentage of total particles at submicron range (Y_1), mean diameter particles (Y_2) and the specific surface area (Y_3), as particle characteristics.

Determination of Particle Size and Specific Surface Area The particle size was determined by laser diffraction using a Mastersizer (Malvern laser diffraction particle size analyzer, Malvern Instruments, U.K.). The Mastersizer comprises of Helium-Neon laser as a light source. This is then focused by a Fourier lens to a detector, which consists of a large number of photosensitive elements radiating outward from the center. The intensity of the scattered light is measured. The volumetric particle size distribution and specific surface area is calculated by using an optical model and mathematical deconvolution procedure. For particle size analysis, the samples were dispersed in Milli-Q water. Measurements were carried out at 30°C using a Helium-Neon laser at an angle of 90°.

Determination of Span Value The size distribution (polydispersity) was measured in terms of Span factor expressed as

$$\text{Span} = (D_{90\%} - D_{10\%})/D_{50\%} \quad (1)$$

where $D_{90\%}$, $D_{10\%}$ and $D_{50\%}$ are the diameters where the given percentage of particles is smaller than that size.[22,23] A low value of span indicates a narrow size distribution and low polydispersity.[24]

Table 1. Level of the Investigated Variables

Code unit	Independent Variables		
	PLGA concentration (X_1) ; % w/v	Dispersant concentration (X_2) ; % w/v	Phase volume ratio (X_3) ; o/w
−1	2	4	1 : 5
+1	4	8	2 : 5

Table 2. Design Points for Investigated Variables

Formula	PLGA concentration (X_1) ; % w/v	Dispersant concentration (X_2) ; % w/v	Phase volume ratio (X_3) ; o/w
1a	2	4	1 : 5
2a	4	4	1 : 5
3a	2	8	1 : 5
4a	2	4	2 : 5
5a	4	8	1 : 5
6a	2	8	2 : 5
7a	4	4	2 : 5
8a	4	8	2 : 5
1b	2	4	1 : 5
2b	4	4	1 : 5
3b	2	8	1 : 5
4b	2	4	2 : 5
5b	4	8	1 : 5
6b	2	8	2 : 5
7b	4	4	2 : 5
8b	4	8	2 : 5

Data Analysis Analysis of data and multiple regression analysis were carried out using software STATGRAPHICS Plus version-3.0. Results of analysis of variance (ANOVA) and RSM are summarized in Table 4.

RESULTS AND DISCUSSION

Preparation of nanoparticles by solvent-displacement technique, may involve complex interfacial hydrodynamic phenomena. Addition of polymer solution in aqueous phase resulted in emulsification of the organic solution in the form of nanodroplets, due to some kind of interface instability arising from rapid diffusion of acetone across the interface and

marked decrease in the interfacial tension. The mechanism of nanoparticles formation could be explained in terms of interfacial turbulence or spontaneous agitation of the interface between two equilibrated liquid phases, involving flow, diffusion and surface processes.[25] The molecular mechanism of interfacial turbulence could be explained by the continuous formation of eddies of solvent (acetone) at the interface or due to thermal inequality in the system. Since dispersed droplets contain polymer, these will tend to precipitate and form nanoparticulate suspension because of the presence of non-solvent medium containing dispersant.[26] The effects of different combinations previously mentioned variables X_1, X_2 and X_3, on the responses Y_1, Y_2 and Y_3 are shown in Table 3 and results of ANOVA analysis is shown in Table 4. The multiple regression analysis[27] yielded the following regression equations, which suggest an empirical relationship between the values of responses and the independent variables in coded unit:

$$Y_1 = 72.18\,(p<0.05) + 6.00\,(p<0.05)\,X_1 + 1.43\,(p<0.05)\,X_2 + 11.75\,(p<0.05)\,X_3 - 3.71\,(p<0.05)\,X_1X_2 + 3.23\,(p<0.05)\,X_2X_3 + 0.89\,(p<0.05)\,X_1X_3 + 0.19\,(p=0.7263)\,X_1X_2X_3 \quad (2)$$

$$Y_2 = 764\,(p<0.05) - 53\,(p<0.05)\,X_1 - 48\,(p<0.05)\,X_2 - 121\,(p<0.05)\,X_3 + 26\,(p<0.05)\,X_1X_2 - 14\,(p=0.0624)\,X_2X_3 + 3\,(p=0.6507)\,X_1X_3 + 8\,(p=0.2562)\,X_1X_2X_3 \quad (3)$$

$$Y_3 = 8.133\,(p<0.05) + 0.57\,(p<0.05)\,X_1 + 0.29\,(p=0.058)\,X_2 + 1.33\,(p<0.05)\,X_3 - 0.55\,(p<0.05)\,X_1X_2 + 0.59\,(p<0.05)\,X_2X_3 + 0.15\,(p=0.1349)\,X_1X_3 + 0.11\,(p=0.41)\,X_1X_2X_3 \quad (4)$$

$$Y_1 = 72.18 + 6.00X_1 + 1.43X_2 + 11.75X_3 - 3.71X_1X_2 + 3.23X_2X_3 + 0.89X_1X_3 \quad (5)$$

$p<0.05$ $R^2=0.9941$ Adjusted $R^2=0.9890$

$$Y_1 = 72.18 - 2.28X_2 + 12.64X_3 + 3.23X_2X_3 \quad (6)$$

$$Y_1 = 66.17 + 5.14X_2 + 10.85X_3 + 3.23X_2X_3 \quad (7)$$

$$Y_2 = 764 - 53X_1 - 48X_2 - 121X_3 + 26X_1X_2 \quad (8)$$

$p<0.05$ $R^2=0.9834$ Adjusted $R^2=0.9689$

$$Y_2 = 711 - 22X_2 - 121X_3 \quad (9)$$

$$Y_2 = 817 - 74X_2 - 121X_3 \quad (10)$$

$$Y_3 = 8.133 + 0.57X_1 + 1.33X_3 - 0.55X_1X_2 + 0.59X_2X_3 \quad (11)$$

$p<0.05$ $R^2=0.9736$ Adjusted $R^2=0.9506$

Equations (2)−(4) represent full model. The full models were reduced considering the *p*-value of each factor, shown in Table 4. Equations. (5)−(11) represent reduced model having significant coefficient at 95% confidence level ($p \leq 0.05$). The predicted

Table 3. Observed Response and Predicted Values of Response Variables

Run No.	Percentage of particles at submicron range ; (Y_1)			Mean diameter d_{vs} (nm) ; (Y_2)			Specific surface area (M^2/gm) ; (Y_3)		
	Observed response	Predicted response	Residual value	Observed response	Predicted response	Residual value	Observed response	Predicted response	Residual value
1a	52.22	59.41	−7.19	1000	1012	−12	5.97	6.273	−0.303
2a	63.15	71.05	−7.9	870	854	16	6.88	8.513	−1.633
3a	56.52	57.23	−0.71	870	864	6	6.91	6.193	0.717
4a	68.11	68.67	−0.56	770	770	0	7.77	7.753	0.017
5a	66.8	60.03	6.77	820	810	10	7.32	6.233	1.087
6a	83.86	85.41	−1.55	600	622	−22	9.98	10.033	−0.053
7a	91.54	89.87	1.67	610	612	−2	9.9	9.993	−0.093
8a	92.93	91.77	1.16	530	568	−38	11.23	10.073	1.157
1b	54.2	59.41	−5.21	990	1012	−22	6.08	6.273	−0.193
2b	64.5	71.05	−6.55	820	854	−34	7.29	8.513	−1.223
3b	58.32	57.23	1.09	910	864	46	6.6	6.193	0.407
4b	69.58	68.67	0.91	810	770	40	7.44	7.753	−0.313
5b	67.72	60.03	7.69	810	810	0	7.38	6.233	1.147
6b	86.56	85.41	1.15	590	622	−32	10.15	10.033	0.117
7b	87.84	89.87	−2.03	630	612	18	9.57	9.993	−0.423
8b	91.04	91.77	−0.73	600	568	32	10.07	10.073	−0.003

* *p*-value in each case is <0.05. Predicted values calculated through model Eqs. (5), (8) and (11).

Table 4. Response Surface Data and Analysis of Variance*

Parameters	Percentage particles at submicron range ; (Y_1)		Mean diameter d_{vs} (nm) ; (Y_2)		Specific surface area (M^2/gm) ; (Y_3)	
	T-statistic	*p*-value	T-statistic	*p*-value	T-statistic	*p*-value
Model	F Ratio=194.09	0.0000	F Ratio=42.24	0.0000	F Ratio=67.95	0.0000
I	191.048	0.0000	85.8828	0.0000	115.05	0.0000
X_1	15.9056	0.0000	6.03172	0.0003	−7.9961	0.0000
X_2	2.68103	0.0279	2.20253	0.0588	−7.24355	0.0001
X_3	31.1049	0.0000	14.0432	0.0000	−18.3441	0.0000
X_1 X_2	−4.91148	0.0012	−2.91687	0.0194	4.0451	0.0037
X_2 X_3	6.04986	0.0003	4.47039	0.0021	−2.16366	0.0624
X_3 X_1	2.37055	0.0452	1.66301	0.1349	0.47036	0.6507
X_1 X_2 X_3	0.362617	0.7263	0.867947	0.4107	1.22294	0.2562
Error MS	2.28389		0.143513		0.0070625	
Model MS	443.278	<0.0001	6.06235	<0.0001	0.047992	<0.0001
R^2	99.4146		97.3658		98.346	
Adjusted R^2	98.9024		95.0609		96.8987	
Durbin-Watson statistics	1.32502		1.26781		1.53982	

* MS indicates mean square and R^2 indicates determination coefficient.

values of three responses were calculated by using the mathematical model from Eqs. (5), (8) and (11); tabulated in Table 3. Results showed that percentage of particles at submicron range to be fitted with a multiple linear regression model Eq. (1) to describe relation between the response and three independent variables along with their interactions. Higher *p* value ($p=0.7263$) belonging to $X_1X_2X_3$, indicated $X_1X_2X_3$ to be insignificant at 95% or higher confidence limit (Table 4). So Eq. (2) was reduced to Eq. (5). The goodness of fit of the model was checked by adjusted determination co-efficient (adjusted R^2). The determination co-efficient (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. The results showed that the value of determination coefficient ($R^2=0.9941$) was as high as the value of the adjusted determination co-efficient (adjusted $R^2=0.9890$) which indicated a high significance of the model.

The model equation showed that polymer concentration, dispersant concentration and phase volume ratio had significant effect on percentage of particle at submicron range. However, during fabrication of particles it has been seen that when the amount of polymer was increased above the higher level selected in the present experimental design (>4%), agglomeration of polymer occurred. Therefore fixing the concentration of polymer at highest level (+1) and lowest level (−1), Eq. (5) was further reduced to Eqs. (6) and (7). Figures 1, 2, 3 and 4 represent the response surfaces and contour plots of Eqs. (6) and (7), respectively. A significant synergistic interaction between percentage of dispersing agent and phase volume ratio was reflected by the pattern of lines in Figs. 2 and 4 when the polymer concentration was fixed at upper level and lower level, respectively.

Results showed that mean diameter, to be fitted with a multiple linear regression model Eq. (3) to describe relation between the response and three independent variables along with their interactions. Higher *p* values, 0.0624, 0.6507 and 0.2562 belonging to X_2 X_3, X_1 X_3 and X_1 X_2 X_3, were noticed (Table 4). Since *p*-value is greater than 0.05, the terms were not statistically significant at 95% or higher confidence level. Therefore model Eq. (3) was reduced to Eq. (8). The value of adjusted determination co-efficient (adjusted $R^2=0.9689$) indicated a high significance of the model.

Since agglomeration of polymer occurred at concentration greater than 4% of polymer possibly due to higher density of internal phase, fixing the concentration of PLGA at highest level (+1) and lowest level (−1) Eq. (8) was further reduced to Eqs. (9) and (10). Figures 5, 6, 7 and 8 represent the response surfaces and contour plots of Eqs. (9) and (10) respectively.

From the pattern of lines in Figs. 6 and 8 it was seen that mean diameter was decreased when both the

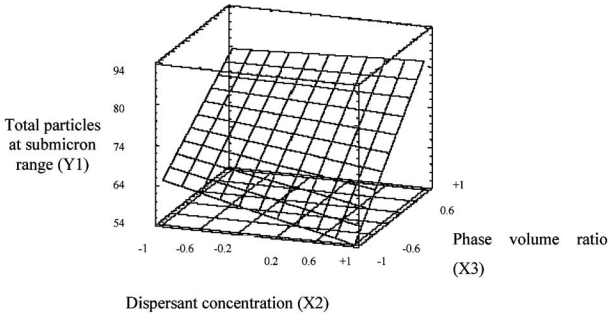

Fig. 1. Surface Plot for Total Particles at Submicron Range Fixing Polymer Concentration at Higher Level

Three-dimensional surface plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on total particles at submicron range (Y_1) fixing polymer concentration (X_1) at higher level.

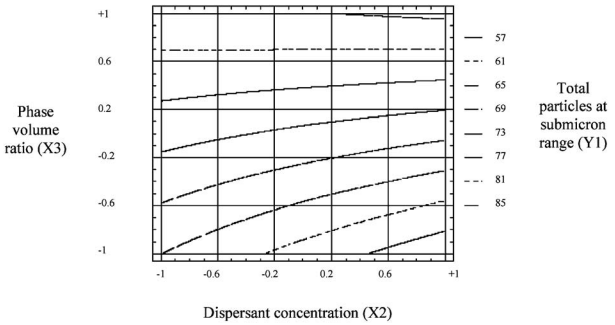

Fig. 2. Contour Plot for Total Particles at Submicron Range Fixing Polymer Concentration at Higher Level

Contour plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on total particles at submicron range (Y_1) fixing polymer concentration (X_1) at higher level.

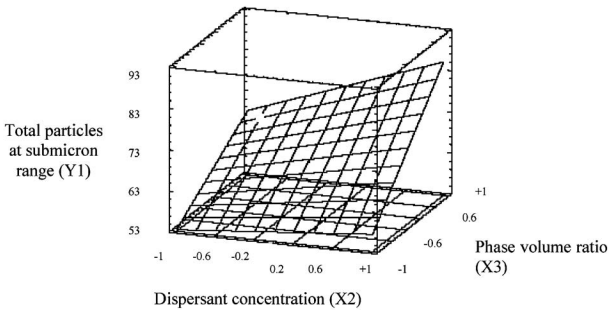

Fig. 3. Surface Plot for Total Particles at Submicron Range Fixing Polymer Concentration at Lower Level

Three-dimensional surface plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on total particles at submicron range (Y_1) fixing polymer concentration (X_1) at lower level.

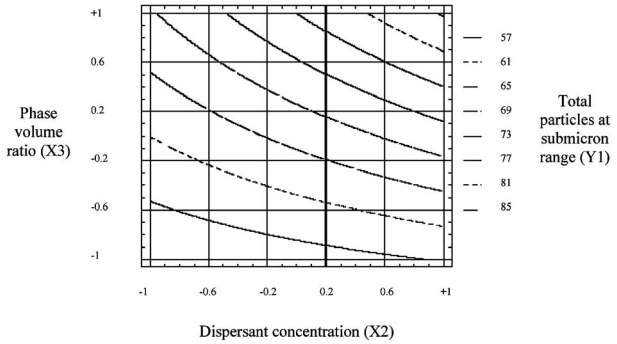

Fig. 4. Contour Plot for Total Particles at Submicron Range Fixing Polymer Concentration at Lower Level

Contour plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on total particles at submicron range (Y_1) fixing polymer concentration (X_1) at lower level.

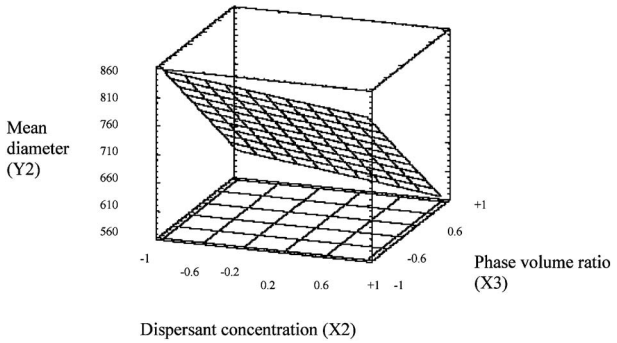

Fig. 5. Surface Plot for Mean Diameter Fixing Polymer Concentration at Higher Level

Three-dimensional surface plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on mean diameter (Y_2) fixing polymer concentration (X_1) at higher level.

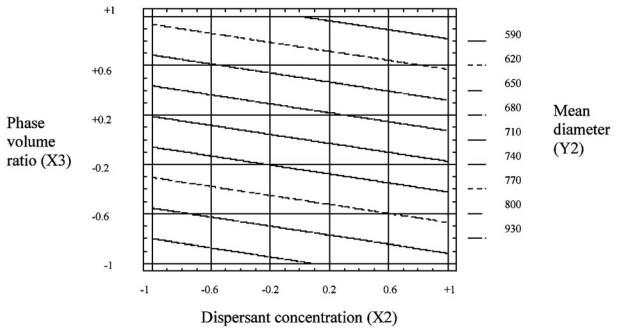

Fig. 6. Contour Plot for Mean Diameter Fixing Polymer Concentration at Higher Level

Contour plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on mean diameter (Y_2) fixing polymer concentration (X_1) at higher level.

dispersant concentration and phase volume ratio are increased simultaneously. Higher concentration of dispersant resulted in smaller droplets with increased stability of the primary emulsion during mixing of organic phase to aqueous phase and subsequently resulted in smaller particle size. In case of high phase volume ratio, amount of organic phase was increased. Due to the increased amount of organic solvent, viscosity of organic phase was decreased. Less viscous

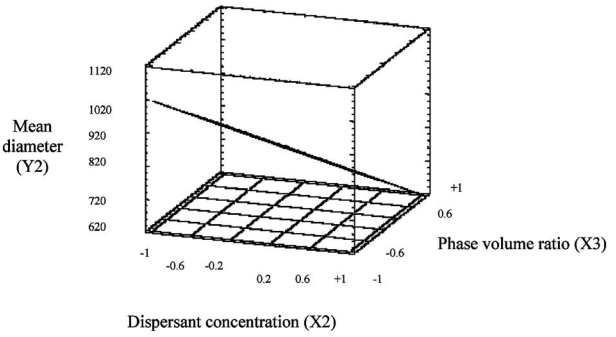

Fig. 7. Surface Plot for Mean Diameter Fixing Polymer Concentration at Lower Level

Three-dimensional surface plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on mean diameter (Y_2) fixing polymer concentration (X_1) at lower level.

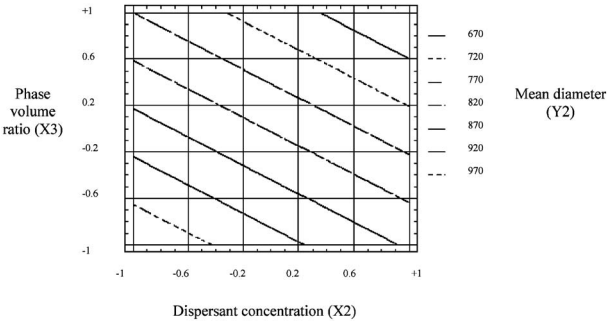

Fig. 8. Contour Plot for Mean Diameter Fixing Polymer Concentration at Lower Level

Contour plot for effect of concentration of dispersant (X_2) and phase volume ratio (X_3) on mean diameter (Y_2) fixing polymer concentration (X_1) at lower level.

organic phase resulted in smaller globules containing lower amount of polymer in primary emulsion droplets and finally smaller particles were formed. An antagonistic interaction between dispersant concentration and phase volume ratio has been observed which might be necessary to reduce the mean diameter.

Relationship between specific surface area and three independent variables along with their interactions was described by a multiple linear regression model Eq. (4). Higher p values belonging to X_2, X_1 X_3, and X_1 X_2 X_3 were noticed and hence model Eq. (4) was reduced to Eq. (11). The value of adjusted determination co-efficient (adjusted $R^2=0.9506$) indicated a high significance of the model.

The model showed concentration of PLGA and phase volume ratio and their simultaneous change had significant effect on specific surface area of resulted particles. PLGA at low concentration produced smaller particles due to less viscosity of internal phase in primary emulsion resulting in increased specific surface area. Increased phase volume ratio resulted in higher specific surface area due to small particle size which is explained earlier. Simultaneous change of concentration of PLGA and polysorbate 20 also had significant influence on specific surface area. Increased amount of polysorbate 20 resulted in smaller droplets of dispersing phase that finally formed small particles with high specific surface area. Average span value was calculated to be 0.8347 ± 0.1220, which is below 1, indicating uniformity in particle size distribution.

CONCLUSION

The work presented here describes a detailed analysis of some formulation variables that might have influence individually or jointly on particle characteristics by applying factorial design and RSM. Formation of nanoparticles was related to the interfacial area generated by emulsion formation and reduction of globule size due to fast solvent diffusion. All three factors were important to control particles at submicron range and their mean diameter. Simultaneous change of PLGA concentration and polysorbate 20 concentration had significant effect on specific surface area. The application of RSM in the formulation design of nanoparticles has profound importance in the manufacture of nanoparticulate drug delivery system, since it significantly reduces the number of trial experimentation, reducing cost, time and resources, which will be beneficial for economical industrial manufacturing. However, the present study has been conducted on blank nanoparticles without incorporation of drug. The resultant effect of incorporating drug might alter the different dependent variables. This phenomenon is under study at present.

Acknowledgements One of the authors, Nita Mondal is grateful to UGC, Govt. of India [F. 10–17 /2004 (SA–I)] for financial assistance to carry out the research work. Authors are also grateful to Sun Pharmaceuticals Advanced Research Centre, (Vadodara, India) for sending PLGA as gift sample and to East India Pharmaceutical Works Ltd (Kolkata, India) to carry out particle size analysis.

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