

Original Article

Development, optimization and evaluation of anti-tubercular drugs loaded pulmonary solid lipid nanoparticles for management of tuberculosis using Box-Behnken design

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ABSTRACT

The main aim of the study is to develop, optimize and characterize the anti-tubercular drugs loaded solid lipid nanoparticles (SLNs) for pulmonary delivery. In this particular study, the SLNs were developed by modified microemulsification method using Box-Behnken design. The Box-Behnken design shows that the low lipid concentration (2.25%), low surfactant concentration (5 ml), and high homogenization speed (5000 rpm) are the effective factors to be considered for the present study. The optimized formulation shows low particle size (241.6 nm), optimum polydispersity index (0.122), and entrapment efficiency 61.28% for rifampicin, and 66.13% for isoniazid with good release characteristics. The spray drying of resultant SLNs showed good powder flow properties.

Keywords: Box-Behnken design, isoniazid, pulmonary drug delivery, rifampicin, solid lipid nanoparticles

INTRODUCTION

Tuberculosis (TB) is a widespread infectious disease that has been identified as second most disease responsible for high mortality rate across the world due to infectious diseases.^[1] Despite its ubiquity, the risk of acquiring TB has varied markedly over both time, place and among members of specific populations.^[2] From the early 16th and 17th centuries to the present day of life, TB stills a global health threat with some new emergence of resistance. This type of emergence poses a vital challenge to control TB cases across the World. Mortality and morbidity rates are high due to this new face of TB. The newer nanotechnologies based drug delivery approaches involving micrometric and nanometric carriers are much needed at this stage. These delivery systems would provide more advantages over conventional systems of treatment by producing enhanced therapeutic efficacy, uniform

distribution of drug molecule to the target site, sustained and controlled release of drug molecules, and lesser side effects. TB is a major reason for billions of active TB cases and deaths across the world. However, there is effective and cheap treatments are available by the government and non-government healthcare authorities.^[3] Over more than five decades, various TB treatment programs still failed to decrease the prevalence of the disease. TB is mainly caused by rod-shaped microorganism

Mycobacterium TB

TB is an airborne infection which is a main reason for high mortality and morbidity. Disease mainly affects the peoples in both developing as well as in developed countries.^[3] Mostly patients having active pulmonary TB may have mild/excessive coughing, fever, fatigue, loss of appetite, loss of weight, night sweats, and bloody sputum.^[4] It is expected that more than 200 million peoples will be affected by this disease and that expected 35 million peoples will die due to disease in this decade if effective control and preventive measures are not taken by the concerns authorities.^[1]

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Several approaches have been employed to deliver the anti-tubercular drugs into the target site such as anti-tubercular drugs loaded liposomal preparation,^[5] niosomes,^[6] lipid microsphere,^[7] porous nanoaggregates,^[8] oral nanoparticles,^[9] microspheres,^[10] and microemulsion.^[11] There are various disadvantages of these novel drug delivery systems such as low drug loading, drug leakage problems, stability issues, and drug release problems. Solid lipid nanoparticles (SLNs) are the best alternative to conventional drug delivery systems.^[12] Solid lipid micro/nanoparticles (SLNs) are colloidal aqueous dispersions comprises melted bio-degradable lipids in water or aqueous solution of surfactant and size ranging between 50 and 1000 nm.^[4] Solid lipid nanoparticles are a emerging field of nanotechnology with potential applications in various areas such as ineffective drug delivery, clinical medicines, cosmeceuticals, and in research also.^[13] SLNs containing lipid concentrations up to 2.5% do not show any cytotoxic effects *in vitro*.^[14] Commonly used lipids are triglycerides, fatty acids, partial glycerides, fats, and waxes. The biggest advantages of SLNs are the use of lipids in the preparation are generally physiological lipids which have the less chances of acute/chronic toxicities.^[15] Lipophilic drugs are better suited for solid lipid nanoparticles.^[13] Various advantages of SLNs are that they are effective in drug targeting purposes. It has high drug stability, high drug loading efficiency, or high % age encapsulation efficiency, ability to encapsulate both lipophilic and hydrophilic drug substances, less or no use of organic solvents, increased bioavailability of entrapped bioactive compounds, less biotoxicity of carrier system, and better protection of incorporated labile compounds.

The main objective of the present study is to develop the SLNs based pulmonary drug delivery system. In this study, the glycerol monostearate has been used as solid lipid base, and tween 80 as surfactant base as the non-ionic surfactants can improve the dissolution of poor aqueous soluble compounds. The Box-Behnken design was used to optimize and evaluate the effect of different processing variables on characteristics of the SLNs. To improve the stability of prepared SLN system and to improve the patient compliance, the spray drying has been done so as to deliver the therapeutic drug concentration at the target site, i.e., lungs through pulmonary route.

MATERIALS AND METHODS

Materials

Isoniazid and Rifampicin was received as a gift sample from BV Patel Centre (Ahmadabad, India), Glyceryl monostearate, Tween 80, heparin, L-leucine, mannitol, Disodium hydrogen phosphate, sodium hydroxide were obtained from CDH Laboratory Reagents (New Delhi, India), HPLC grade acetonitrile and methanol from Rankem Laboratory reagents (New Delhi, India). All the other chemicals used were of analytical grade.

Methods

Preparation of anti-tubercular drugs loaded SLNs using modified microemulsification method^[16]

SLNs incorporating rifampicin and isoniazid were prepared by modified emulsification method reported by Singh *et al.*^[16] Briefly, the

lipidic phase (containing lipid and polysorbate 80) and the aqueous phase (water) were heated ~10° above the lipid melt temperature of 70°C. The rifampicin was added to the lipidic phase under magnetic stirring by using magnetic stirrer (0683, SPINOT Ambala, Haryana, India) at 1500 rpm. The isoniazid was added to aqueous phase separately. The aqueous phase was added to lipid-surfactant mixture containing rifampicin under magnetic stirring at 1500 rpm. Stirring was continued for 2–10 min and the hot microemulsion, thus formed spontaneously and stirring was continued at 5000 rpm for 1.5 h. In the aqueous medium, SLNs were formed by crystallization of the molten lipid droplets present in the microemulsion.

Experimental designs and analysis

Box-Behnken statistical screening design was used to optimize the formulation parameters. Response surface methodologies, such as the Box-Behnken and Central Composite Design (CCD), model possible curvature in the response function. The Box-Behnken design was specifically selected since it requires fewer runs than a CCD in cases of three or four variables. This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube, and center point replicates, whereas the “missing corners” help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases. A design matrix comprising 17 experimental runs was constructed.

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

where, Y is the measured response associated with each factor level combination; b₀ is an intercept, b₁ to b₃ are regression coefficients computed from the observed experimental values of Y from experimental run; and A, B, and C are the coded level of independent variables representing the linear terms. The dependent and independent variables selected are shown in Table 1 along with their low and high levels for the preparation of final optimized formulation. The lipid concentration, surfactant concentration and homogenization speed used for SLNs formation were taken as independent variables. These three independent variables were analyzed by design of two responses which were particle size and polydispersity index (PDI) of SLNs, these two responses were dependent variables. Table 1 is showing the different variables used in optimization of formulation by Box-Behnken design.

Table 1: Different variables used in optimization of formulation by Box-Behnken design

	Factors	Levels	
		Low level	High level
X ₁	Lipid concentration (% age)	1%	4%
X ₂	Surfactant concentration (ml)	5 ml	15 ml
X ₃	Homogenization speed (rpm)	1000 rpm	3000 rpm
	Dependent variables	Constraints	
Y ₁	Particle size (nm)	Minimum	
Y ₂	PDI	Optimum	

PDI: Polydispersity index

Design matrix comprising 17 experimental runs with range of three variables, i.e., lipid concentration (X_1), surfactant concentration (X_2), and homogenization speed (X_3) and the respective observed responses, i.e., particle size (Y_1) and PDI (Y_2). The responses obtained after the preparation of these 17 formulations were filled in the design. This data were analyzed by the design of experiment (Design Expert® Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN). The best-fitting model was selected. The run which showed the minimum particle size range of the SLNs with optimum PDI was found to be optimized. Finally, optimized was selected for further characterization.

Characterization of optimized SLN formulation

The optimized formulation was characterized for different parameters such as particle size, zeta potential, entrapment efficiency, *in vitro* drug release study, and surface morphology.

Particle size distribution and PDI

Particle size and particle size distribution (PDI) of optimized formulations were determined by laser diffractometry using Beckman Coulter Delsa TM Nano C Particle Analyzer. The cuvettes used during the analysis were cleaned with distilled water to reduce any kind of contaminations. For particle size analysis and PDI, 2 ml of formulation was taken into the cuvette. The particle size was measured by selecting particle size SOP in software, i.e., Beckman Coulter Delsa TM Nano C Particle Analyzer.

Zeta potential

Zeta potential was measured by Zetasizer (Beckman Coulter Counter, USA), working on the principle of electrophoretic light scattering, which determines electrophoretic movement of charged particles under an applied electric field from Doppler shift of scattered light, for zeta potential determination. Diluent used was water and temperature was 27.7°C during zeta potential determination.

Surface morphology

The prepared SLNs of rifampicin and isoniazid were subjected to check morphological characters such as size/shape. The aliquots were taken to prepare slide and covered it with coverslip. After this, the slide was examined under Digital Motic Microscope (DMWB series) to visualize the morphological characteristics.

Determination of entrapment efficiency (EE)

The rifampicin and isoniazid loaded SLNs were subjected to centrifugation at 25,000 rpm for 1 h at controlled temperature of 4°C. Then, 1 ml supernatant was taken and diluted up to 10 ml with water in case of isoniazid and with methanolic phosphate buffer solution (PBS) in case of rifampicin. Then, it was analyzed at 262.96 nm for isoniazid and at 478.30nm for rifampicin using spectrophotometer (Perkin Elmer, Japan). Entrapment efficiency was calculated using following formula:

$$\% EE = \frac{\text{Total Amount of drug added} - \text{Amount of drug in supernatant}}{\text{Total amount of drug added}} \times 100$$

In vitro drug release study

The *in vitro* release study, the dialysis tubing method was used. In case of SLNs, the anti-tubercular drugs loaded SLNs were transferred into pre-soaked dialysis membrane or bag. Both ends of dialysis membrane or bag were tied with the help of threads. One end of the bag was tied withstand while the other end was suspended in beaker containing 100 ml of methanolic PBS as a media. The beaker was placed on magnetic stirring with hot plate. The 50 rpm was set for rotation of magnetic bead. The temperature of the whole system was kept constant at 37°C. Samples were collected (1 ml) at different time intervals of 24 h for SLNs and sink conditions were also maintained by replacing the media in same volume. Volumes were made up to 10 ml with methanolic PBS and were assayed spectrophotometrically at 262 nm for isoniazid and 478 nm for rifampicin.

Spray drying of optimized SLNs

The optimized formulation of SLNs was subjected to spray drying after addition of different concentrations of mannitol and L-leucine. L-leucine was added to obtain anti-adherent property so that lipidic formulation does not produce sticky mass. Formulations were prepared by varying the concentration of the mannitol and leucine. The best formulation with added L-leucine and mannitol known concentration was selected. The 1% L-leucine and 1.5% mannitol were found best-fitted concentrations.

Characterization of spray dried powders

Density profile

The spray dried formulations were taken and the bulk volume was noted. The formulations were then tapped 100 times and the tapped volume was noted. Further, the Carr's index was calculated using formula:

$$C = 100[(V_b - V_t)/V_b]$$

Hausner's ratio using formula:

$$H = 100/(100 - C).$$

Where C=Carr's index, V_b =Bulk volume, V_t =Tapped volume, H=Hausner's ratio.

Angle of repose

It was determined by fixed funnel method. The pile of the powders was carefully built up by dropping the powder material through a funnel tip from a height of 2 cm. The angle of repose was calculated by:

$$\tan \theta = h/r$$

Where, θ is angle of repose.

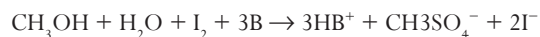
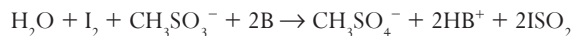
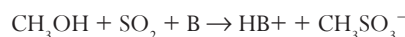
h is height of the particles pile.

r is distance from the center of the pile to the edge.

Moisture content

The moisture content of the dry powders was performed by the Karl Fisher volumetric titration method. This method is designed to

determine the water content in substances, utilizing the quantitative reaction of water with iodine and sulfur dioxide in the presence of a lower alcohol, such as methanol and an organic base (B), such as imidazole or pyridine, as shown in the following formula:



In the coulometric titration method, first, iodine is produced by electrolysis of the reagent containing iodide ions. Then, the moisture content in a sample is determined by measuring the quantity of electricity which is required for the electrolysis (i.e., for the production of iodine), based on the quantitative reaction of the generated iodine with water. The water content was measured in triplicate on approximately.^[17]

Scanning electron microscopy (SEM)

SEM was employed for visualization of shape and size of the prepared SLNs and nanoemulsion. The sample was visualized under a SEM. The magnification giving best resolution was selected. From the SEM images, aerodynamic diameter was calculated.

RESULTS AND DISCUSSION

Optimization of SLNs using design of experts (DOE)

All the responses observed for 17 formulations were simultaneously fitted to quadratic models using Design Expert® software and the response was analyzed by drawing different contour plots and 3-D graph. The effect of different variables was observed on particle size, PDI. Table 2 is showing the different formulations that were prepared according to runs by DOE.

Response Y1 (particle size)

Response Y1 is the response regarding particle size. To analyze this response, there is no need of transformation, the best-fitted model was observed to analyze the response was quadratic model and the comparative values of R², SD and % C.V. are given in Table 3.10 along with the regression equation generated for this response. Only statistically significant ($P < 0.05$) coefficients are included in the equations. Table 3 is showing the summary of results of regression analysis for responses Y1, for fitting to quadratic model.

Response Y2 (PDI)

Variation in the PDI is a function related to the rate at which solid lipid nanocarriers are dispersed in the prepared suspension. Thus a response must be required to be observed for PDI. The best-fitted model for response Y2 is the quadratic model, and the comparative values of R², SD and % C.V. are given in Table 4, along with the regression equation generated for this response. Only statistically significant ($P < 0.05$) coefficients are included in the equations.

On the basis of regression equations, allusion can be drawn from the magnitude and mathematical sign of each coefficient. The regression equation clearly explains that the lipid, surfactant concentration is directly proportional to the PDI, and the homogenization speed is inversely proportional to the PDI. Coefficients with higher order terms or more than one factor term in the regression equation represent quadratic relationships or interaction terms, respectively. It also shows that the relationship between responses and factors is not always linear. Used at different levels in the experiment or when more than one factors are changed simultaneously, a factor can produce different degree of responses.

Response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are very useful to study the interaction effects of the

Table 2: Different formulations those were prepared according to runs by DOE

1	Run	Independent variables			Response	
		A. Lipid conc. (%)	B. Surfactant conc. (ml)	C. Homogenization speed (rpm)	Response Y1 particle size (nm)	Response Y2 PDI
3	1	1	15	3000	241.6	0.122
11	2	2.5	5	5000	316.7	0.209
4	3	4	15	3000	507.4	0.247
8	4	4	10	5000	478.5	0.413
12	5	2.5	15	5000	382.3	0.385
5	6	1	10	1000	201.9	0.225
7	7	1	10	5000	198.6	0.229
6	8	4	10	1000	453.6	0.219
15	9	2.5	10	3000	368.9	0.334
16	10	2.5	10	3000	368.9	0.334
17	11	2.5	10	3000	368.9	0.334
9	12	2.5	5	1000	322.5	0.152
14	13	2.5	10	3000	368.9	0.334
2	14	4	5	3000	421.8	0.397
10	15	2.5	15	1000	341.7	0.211
13	16	2.5	10	3000	368.9	0.334
1	17	1	5	3000	181	0.261

DOE: Design of experts, PDI: Polydispersity index

Table 3: Summary of results of regression analysis for responses Y1, for fitting to quadratic model

Quadratic model	R ²	Adjusted R ²	Predicted R ²	S.D	% age C.V
Response Y1 Particle size	0.9961	0.9911	0.9378	9.03	2.61

Regression equation for quadratic model:
 $SLNs \text{ particle size (Y1)} = +3.68.90 + 129.78A + 28.88B + 7.05C + 6.25AB + 7.05AC + 11.60BC - 19.30A^2 - 11.65B^2 - 16.45C^2$

Table 4: Summary of results of regression analysis for responses Y2, for fitting to quadratic model

Quadratic model	R ²	Adjusted R ²	Predicted R ²	S.D	% age C.V
Response Y2 Particle size	0.7030	0.3211	-3.7525	0.071	25.48%

Regression equation for quadratic model:
 $SLNs \text{ PDI (Y2)} = +0.33 + 0.055A - 6.750E - 0.03B + 0.054C - 2.750E - 0.03AB + 0.048AC + 0.029BC - 0.023A^2 - 0.055B^2 - 0.040C^2$

SLN: Solid lipid nanoparticles, PDI: Polydispersity index

factors on the responses. These types of plots are useful in the study of the effects of two factors on the response at 1 time, while the third factor was kept at a constant level.

Contour plots for particle size

The interaction between the two independent variables at constant value of third independent variables is shown in Figure 1.

- Lipid concentration (A) and surfactant concentration (B) at three constant (low, medium, and high) levels homogenization speed (C).
- Lipid concentration (A) and homogenization speed (C) at three constant (low, medium, and high) levels of surfactant concentration (B).
- Surfactant concentration (B) and homogenization speed (C) at three constant (low, medium, and high) levels of lipid concentration (A).

3-D response surface analysis response Y1 (particle size)

Contour plot analysis can be further explained by 3D-point view in all probable case of interactions, low level of lipid (A), and surfactant (B), as well as high levels of homogenization speed (C), was providing favorable range of minimum particle size. Surface response curves at the intermediate level are given in Figure 2a-c.

Effect of variables on particle size

- Effect of lipid concentration on particle size: The contour plot shows that at low lipid concentration, the prevalence of blue region was high. With the gradient increase in lipid concentration, the acceptable blue regions were decreasing which shows that the size is increasing significantly. By keeping the surfactant ratio constant, the lipid concentration varied and concluded that the particle size was found increases with increase in lipid concentration. This represents that at low to high lipid concentration, particle size increases due to the accumulation of vesicles which forms aggregates and resultant the size increases.
- Effect of surfactant concentration on particle size: The contour plot shows that at low surfactant concentration, the prevalence

of blue region is high as compared to green and red regions. By increasing the surfactant concentration, the corresponding blue region goes on decreasing, and green and red regions goes on increasing. By keeping the concentration of lipid constant, concentration of surfactant varied and concluded that the particle size was found increasing with increase in concentration of surfactant but at the optimum levels of surfactant in formulation, the surfactant molecules stabilizes the lipid particles by providing the protective thick layer over them but further increase in surfactant concentration in formulation may increase the coating over lipid particles which in turns increase the particle size. Further, the very less use of surfactant and high levels of lipid may increase the particle size.

- Effect of homogenization speed on particle size: The contour plot represents that the homogenization speed is inversely proportional to the particle size. This means that by increasing the homogenization speed, the particle size of formulation decreases. The contour plots show that at low homogenization speed the acceptable blue region is fewer whiles the green and red regions are high but on increasing the homogenization speed, it was found that the blue region was increased and red and green region goes on decreased. By keeping the lipid, surfactant concentration constant and homogenization speed varied, it was concluded that optimum to high homogenization speed decreases the particle size by breaking the particles into smaller sizes.

Contour plots for PDI

The interaction between the two independent variables at a constant value of third independent variables is shown in Figure 3.

- Lipid concentration (A) and surfactant concentration (B) at three constant (low, medium, and high) levels homogenization speed (C).
- Lipid concentration (A) and homogenization speed (C) at three constant (low, medium, and high) levels of surfactant concentration (B).
- Surfactant concentration (B) and homogenization speed (C) at three constant (low, medium, and high) levels of lipid concentration (A).

3-D Response surface analysis response Y2 (PDI)

Contour plot analysis can be further explained by 3D-point view in all probable case of interactions. Surface response curves at the intermediate level are given in Figure 4a-c.

Effect of variables on PDI

The contour plot analysis for response of PDI showed same results as in particle size, because if large is the particle size more or higher the value of PDI was found. As such the small particle size shows less PDI value. This is due to PDI is directly related to the particle size. Contour plot analysis shows that optimum to the low concentration of lipids and surfactant and optimum to a high level of homogenization speed was found favorable range for minimum PDI value. The particle size and particle size distribution are the critical factors in the performance of SLNs as formulations with a wide range of particle

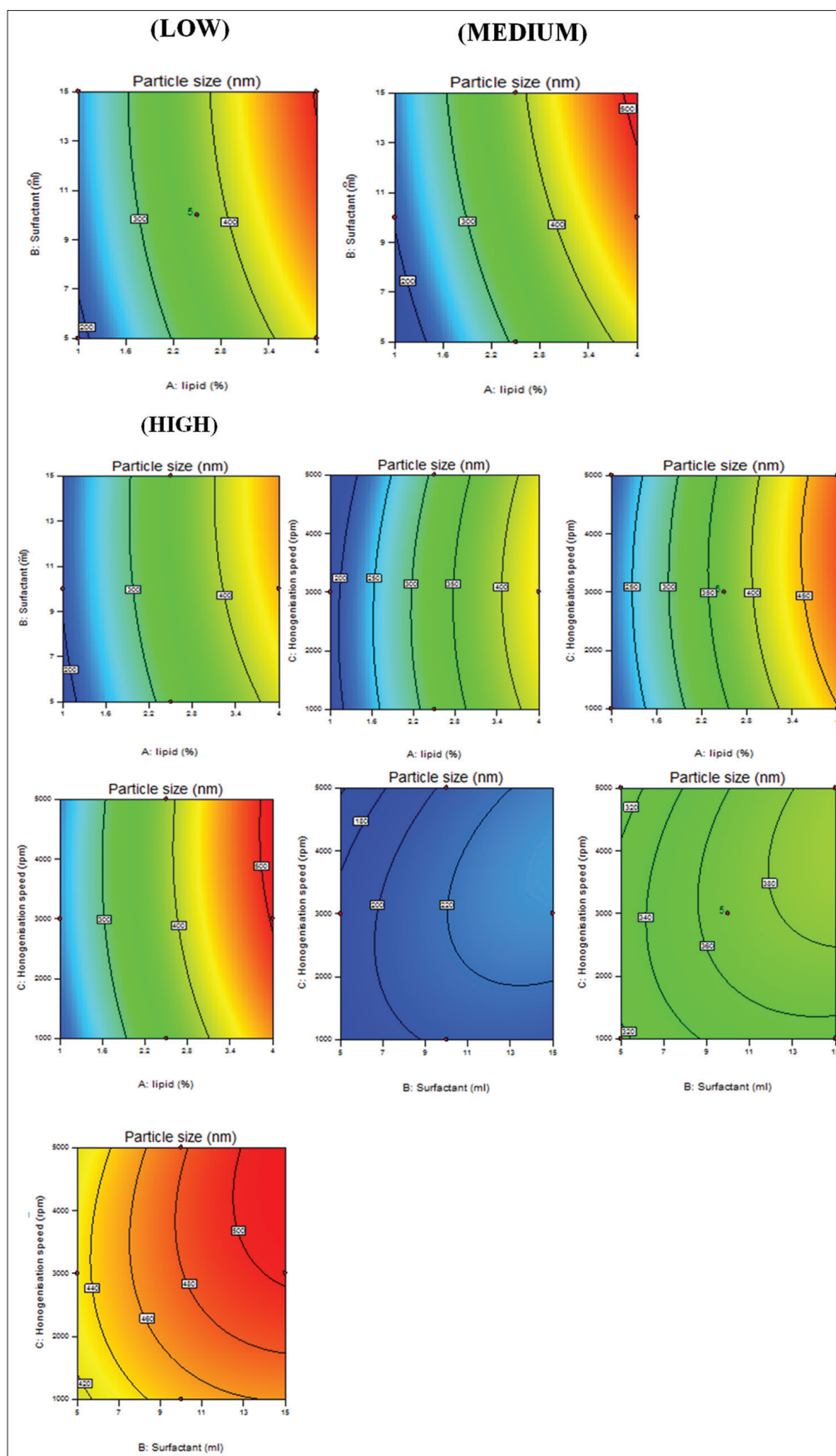


Figure 1: Different contour plots for analysis of response, i.e., particle size

size distribution may show significant affect the drug loading, drug release and other bioavailability issues. The narrow particle size and PDI value are much needed so that particles can be easily taken up by the targeting cells through different biological processes to have targeted drug action.

Validation of DOE

To validate the DOE experiment, the particle size of three checkpoint formulations F1, F2, and F3 [Table 5] were compared with their expected values. There was a significant difference in the observed and the expected values which clearly indicated the validity of the Box-Behnken design. The final selection of the formulation was made, and the optimized formulation was considered for the further study.

Characterization of optimized formulation

Formulation code no. F7 was selected as final optimized formulation as the particle size less than the expected particle

size and the % age encapsulation efficiencies for both the drug molecules is also good.

Particle size and size distribution

The particle size of the optimized SLNs was determined using Zetasizer (Beckman Coulter, USA) was found to be 241.6 nm with PDI of 0.122. The particle size and size distribution of optimized formulation are shown in Figure 5.

Zeta potential

Zeta potential of the optimized formulation was found to be -11.07 mV and is shown in Figure 6. This depicts the high stability of the optimized vesicular formulation.

Surface morphology

Optimized rifampicin/isoniazid loaded SLNs were observed under Motic digital microscope (DMWB B1 series) and SEM for surface morphology. The Motic image of drug loaded SLNs is shown in

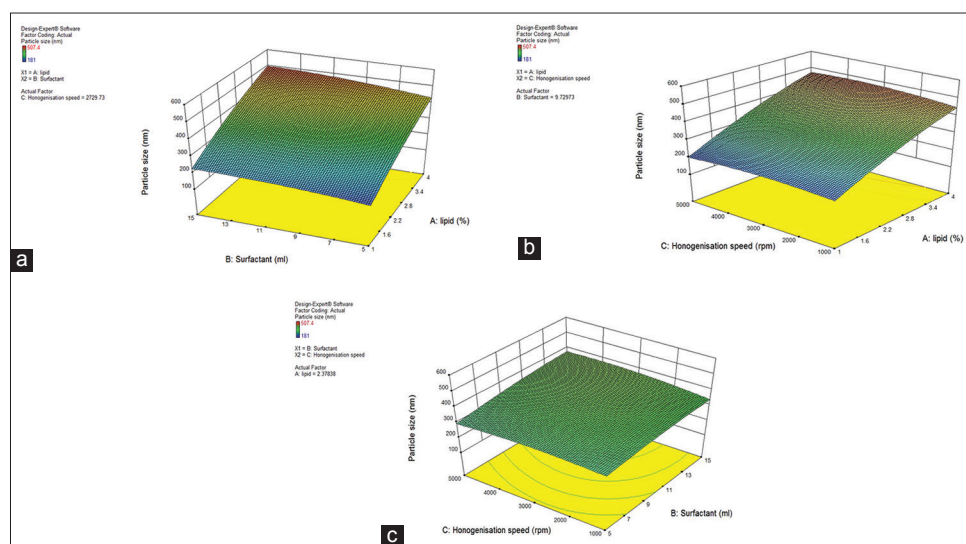


Figure 2: (a) Response surface plot is showing effect of interaction between lipid concentration (A) and surfactant concentration (B) at intermediate levels homogenization speed (C) on particle size of solid lipid nanoparticles (SLNs). (b) Response surface plot is showing effect of interaction between lipid concentration (A) and homogenization speed (C) at intermediate levels of surfactant concentration (B) on particle size of SLNs. (c) Response surface plot showing the effect of interaction between surfactant concentration (B) and homogenization speed (C) at intermediate levels of lipid concentration (A) on the particle size of SLNs

Table 5: Validation of results obtained from DOE

Formulation code	Composition of formulation and parameters				Expected particle size (nm)	Observed particle size (nm)	% age encapsulation of RIF	% age encapsulation of INH
	Lipid concentration (% age)	Surfactant concentration (ml)	Drug content (mg) RIF+INH	Homogenization speed (rpm)				
F1	2.25	5	20	1000	346.7	353.9	42.39	59.23
F2	2.25	10	20	1000	377.1	399.7	35.86	54.78
F3	2.25	15	20	1000	418.2	421.8	49.32	62.86
F4	2.25	5	20	3000	357.4	325.7	58.17	69.42
F5	2.25	10	20	3000	375.6	368.9	41.90	63.93
F6	2.25	15	20	3000	392.9	382.3	52.34	61.43
F7	2.25	5	20	5000	298.9	241.6	61.28	66.13
F8	2.25	10	20	5000	325.4	322.5	59.36	65.18
F9	2.25	15	20	5000	343.9	368.6	54.73	67.82

DOE: Design of experts

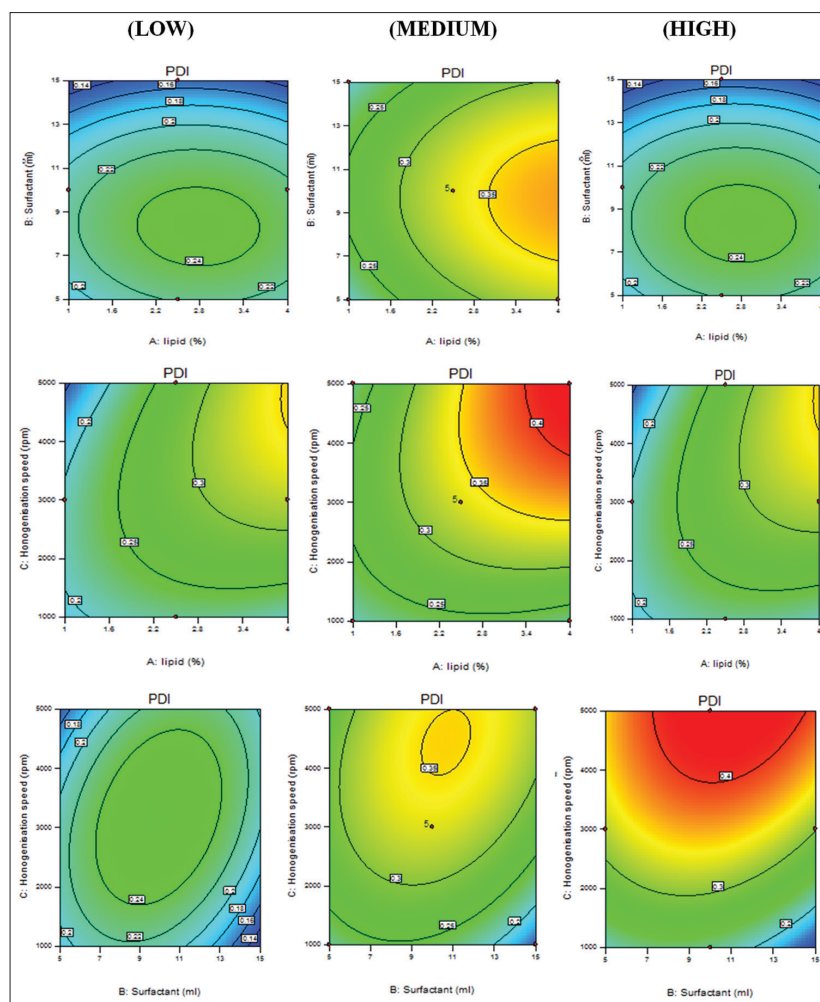


Figure 3: Contour plots for analysis of response (polydispersity index)

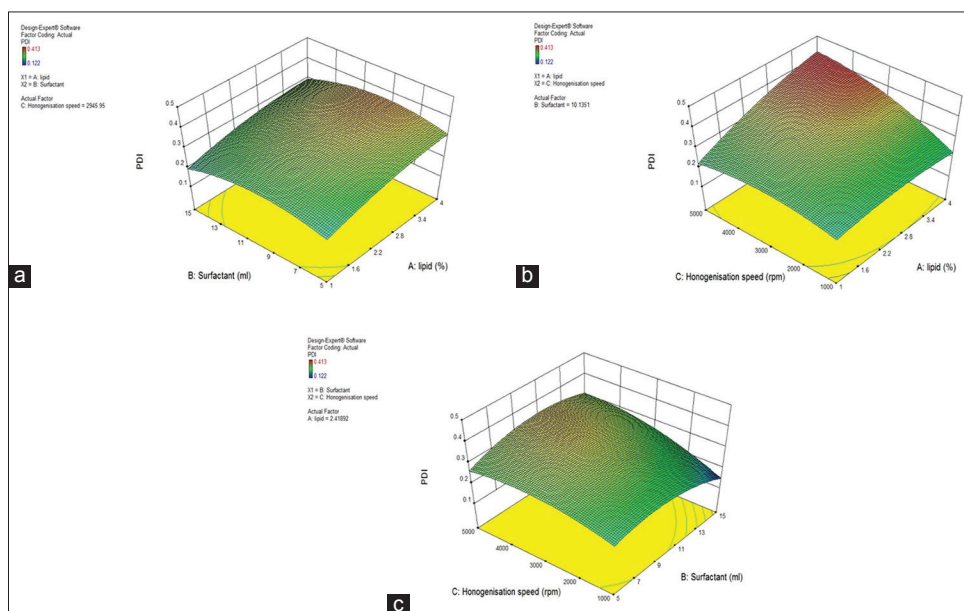


Figure 4: (a) Response surface plot is showing the effect of interaction between lipid concentration (A) and surfactant concentration (B) at intermediate levels homogenization speed (C) on polydispersity index (PDI) of solid lipid nanoparticles (SLNs). (b) Response surface plot is showing effect of interaction between lipid concentration (A) and homogenization speed (C) at intermediate levels of surfactant concentration (B) on PDI of SLNs. (c) Response surface plot showing effect of interaction between surfactant concentration (B) and homogenization speed (C) at intermediate levels of lipid concentration (A) on PDI of SLNs

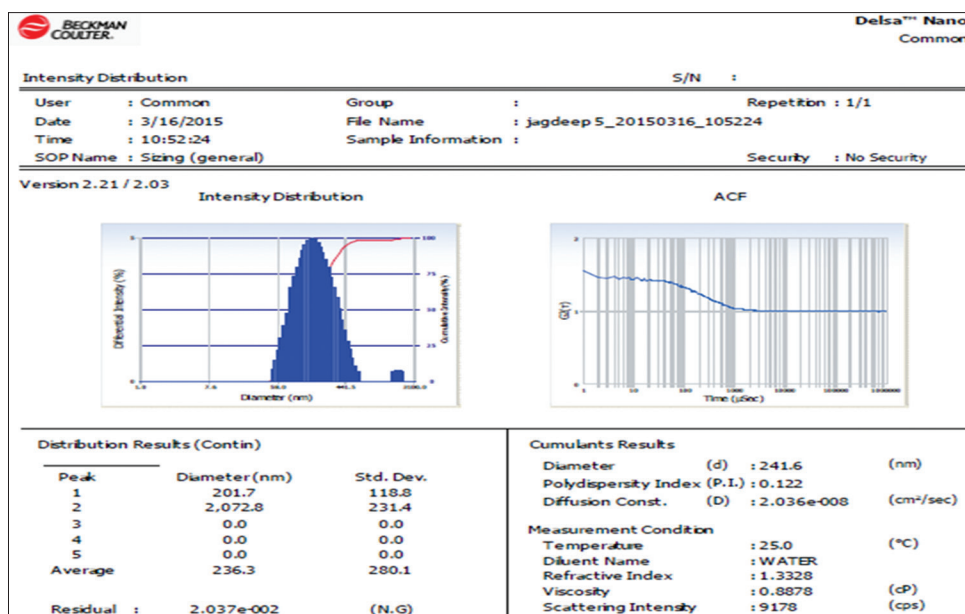


Figure 5: Particle size and size distribution of final optimized formulation

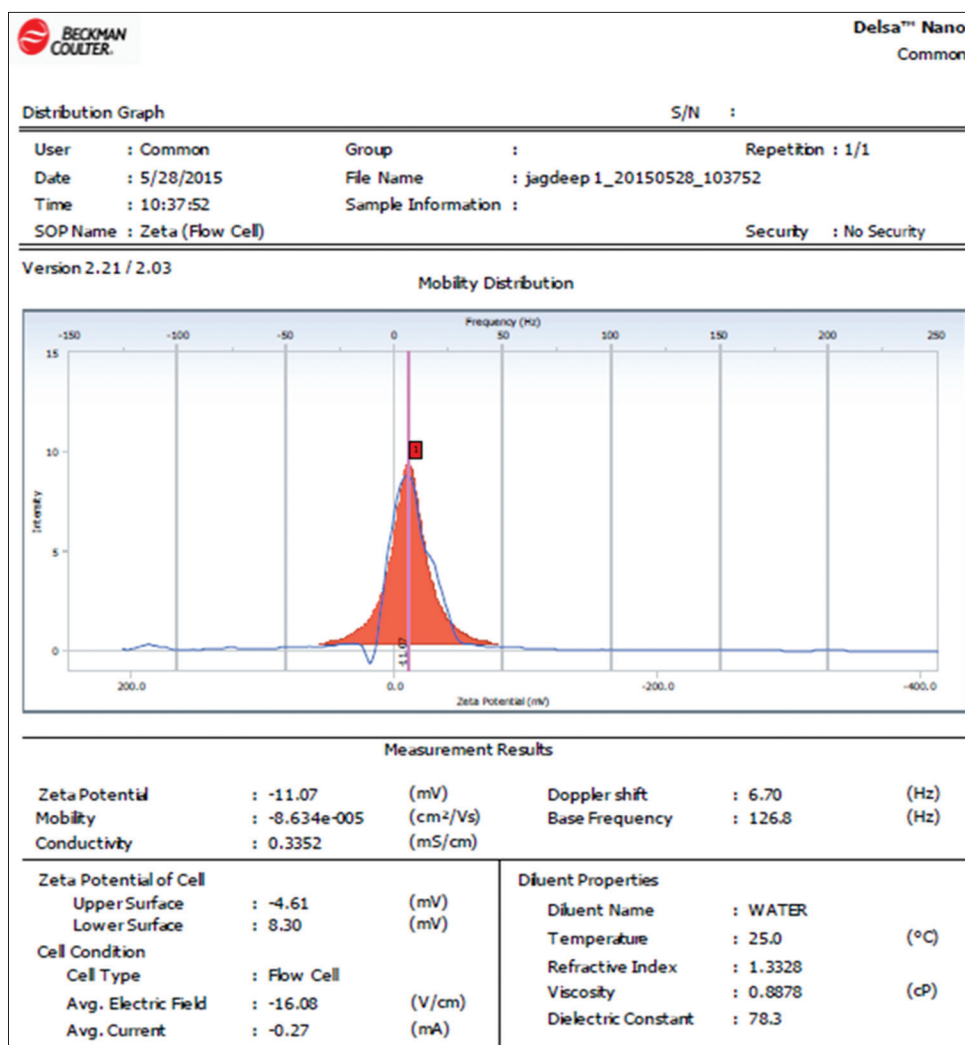


Figure 6: Zeta potential of final optimized formulation

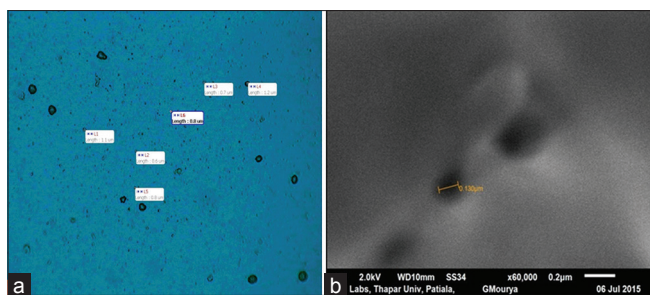


Figure 7: (a) Motic image of final optimized formulation and (b) is the scanning electron microscopy image of final optimized formulation

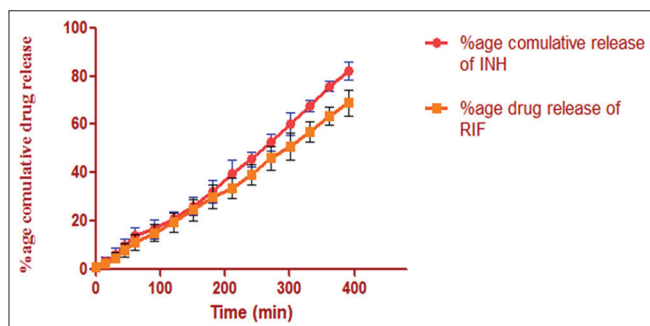


Figure 8: *In vitro* drug release from anti-tuberculosis drug loaded solid lipid nanoparticles

Figure 7a and b is showing the SEM image revealed that uniform size vesicles or particles were formed.

Entrapment efficiency of SLNs

Drug Entrapment of the optimized drug loaded SLNs was found to be 61.28% for RIF and 66.13% for INH, which describes an appreciable drug entrapment in solid lipid nanoparticulate system.

In vitro drug release

The percentage cumulative release of rifampicin from the SLNs was found 79.31%, and isoniazid was found 63.11% in 6.5 h. Figure 8 is showing *in vitro* drug release from anti-TB drug loaded SLNs in phosphate buffer pH 7.4.

CONCLUSION

The present study concluded that the solid lipid nanoparticulate systems are effective against TB as they have good drug loading, controlled drug release for definite period of time and ability to release the drug substance at the target site which will enhance the drug concentration at infected site and will help to reduce the therapy

time and will improve the patient compliance. SLNs are better suited for effective and targeted drug delivery to lungs in TB and able to improve the disease conditions in a better way.

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