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# PREPARATION AND OPTIMIZATION OF ROSUVASTATIN CALCIUM LOADED SOLID LIPID NANOPARTICLES BY CENTRAL COMPOSITE DESIGN

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## ABSTRACT

**Background:** Solid lipid nanoparticles are an alternative carrier system used to load the drug targeting, to improve bioavailability by increasing solubility, permeability and protecting the drug from first pass metabolism. **Objective:** The aim of this investigation was to design and evaluate solid lipid nanoparticles (SLNs) of rosuvastatin calcium. **Materials and methods:** A modified solvent emulsification diffusion technique was used to produce the rosuvastatin loaded solid lipid nanoparticles. A 4-factor, 3-level Central Composite design was applied to study the effect of independent variables (factors) i.e. lipid (A), surfactant concentration (B), stirring speed (C) and drug amount (D) on dependent variables (responses) i.e. drug entrapment efficiency (Y1), drug loading (Y2) and particle size (Y3). 3-D surface response plots were drawn and optimized formulation was selected based on desirability factor. **Results:** The results of optimized formulation showed average particle size of 115.4 nm, entrapment efficiency of 97.16% and drug loading of 60.34%. Transmission electron microscopy (TEM) reveals that particles were spherical in shape with smooth surfaces and uniform distribution. **Conclusion:** Thus, the present study can be useful for the successful design, development and optimization of SLNs for rosuvastatin calcium using a 4-factor, 3-level Central Composite design.

**KEYWORDS:** Central Composite design, Rosuvastatin calcium, Solid lipid nanoparticles, Solvent emulsification diffusion technique etc.

## INTRODUCTION

**Rosuvastatin calcium** belongs to the class of medications called statins and is used as an adjunct to dietary therapy to treat primary Hyperlipidemia, mixed dyslipidemia and Hyper triglyceridemia. It is poorly soluble in water. It has very low oral bioavailability of about 20% due to its first pass metabolism and half life of 19 hours (Gadad A P., *et al* 2016). Rosuvastatin is a selective and competitive inhibitor of hydroxyl methyl glutaryl-coenzyme A reductase (HMG-CoA reductase). HMG-CoA reductase is the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. It is also also used for primary and secondary prophylaxis of cardiovascular events in patients with multiple risk factors including diabetes mellitus (Agarwal R., *et al* 2015). These above mentioned points makes rosuvastatin promising drug for formulation into SLNs for enhancement of its oral bioavailability.

Solid lipid nanoparticles (SLNs) introduced in 1991, offer an alternative drug delivery systems with lower acute and chronic toxicity, good tolerability and bidegradability to tradition colloidal carriers such as – fat emulsions, liposomes, polymeric micro and nanoparticles. They have potential to carry lipophilic and hydrophilic drugs. SLN offer unique properties such as small particle size, wide surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals. Therfore, they can be considered as better alternative to liposomes, microemulsions, nanoemulsions, self emulsifying drug delivery system and polymeric nanoparticles (Ekambaram P., *et al* 2012).

In the present research work, rosuvastatin loaded solid lipid nanoparticles were prepared by modified solvent emulsification diffusion technique. The formulation was optimized by using 4-factor, 3-level Central Composite design. The optimized formulation was evaluated for various parameters like particle size analysis, polydispersity index, zeta potential, entrapment efficiency, drug loading capacity, TEM analysis etc.

To optimize the production of these SLNs, a statistically experimental design methodology was employed properly. After selecting the critical variables affecting particle size, entrapment efficiency and drug loading, the response methodology of the Central Composite

design (version 8.0.7.1, stat-ease, Inc, Minneapolis, Minnesota, USA) using a 4 factor, 3 level, was employed to optimize the level of particle size, entrapment efficiency and drug loading variables. The Central Composite design (CCD) is one of the most efficient designs of response surface experimental methodology to study the effect of formulation components on responses for exploring quadratic response surfaces and the second order polynomial model (Jakhar J.K., *et al* 2014).

## MATERIALS AND METHODS

### Materials

Rosuvastatin calcium was generously donated by Sun Pharmaceuticals Ltd. Gurgaon, Haryana, India, Stearic acid (Acros organics, U.S.A), Poloxamer 407(BASF, USA) and Tween 80 LR were supplied by CDH Ltd New Delhi, India. All other solvents and chemicals were of analytical grade. Double distilled water was used throughout the studies.

### Preparations of SLNs

In a Preliminary laboratory study, various factors like lipid concentration (Stearic acid, 20-100 mg), surfactant concentration [Poloxamer 407& Tween 80 in 1:1 (% w/v), 0.5-2.5%], stirring speed (2000-4000 rpm), drug amount (Rosuvastatin, 10-50 mg) chloroform: methanol ratio (1:1, 2.5% v/v) as the solvent of drug and lipids and sonication time 5 min were fixed and their effect on particle size, entrapment efficiency were determined. The design matrix was built by the statistical software package, Design-Expert (version 8.0.7.1, Stat Ease, Inc., Minneapolis, Minnesota, USA) and Table 1 shows the factors and their respective levels. In the current study, all of the experiments were performed in triplicate and the averages were considered as the response.

Rosuvastatin loaded SLNs were prepared by slight modification of the previously reported solvent emulsification diffusion technique (Yasir M., *et al* 2013).

The Drug loaded SLNs were prepared by the modified solvent emulsification – diffusion technique. Accurately weighed amount of lipid (20-100mg) was dissolved in a 2.5 mL (2.5%v/v) mixture of methanol and chloroform (1:1) as the internal oil phase. Drug (10-50 mg, ratio of drug to lipid 1:2) was dispersed in the above solution. This organic phase was the added drop by drop into a homogenizer tube containing 22.5 mL of an aqueous solution of surfactant & co surfactant Poloxamer 407 & Tween 80 in 1:1% w/v (0.5-2.5% w/v) as external aqueous phase and homogenized for 30 min at stirring speed (2000-4000 rpm)

(Remi Instruments Pvt. Ltd, India) to form a primary emulsion (o/w). The above primary emulsion was then poured in to 75 mL of ice cold water (2-3°C) containing surfactant (0.5-2.5% w/v) and stirred to extract the organic solvent into the continous phase and for proper solidification of SLNs. The stirring was continued for 2-2.5h at (2000-4000 rpm) to disperse the SLNs. The SLN dispersion was sonicated for 5 min (1 cycle, 100% amplitude, Bandelin sonoplus, Germany) to produce SLN dispersions of uniform size. The dispersion was then centrifuged at 18,000 rpm for 20 min (Remi Instruments Pvt. Ltd, India) to separate the solid lipid material containing the drug and washed with deionized water time to ensure the complete removal of organic solvent. This was then redispersed in (0.5-2.5% w/v) aqueous surfactant mixture of surfactant & co surfactant Poloxamer 407 & Tween 80 in 1:1% w/v and sonicated for 5 min to obtain the SLNs. The SLN dispersions were lyophilized in the presence of 5% (w/v) mannitol as cryoprotectant.

**Table 1 - Variables and their levels in Central Composite Design.**

| **Variables** | | | | | |
|---|---|---|---|---|---|
| **Independent variables** | **Levels** | | | | |
| | **-2** | **-1** | **0** | **+1** | **+2** |
| A= Lipid [Stearic acid (mg)] | 20 | 40 | 60 | 80 | 100 |
| B= Surfactant [Poloxamer 407 & Tween 80 in 1:1 (% w/v)] | 0.5 | 1 | 1.5 | 2 | 2.5 |
| C= Stirring Speed (rpm) | 2000 | 2500 | 3000 | 3500 | 4000 |
| D= Drug amount [Rosuvastatin calcium (mg )] | 10 | 20 | 30 | 40 | 50 |
| **Dependent variables** | **Goals** | | | | |
| Y1 = Drug entrapment | Maximize | | | | |
| Y2 = Drug loading | Maximize | | | | |
| Y3= Particle size | Mimimize | | | | |

**Experimental design**

According to CCD, a complete design consisted of 29 experimental points that included eight vertex points, six axial points and two replications at the centre point for estimation of pure error sum of squares, were performed to choose the best model among the linear, two factor interaction model and quadratic model due to the analysis of variance (ANOVA) *F*-value (Saini D., *et al* 2015). The obtained *P*-value less than 0.05 was considered to be statistically significant. From the preliminary screening test, it was found that the Lipid [Stearic acid (mg)] concentration (A), Surfactant [Poloxamer 407 & Tween 80 in 1:1 (% w/v)] concentration (B), Stirring speed (rpm) (C) and Drug concentration (D) had a significant effect on the drug entrapment efficiency (Y1), drug loading (Y2) and mean

particles size (Y3) of SLNs. Therefore by fixing the homogenization time (30 min), stirring time (2h) and sonication time (5 min), selected variables (A), (B), (C) and (D) were studied at different levels as -2 (low), -1, 0 (medium), +1,and +2 (high). The coded (factors) and actual values (responses) of the variables are given in Table 2. Predicting the response through the full second order polynomial equation is as shown in Equation:

$$Y= \beta_0 + \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{11}X^2_1+ \beta_{22}X^2_2+ \beta_{33}X^2_3+ \beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3$$

The equation can be used to draw conclusion after considering the magnitude of coefficient and mathematical sign it carries i.e. positive or negative. Where Y was predicted response(s), $\beta_0$ was an intercept, $\beta_1$, $\beta_2$ and $\beta_3$ were linear coefficients, $\beta_{11}$, $\beta_{22}$ and $\beta_{33}$ were squared coefficients and quadratic term, $\beta_{12}$, $\beta_{13}$ and $\beta_{23}$ were interaction coefficients and $X_1$, $X_2$ and $X_3$ were independent variables, which were selected based on the results from a preliminary study. To evaluate the fitness of the second-order polynomial equation, multiple correlation coefficient ($R^2$) and adjusted $R^2$ were employed as quality indicators.

**Optimization of data and validation of response surface methodology (RSM)**

Different batches were prepared with different independent variables at different levels and responses, like % entrapment efficiency, % drug loading and particle size were obtained. The data was substituted to design expert software and polynomial equations were obtained. The models were evaluated in terms of statistically significant coefficients and $R^2$ values. 3-D surface plots were used to assess the relationship between the variables and the responses. The selection of optimum formulations was based on the highest possible value of % entrapment efficiency (Y1) and % drug loading (Y2) and smallest value of particle size (Y3) as shown in Table 1. Finally, four optimized formulations were selected as check point to validate RSM. These formulations were again prepared and evaluated for responses. The resulting observed responses were compared with the predicted responses and % error was calculated. A linear regression plots between actual and predicted responses were plotted (Varshosaz J., *et al* 2010).

**Table 2- Observed responses for 29 runs of rosuvastatin calcium SLNs according to Central Composite design (n=3, mean ± SD).**

| Formu-lation No code | Lipid (Stearic acid) (%) | Surfactant (%) | Stirring Speed (rpm) | Drug (mg) | %Entramentefficiency ± SD (n=3) | % Drug loading ± SD (n=3) | Mean particle size (nm) ± SD (n=3) |
|---|---|---|---|---|---|---|---|
| F1 | 40 | 1 | 2500 | 20 | 89.64 ± 6.56 | 52.45 ± 5.43 | 157.54 ± 11.34 |
| F2 | 80 | 1 | 2500 | 20 | 92.56 ± 3.05 | 46.28 ± 2.87 | 224.54 ± 10.34 |
| F3 | 40 | 2 | 2500 | 20 | 89.17 ± 4.15 | 52.34 ± 6.65 | 154.65 ± 13.64 |
| F4 | 80 | 2 | 2500 | 20 | 92.86 ± 6.34 | 40.43 ± 8.67 | 230.13 ± 18.45 |
| F5 | 40 | 1 | 3500 | 20 | 96.43 ± 7.25 | 52.26 ± 4.65 | 145.43 ± 19.19 |
| F6 | 80 | 1 | 3500 | 20 | 95.15 ± 8.45 | 55.24 ± 5.46 | 225.72 ± 15.21 |
| F7 | 40 | 2 | 3500 | 20 | 94.04 ± 2.48 | 45.34 ± 4.62 | 224.01 ± 16.2 |
| F8 | 80 | 2 | 3500 | 20 | 92.13 ± 7.56 | 46.27 ± 7.87 | 166.65 ± 20.57 |
| F9 | 40 | 1 | 2500 | 40 | 96.34 ± 5.65 | 47.45 ± 4.76 | 234.53 ± 21.32 |
| F10 | 80 | 1 | 2500 | 40 | 89.65 ± 7.54 | 50.45 ± 2.96 | 165.21 ± 26.32 |
| F11 | 40 | 2 | 2500 | 40 | 92.76 ± 3.76 | 46.45 ± 2.64 | 245.02 ± 12.76 |
| F12 | 80 | 2 | 2500 | 40 | 95.67 ± 6.98 | 32.56 ± 3.65 | 178.64 ± 16.32 |
| F13 | 40 | 1 | 3500 | 40 | 96.02 ± 5.74 | 51.23 ± 6.76 | 233.67 ± 17.32 |
| F14 | 80 | 1 | 3500 | 40 | 95.64 ± 5.42 | 46.36 ± 3.26 | 171.32 ± 17.21 |
| F15 | 40 | 2 | 3500 | 40 | 94.12 ± 6.43 | 47.54 ± 4.76 | 236.78 ± 16.34 |
| F16 | 80 | 2 | 3500 | 40 | 96.14 ± 7.67 | 58.71 ± 5.65 | 130.34 ± 17.32 |
| F17 | 20 | 1.5 | 3000 | 30 | 98.45 ± 4.34 | 46.43 ± 6.55 | 264.64 ± 19.42 |
| F18 | 100 | 1.5 | 3000 | 30 | 95.68 ± 8.65 | 43.32 ± 2.75 | 194.45 ± 20.37 |
| F19 | 60 | 0.5 | 3000 | 30 | 94.89 ± 2.76 | 49.54 ± 4.65 | 192.08 ± 18.45 |
| F20 | 60 | 2.5 | 3000 | 30 | 84.65 ± 8.56 | 52.23 ± 2.65 | 196.40 ± 19.54 |
| F21 | 60 | 1.5 | 2000 | 30 | 93.06 ± 5.76 | 45.45 ± 4.76 | 193.34 ± 18.56 |
| F22 | 60 | 1.5 | 4000 | 30 | 96.34 ± 4.70 | 48.69 ± 2.87 | 152.48 ± 12.54 |
| F23 | 60 | 1.5 | 3000 | 10 | 92.65 ± 4.23 | 48.45 ± 2.82 | 198.57 ± 17.87 |
| F24 | 60 | 1.5 | 3000 | 50 | 90.21 ± 8.21 | 47.15 ± 5.87 | 200.34 ± 16.34 |
| F25 | 60 | 1.5 | 3000 | 30 | 92.65 ± 6.34 | 48.34 ± 2.54 | 199.54 ± 15.83 |
| F26 | 60 | 1.5 | 3000 | 30 | 91.65 ± 1.76 | 48.17 ± 3.87 | 200.53 ± 17.37 |
| F27 | 60 | 1.5 | 3000 | 30 | 90.34 ± 1.64 | 49.34 ± 4.12 | 198.53 ± 18.65 |
| F28 | 60 | 1.5 | 3000 | 30 | 92.06± 4.98 | 49.45 ± 4.98 | 199.45 ± 16.29 |
| F29 | 60 | 1.5 | 3000 | 30 | 92.24 ± 7.23 | 49.56 ± 2.75 | 195.56 ± 19.34 |

**Determination of particle size, polydispersity index (PDI) and zeta potential**

Average particle size, polydispersity index (PDI) and zeta potential of rosuvastatin calcium loaded SLN dispersion was measured by photon correlation spectroscopy (PCS) using a Malvern instruments, HAS 3000: Malvern, U.K.). All samples were diluted in 1:10 ratio with double distilled water to get optimum counts. The analysis was performed at 25°C with the angle of detection 90° (Abdul Hasan Sathali A., *et al* 2013).

**Determination of entrapment efficiency and drug loading**

To determine the % entrapment efficiency and % drug loading a fixed quantity of SLNs dispersion (10 ml) was taken in a centrifuge tube and centrifuged at 18,000 rpm for 20 min at

room temperature (Remi Instruments Pvt. Ltd, India), the lipid portion was isolated and the absorbance of the drug in the supernatant was determined spectrophotometically at 244 nm (Shimadzu 1800, Japan). The % entrapment efficiency and % drug loading were calculated by using the following equation:

Drug Entrapment Efficiency (%) = $(Wt - Ws) / Wt \times 100$

Drug Loading (%) = $(Wt - Ws) / (Wt - Ws + W_L) \times 100$

Where Wt is the total weight of drug used, Ws weight of drug in the supernatant, and $W_L$ is the weight of the lipid used in preparinf the SLNs (Yasir M., *et al* 2014).

**Transmission electron microscopy (TEM)**

Transmission electron microscopy (TEM) was used to investigate the surface morphology of the particles. The SLNs sample was observed in the form of aqueous dispersion using (TEM, Philips CM 10, Holland). For evaluation a drop (approx. 10µL) of the SLNs dispersions was suitably diluted with distilled water (1:100), filtered (0.22µm) and applied on carbon coated grid with 2% phosphotungstic acid solution (PTA) and it was left for 30 sec. The dried coated grid was taken on a slide and covered with a cover slip and placed on 400-mesh copper grids with films for observation of surface morphology (Yasir M., *et al* 2014).

**RESULT AND DISCUSSION**

A four factor, three level Central Composite design (version 8.0.7.1, stat-ease, Inc, Minneapolis, Minnesota, USA) was employed to optimize the formulation (Table 1). For four factors, the central composite design offers some advantage in requiring a fewer number of runs over the three level full factorial designs. In full factorial designs, as number of factors increase there is increase in number of trial runs exponentially, such as $4^3$=64, but with central composite design optimization can be completed with 29 experiments with six centre points.

Total 29 runs with six centre points were generated and their responses are shown in Table 2 and 3. The ranges of Y1, Y2 and Y3 for all formulations were 84.65 ± 8.56% to 98.45 ± 4.34, 32.56 ± 3.65 to 58.71 ± 5.65 and 130.34 ± 17.32 to 245.02 ± 12.76. As per results, entrapment efficiency (Y1), drug loading (Y2) and particle size (Y3) were fitted with quadratic model and insignificant lack of fit ($P > 0.05$). The positive sign of the factors represent a synergistic effect on the response, while a negative sign means an antagonist relationship. It was observed that all the four independent variables, i.e. the Lipid [Stearic

acid (mg)] concentration (A), Surfactant [Poloxamer 407 & Tween 80 in 1:1 (% w/v)] concentration (B), Stirring speed (rpm) (C) and Drug concentration (D) had a significant effect on the three responses, i.e. drug entrapment efficiency (Y1), drug loading (Y2) and mean particles size (Y3).

**Response analysis through polynomial equation**

The second order polynomial equation relating the response of drug entrapment efficiency (Y1) is given below:

$$Y1 = +91.78 + 0.59A - 0.46B + 1.87C + 0.45D - 0.20AB - 1.17AC + 0.20AD + 0.19BC - 0.41BD - 0.45CD + 1.34A^2 + 0.85B^2 - 0.76C^2 + 0.49D^2 \quad (1)$$

The model F-value of 29.01 implied that the model was significant ($p < 0.0001$). The 'Lack of Fit F-value' of 0.03 implied that the Lack of Fit is not significant ($p = 0.7854$). In this case as Table 3 shows, ANOVA test indicates that A, B, C, D, AC, BD, CD, $A^2$, $B^2$, $C^2$, $D^2$ were the significant model terms. Positive coefficients of A, C, D, AD, BC, $A^2$, $B^2$ and $D^2$ in equation (1) indicate the synergistic effect on % entrapment efficiency, while B, AB, AC, BD, CD and $C^2$ indicate the antagonistic effect on % entrapment efficiency. The "Pred R-Squared" of 0.8670 was in reasonable agreement with the "Adj R-Squared" of 0.9340, indicating the adequacy of model to predict the response of entrapment efficiency. The 'Adeq Precision' of 25.024 indicated an adequate signal. Therefore this model was used to navigate the design. The 3-D surface plots for percent drug entrapment are shown in Fig.1.

The effect of lipid concentration on % entrapment efficiency depends on the extent of drug solubility in lipid. An increase in % entrapment efficiency from 89.64 (F1) to 92.56 (F2) was observed on increasing the lipid concentration from 40 to 80 mg as shown in Table 2. This is probably due to large amount of lipid present for drug entrapment. On further increasing lipid concentration the entrapment efficiency decreased. This is due to expulsion of drug from particle surface (Ghada A., *et al* 2009). A decrease in % entrapment efficiency from 93.06 (F21) to 84.65 (F20) was observed on increasing surfactant concentration and stirring speed as shown in Table 2 the possible mechanism of this behaviour could be that as the particle size decrease on increasing the stirring speed, the surface area increase. As the surfactant concentration increase at the constant amount of lipid, the surface of the formed SLNs is too small to absorb all surfactant molecules, which will result in the formation of micellar solution of the drug. Hence, the solubility of the drug in water phase

will be increased. Therefore, the drug could partition from SLNs into the formed micelles in the water phase during stirring or washing time (Waree T., *et al* 2007).

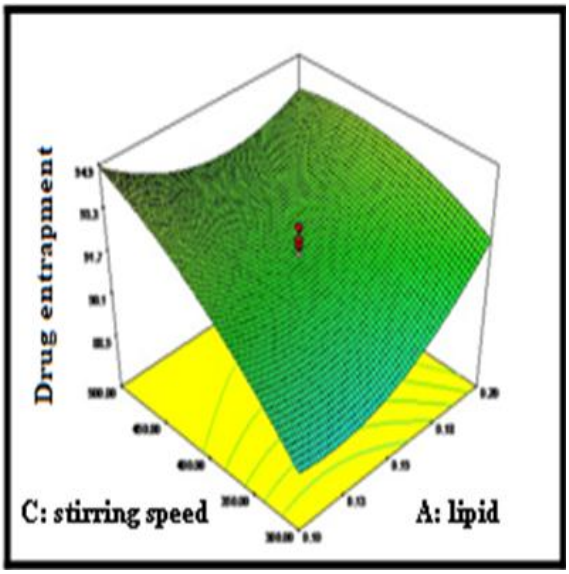
The model proposed the following second-order polynomial equation relating the response of % drug loading (Y2) is given below:

$$Y2 = + 48.09 – 0.67A + 1.51B - 1.94C - 0.72D - 4.05AB + 4.40AC + 1.59AD - 0.66BC + 1.42BD – 0.81CD + 1.24A^2 + 0.45B^2 - 0.81C^2 + 0.39D^2 \quad (2)$$

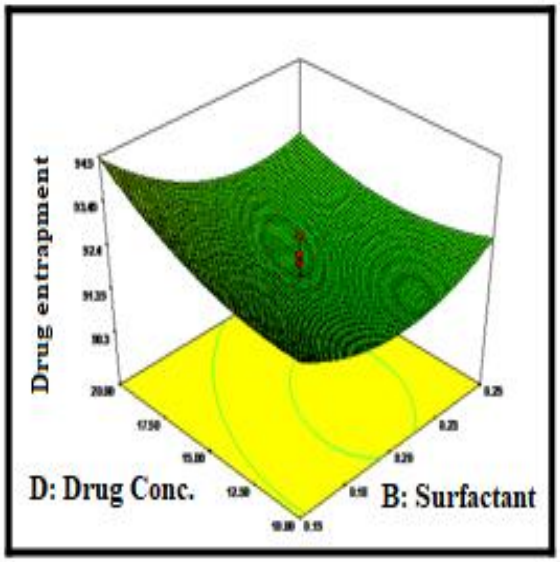
Where, Y2 is the drug loading of nanoparticles, A is lipid concentration, B is surfactant concentration, C is the stirring speed and D is the drug concentration. The model F-value of 53.32 implied that the model was significant ($p < 0.0001$). The 'Lack of Fit F- value' of 0.03 implied that the Lack of Fit was not significant ($p = 0.9609$). In this case as Table 3 shows, ANOVA test indicates that A, B, C, D, AB, AC, AD, BC, BD, CD, $A^2$, $B^2$, $C^2$ and $D^2$ are significant model term. Positive coefficients of B, AC, AD, BD, $A^2$, $B^2$ and $D^2$ in equation (2) indicate the synergistic effect on % drug loading, while negative coefficients of A, C, D, AB, BC, CD and $C^2$ indicate the antagonistic effect on % drug loading. The "Pred R-Squared" of 0.9351 was in reasonable agreement with the "Adj R-Squared" of 0.9492. The 'Adeq Precision' of 36.982 indicated an adequate signal. Therefore this model was used to navigate the design space.

**Table 3: Regression analysis for entrapment efficiency, drug loading and particle size.**

| Factor | Entrapment efficiency | | Drug loading | | Particle size | |
|---|---|---|---|---|---|---|
| | CE | P-value | CE | P-value | CE | P-value |
| **Intercept** | 91.78 | | 48.09 | | 197.92 | |
| A | 0.59 | 0.0001 | -0.67 | 0.0001 | 33.38 | 0.0001 |
| B | -0.46 | 0.0142 | 1.51 | 0.0001 | -0.078 | 0.0001 |
| C | 1.87 | 0.0034 | -1.94 | 0.0001 | 0.57 | 0.0001 |
| D | 0.45 | 0.0023 | -0.72 | 0.0131 | 5.29 | 0.0001 |
| AB | -0.20 | 0.0001 | -4.05 | 0.0001 | 2.01 | 0.0001 |
| AC | -1.17 | 0.0014 | 4.40 | 0.0001 | -2.99 | 0.0001 |
| AD | 0.20 | 0.0025 | 1.59 | 0.1613 | 0.24 | 0.0001 |
| BC | 0.19 | 0.0056 | -0.66 | 0.0001 | -1.29 | 0.0001 |
| BD | -0.41 | 0.0365 | 1.42 | 0.0001 | 0.43 | 0.0001 |
| CD | -0.45 | 0.0256 | -0.81 | 0.0372 | -0.11 | 0.0001 |
| $A^2$ | 1.34 | 0.0001 | 1.24 | 0.0001 | -0.11 | 0.0001 |
| $B^2$ | 0.85 | 0.0001 | 0.45 | 0.0001 | -1.16 | 0.0001 |
| $C^2$ | -0.76 | 0.0021 | 0.71 | 0.0001 | -0.76 | 0.0001 |
| $D^2$ | 0.49 | 0.0001 | 0.39 | 0.0881 | 2.63 | 0.0001 |
| **Lack to fit values** | | | | | | |
| F-value | 0.03 | | 0.03 | | 0.93 | |
| P-value | 0.7854 | | 0.9609 | | 0.5831 | |




**(a)**

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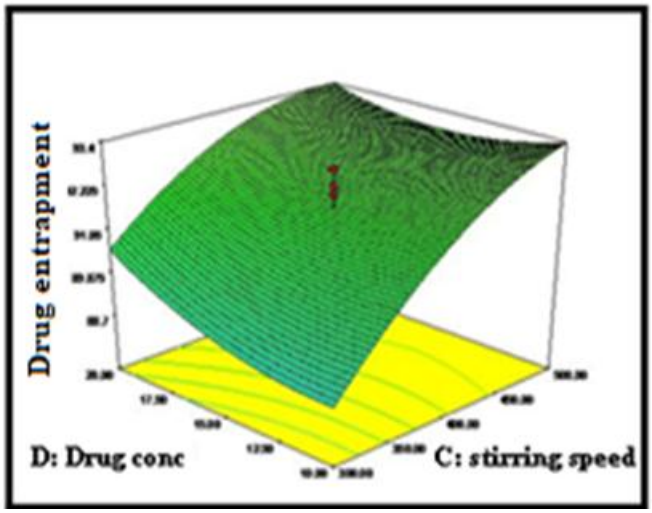



**(c)**

**Fig. 1- (a)-(c) - 3-D surface response plots showing relative effects of process parameters on drug entrapment efficiency.**

The 3-D surface plots for percent drug loading are shown in Fig.2. The effect of lipid concentration on % drug loading is concentration dependent. A decrease in % drug loading from 46.45 (F11) to 32.56 (F12) was observed on increasing the lipid concentration from 40 mg to 80 mg while the stirring speed shows the favourable effect.

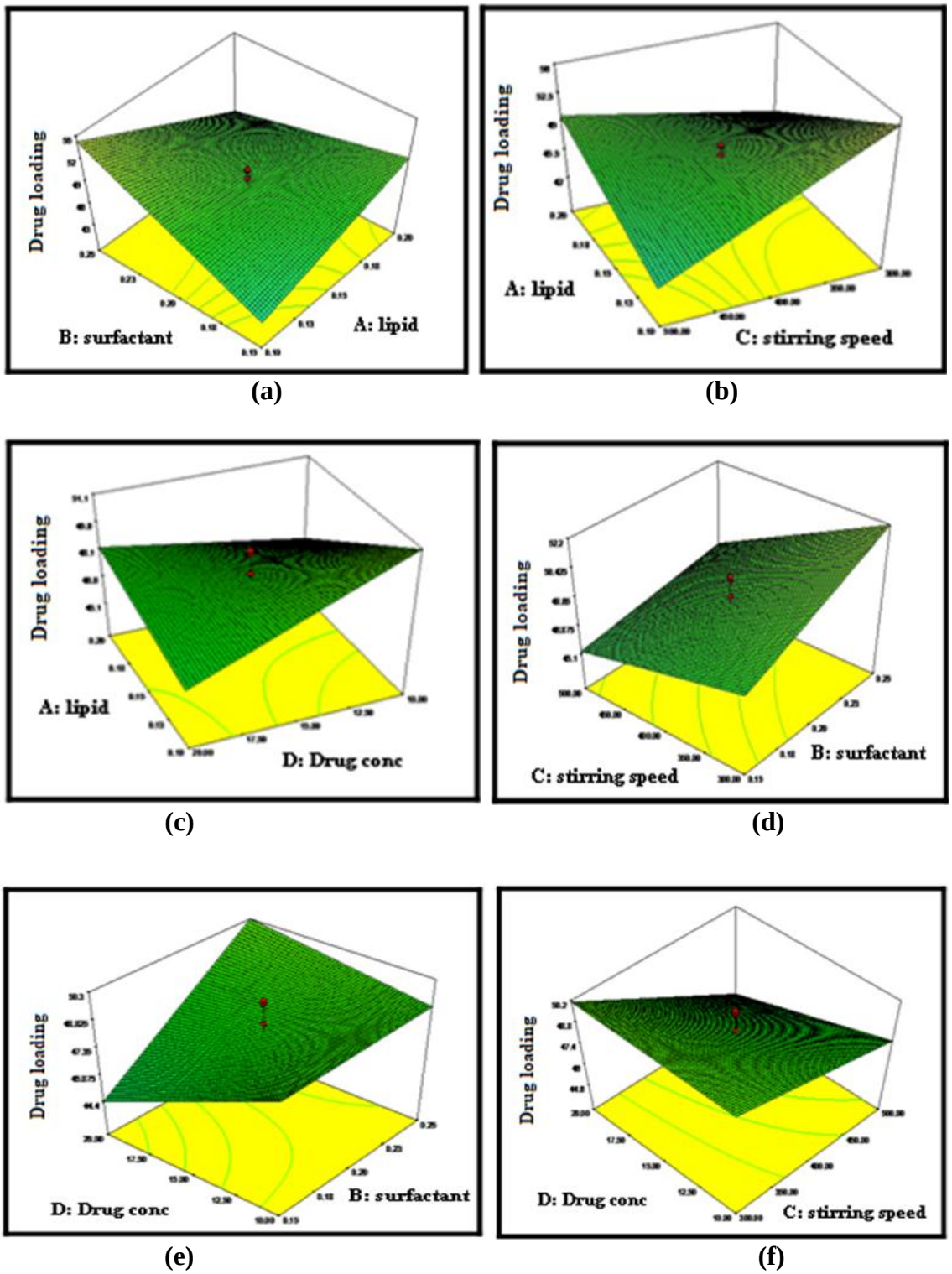



**Fig. 2- (a)-(f) - 3-D surface response plots showing relative effects of process parameters on drug loading.**

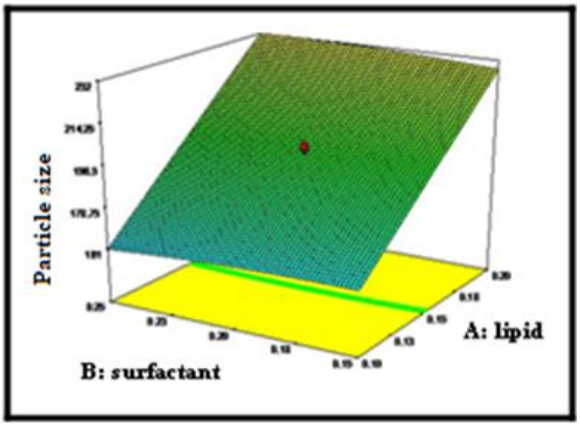
The model proposed the following second-order polynomial equation relating the response of particle size (Y3) is given below:

$$Y3 = + 197.92 + 33.38A - 0.078B + 0.57C + 5.29D + 2.01AB - 2.99AC + 0.24AD - 1.29BC + 0.43BD - 0.11CD - 0.11A^2 - 1.16B^2 - 0.76C^2 + 2.63D^2 \quad (3)$$

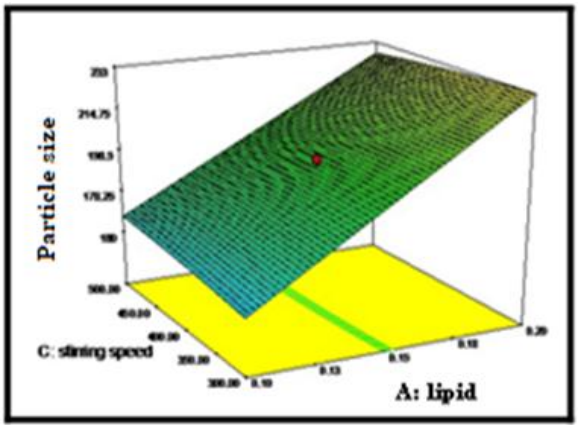
Where, Y3 is the particle size of nanoparticles, A is lipid concentration, B is surfactant concentration, C is the stirring speed and D is the drug concentration. The model F-value of 405.79 implied that the model was significant ($p < 0.0001$). The 'Lack of Fit F-value'of 0.93 implied that the Lack of Fit is not significant ($p = 0.5831$).

In this case as Table 3 shows, the ANNOVA test indicates that A, D, AB, AC, BC, $B^2$ and $D^2$ are significant model terms. Positive coefficients of A, C, D, AB, AD, BD and $D^2$ in equation (3) indicate the synergistic effect on particle size whereas, negative coefficients of B, AC, BC, CD, $A^2$, $B^2$ and $C^2$ shows the antagonistic effect on particle size. The "Pred R- Squared" of 0.9890 was in reasonable agreement with the "Adj R-Squared" of 0.9951. The 'Adeq Precision' of 83.713 indicated an adequate signal. Therefore this model was used to navigate the design space. The 3-D surface plots for particle size are shown in Fig.3.

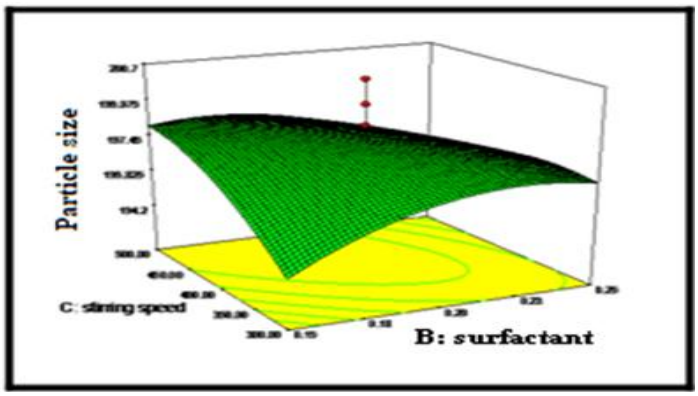
An increase in particle size from 157.54 (F1) to 224.54 (F2) was observed on increasing the lipid concentration from 40 to 80 mg as shown in Table 2. This was probably due to aggregation of particles because of the concentration of surfactant was constant and not enough to form a protective layer on each particle (Pandita D., *et al* 2009). A decrease in particle size from 264.64 nm (F17) to 130.34 nm (F16) was observed on increasing surfactant concentration and stirring speed. The possible mechanism of this behaviour could be that as the particle size decrease on increasing stirring speed. For stabilization of SLNs, the surfactant forms a coating layer on liquid nanoparticles so that they do not coalesce (Alok PS., *et al* 2012).




**(a)**




**(b)**




**(c)**

**Fig. 3- (a)-(c) - 3-D surface response plots showing relative effects of process parameters on particle size.**

**Optimization of data and validation of response surface methodology (RSM)**

The results of the 29 formulations prepared using the experimental design criteria followed for generating the optimized formulation are based on selecting the individual variable and defining their goal and limits. Point prediction software of design expert® was used to determine the optimum values of the factors for maximum entrapment efficiency, drug loading and minimum particle size of nanoparticles. Four formulations (OR1 – OR4) were selected from point prediction software of design expert® and their responses i.e. entrapment efficiency, drug loading and particle size were evaluated. The composition of all optimum check point formulations, their actual and predicted values for the responses and the prediction error are shown in Table 4. The low value of % prediction error assures the validity of generated equations and thus depicts the domain of applicability of RSM model. Finally, the optimum values Stearic acid (80 mg), Surfactant (2.25%), Stirring speed (4000 rpm) and drug (40mg) were selected.

**Table 4- Point prediction check point for optimization, actual value, experimental value and % error (n=3, mean ± SD).**

| Formulation code | Optimized composition | Response | Actual Value | Predicted value | % Error |
|---|---|---|---|---|---|
| OR1 | 80:2:3500:40 | Y1<br>Y2<br>Y3 | 96.14 ± 7.67<br>58.71 ± 5.65<br>130.34 ± 17.32 | 95.97 ± 5.43<br>57.92 ± 4.92<br>130.73 ± 14.72 | 0.17<br>-0.79<br>-0.39 |
| **OR2** | **80:2.25:4000:40** | **Y1<br>Y2<br>Y3** | **97.16 ± 3.73<br>60.34 ± 2.51<br>115.49 ± 2.97** | **94.48 ± 0.88<br>59.01 ± 1.34<br>118.67 ± 1.24** | **2.68<br>1.33<br>-3.18** |
| OR3 | 90:1.5:3500:40 | Y1<br>Y2<br>Y3 | 95.25 ± 5.65<br>57.53 ± 2.56<br>141.65 ± 3.89 | 95.84 ± 4.56<br>56.76 ± 2.77<br>140.41 ± 2.34 | -0.59<br>0.77<br>1.24 |
| OR4 | 90:2:4000:40 | Y1<br>Y2<br>Y3 | 96.77 ± 3.67<br>59.47 ± 1.78<br>124.12 ± 4.67 | 96.23 ± 3.56<br>58.89 ± 1.26<br>126.31 ± 3.95 | 0.54<br>0.58<br>-2.19 |

**[Where, Y1 = Drug entrapment, Y2 = Drug loading and Y3= Particle size].**

A further decrease in particle size and enhancement in % entrapment efficiency and % drug loading was observed. The optimized formulation (OR2) was used for further studies (Table 4).

**Determination of particle size, polydispersity index (PDI) and zeta potential**

A particle size, size distribution and zeta potential curve of optimized formulation (OR2) are shown in Fig. 4 and Fig. 5 respectively. The average particle size, PDI and zeta potential were found to be 115.4 nm, 0.456 and – 18.4 mV respectively.

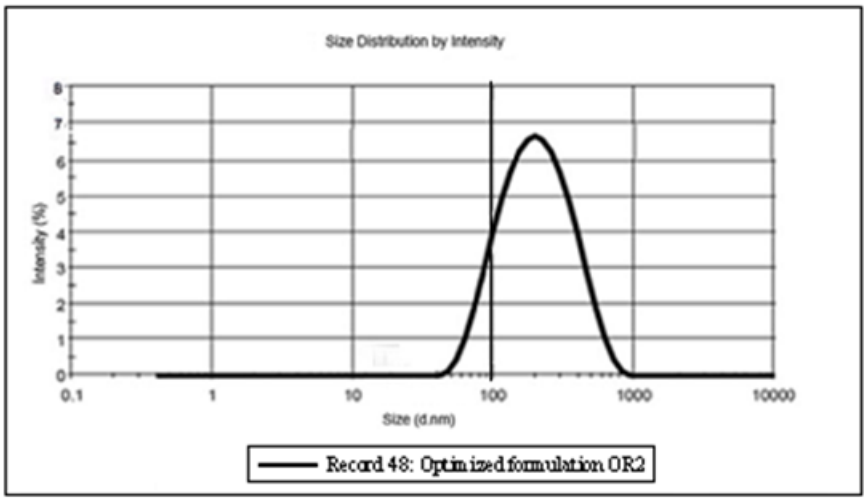



**Fig 4. Particle size distribution curve of optimized formulation (OR2) of rosuvastatin SLNs.**

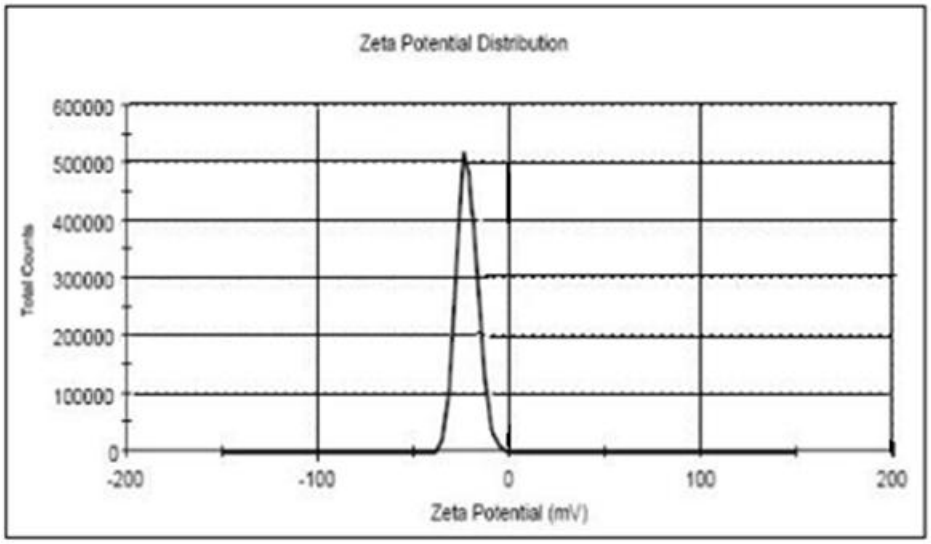



**Fig 5. Zeta potential curve of optimized formulation (OR2) of rosuvastatin SLNs.**

**Determination of entrapment efficiency and drug loading**

The entrapment efficiency and drug loading of optimized formulation (OR2) were found to be 97.16% and 60.34% respectively.

**Transmission electron spectroscopy (TEM)**

The TEM image revealed that the particle size of the optimized formulation (OR2) SLNs were spherical in shape (Fig. 6).

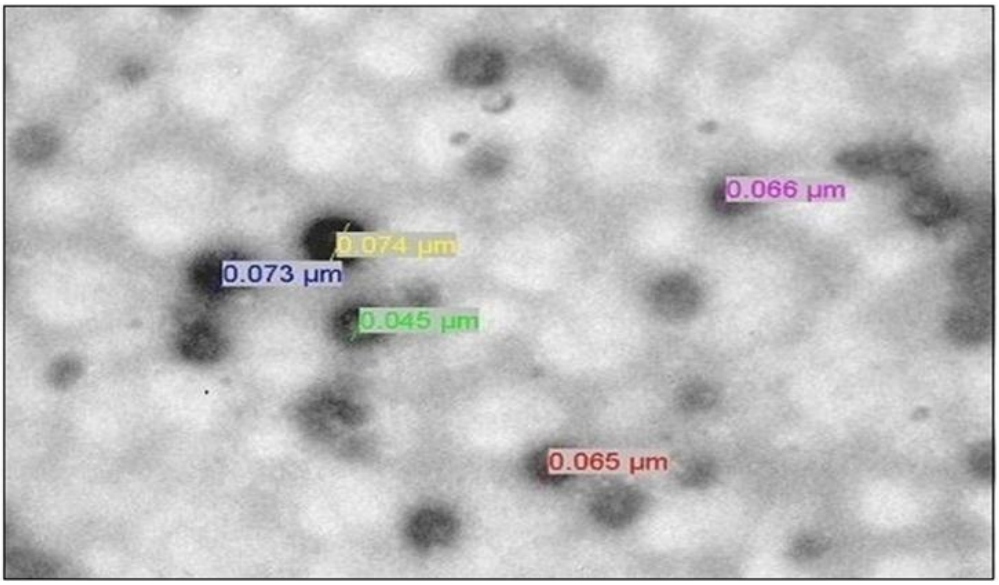



**Fig. 6 Transmission electron microscopy (TEM) image of optimized formulatin (OR2) SLNs.**

## CONCLUSION

In this study, the rosuvastatin loaded SLNs were designed and prepared by modified emulsification diffusion technique. The SLNs were optimized using 4-factor 3 level central composite statistical design. The optimized formulation (OR2) exhibited particle size 115.4 nm, entrapment efficiency 97.16% and drug loading 60.34%. The morphology of optimized SLNs was spherical in shape.

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## CONFLICT OF INTEREST

All authors have none to declare.

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