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Formulation and Development of Stable Metaxalone Nanosuspension Using 3^2 Factorial Design

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ABSTRACT

Nanosuspensions are the dispersions of nanosized particles in a suitable vehicle prepared using surfactants or solubilizers to aid in nanosize distribution. Nanosuspension is best suited for dosage form development of poorly soluble drugs. According to the biopharmaceutical classification system, drugs with poor solubility fall either in BCS class II or BCS class IV. BCS class II drugs show poor solubility and good permeability; hence their bioavailability problems can be overcome by improving their solubility. Metaxalone is one such BCS class II drug from an oxazolidin-2-one class of centrally acting muscle relaxant drugs, indicated for relief of discomforts associated with acute, painful musculoskeletal conditions. Therefore, in present investigation, nanosuspension of Metaxalone has been formulated as an attempt to improve solubility and hence the overall bioavailability of Metaxalone. Media milling technique has been employed for nanosuspension preparation. Surfactant concentration (Poloxamer 407) and stirring time has been optimized using 3^2 factorial design to achieve desired particle size and saturation solubility responses as dependent variables. The particle size (PS) of 215.3 nm and maximum saturation solubility (SS) of 2805µg/ml was obtained as suggested solutions from factorial design which was further confirmed using check point analysis. Interaction of surfactant concentration and stirring time and their effect on particle size and saturation solubility was predicted using the contour plots and response surface plots. The optimized formulation showed around 99% metaxalone *in vitro* dissolution in comparison to around 46% dissolution from SKELAXIN® tablet at 30 minutes. These methodologies could therefore be employed successfully to improve solubility of any BCS class II drug and to predict effects and interactions of many experimental variables at the same time.

Keywords: Nanosuspension, metaxalone, particle size, Poloxamer.

INTRODUCTION

Nanosuspensions are the dispersions of nanosized particles of pure drug in a suitable vehicle which may be stabilized using surfactant or solubilizers. [1] Nanosuspension is best suited for dosage form development of poorly soluble drugs. According to the Biopharmaceutical Classification System (BCS), drugs with poor solubility fall either in BCS class II or BCS class IV. BCS class II drugs showed poor solubility and good permeability, which means that their bioavailability problems can be overcome by improving the solubility. BCS class IV drugs are characterized by simultaneously poor solubility and

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low permeability and in this case, improving the solubility may or may not little improve the bioavailability. Therefore, in the present investigation, the attempts to find improved formulations are mainly targeting BCS class II drugs. [2]

Metaxalone is an oxazolidin-2-one class of centrally acting muscle relaxant, indicated for relief of discomforts associated with acute, painful musculoskeletal conditions. Literature reported Metaxalone as practically insoluble in water as per US Pharmacopeia definition and it is BCS class II drug. [3-4] This drug has annual US sales of $300 M as on January 2016. [5] Metaxalone has low oral bioavailability, fed and fasted absorption variability and differential oral bioavailability based on age. [3]

To the best of our knowledge, no information is available in the literature on the improvement of Metaxalone dissolution and bioavailability using nanosuspension technology. Hence, present study was aimed to explore stable nanosuspension formulation development using 3^2 factorial design [6], for dissolution improvement compare to current marketed formulation of metaxalone.

Present work describes the formulation and development of stable nanosuspension dosage form of Metaxalone as a model drug by media milling technique. The 3^2 factorial design was employed for optimization of the formulation variables such as concentration of poloxamer 407 and stirring time as independent variables. The particle size (PS) and maximum saturation solubility (SS) were selected as dependent variables. Formulation thus optimized was subjected to *in vitro* dissolution test to evaluate improvement of dissolution in comparison to current marketed formulation of metaxalone, SKELAXIN® tablets.

MATERIALS AND METHODS

Metaxalone, poloxamer 407, polysorbate 80, polyvinylpyrrolidone K30, methanol, tween 20, tween 80 and mannitol were used from Amneal Pharmaceuticals private ltd. Ahmedabad, India. All other chemicals used were of analytical grade.

Method of analysis

UV visible spectrophotometric method was used for analyzing metaxalone concentration as described in SBOA of USFDA for SKELAXIN® tablet. [7] Instrument used for analysis was UV-visible spectrophotometer (UV-1700) of Shimadzu AS, Japan, metaxalone was measured at 280nm and 80% methanol/water as diluent.

Method for preparation of Nanosuspension

Nanosuspension was prepared by media milling technique [8], zirconium oxide beads were used as milling media. In 20 ml glass vial, weighed quantities of zirconium oxide beads were taken and 5 ml distilled water was added in this vial, surfactant and drug were incorporated and comminution was carried out on magnetic stirrer for particular period of time. Batch volume, vessel size, magnetic bead size and stirring speed were kept constant. Simple flow diagram for preparation method of nanosuspension adopted in this study is represented in Figure 1.

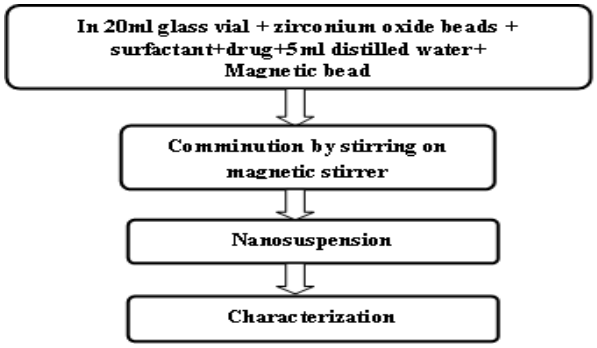

Fig. 1: Flow chart for preparation of metaxalone nanosuspension

Optimization of Formulation Parameters

In the present study, the preliminary parameters were the selection of surfactant, ratio of beads, concentration of drug and concentration of beads in formulation development of metaxalone nanosuspension.

Preliminary parameters were optimized by varying one parameter at a time, while keeping others constant, so that the effect of varied parameter could be evaluated. Each batch was repeated thrice (n = 3) for the confirmation of repeatability. The parameters were optimized to achieve minimum particle size.

Selection of surfactant

Poloxamer 407, polyvinylpyrrolidone K-30 (PVP K-30) and polysorbate 80 at 1% w/v concentration were tried. Suitable surfactant was selected on the basis of the particle size and polydispersivity index (PDI) values.

Ratio of beads

The ratio of beads, ranging from 100:0 to 0:100 of larger: smaller beads were tried. Diameter of smaller beads ranged from 0.4 to 0.7 mm and that of larger beads varied from 1.2 to 1.7 mm.

Concentration of drug

Drug Metaxalone was tried at 0.5%, 0.75% and 1.0% w/v concentrations. As the concentration of drug increased the amount of material required to comminute increased.

Concentration of beads

Depending on the feasibility of stirring on magnetic stirring, the concentration range of beads was selected. Three concentrations of beads were considered for preliminary study, i.e. 80%, 100%, 120% w/v of batch size of 10 ml nanosuspension.

Factorial design for optimization of key parameters

Both particle size and saturation solubility, important features of nanosuspension have been considered to play significant role in the formulation performance, were taken as response or dependent parameters in this study. Preliminary trials were carried out using Poloxamer-407, PVP K-30 and Polysorbate 80 at 1% w/v concentration, beads ratio of 100:0 to 0:100, concentration of Metaxalone at 0.5%, 0.75% and 1% w/v and concentration of beads at 80%, 100% and

120%. On the basis of results of preliminary trials, concentration of Poloxamer 407 was selected as independent variable X_1. The stirring time using different bead ratio and bead concentration plays important role in stable formulation of nanosuspension, hence stirring time was used as independent variable X_2.Thus concentration of Poloxamer 407 and stirring time were taken as independent variables at three levels. Multiple regression analysis, contour plots and 3D response surface plots were used to study the main and interaction effects of the variables on the particle size (Y_1) and saturation solubility (Y_2). The numbers of experiments required in factorial design studies were dependent on the number of independent variables selected. The responses were measured for each trial and then either simple linear equation (1), or interactive equation (2) or quadratic (3) model was fitted by carrying out multiple regression analysis and F-statistic to identify statistically significant terms. [8]

$Y = b_0 + b_1X_1 + b_2X_2$……. (1)

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2$ ……..……..... (2)

$Y = b_0 + b_1X_1 + b_2X_2 + b_1^2X_{11} + b_2{}^2X_{22} + b_{12}X_1X_2$…..... (3)

Where Y is the dependent variable (particle size or saturation solubility), while b_0 is the intercept, b_i (b_1 and b_2), b_{ij} (b_{12}) represents the regression coefficients for the second order polynomial equation and Xi represents the levels of independent formulation variables. Mathematical modelling was carried out by using equation (3) to obtain a second order polynomial equation. [9] The PS and SS obtained at various levels of the two independent variables (X_1 and X_2) were subjected to multiple regressions to yield a second order polynomial equation.

Results of ANOVA of full model and reduced model were carried out and the F-Statistic was applied to check whether the non-significant terms can be omitted or not from the full model.

F calculated was obtained by following formula:

$$\text{F calculated} = \frac{\frac{\text{SSE2} - \text{SSE1}}{\text{No of pameters of omitted}}}{\text{MS of Error of full model}}$$

$$\text{F tabulated} = \frac{\text{DF of FM of Regression} - \text{DF of RM of Regression}}{\text{Error of FM (E1)}}$$

Contour Plots

Contour plots are diagrammatic representation of the values of the response. They are helpful in explaining the relationship between independent and dependent variables. The reduced models were used to plot two dimension contour plots using NCSS software.

Checkpoint Analysis

A check point analysis was performed to confirm the utility of established contour plots in the preparation of Metaxalone nanosuspension. Three values of independent variables (X_1 and X_2) were selected and corresponding values of particle size and saturation solubility were calculated by substituting the values in the reduced polynomial equation. Metaxalone nanosuspensions were prepared experimentally by taking the amounts of the independent variables (X_1 and X_2) on the same check points. Each batch was prepared three times and mean values were determined. Difference of theoretically computed values of PS and SS and the mean values of experimentally obtained PS and SS was compared by using Student's t test method. [10]

Response Surface Plots

Response surface plots are more helpful in understanding both the main and the interaction effects of variables. The effects of different levels of independent variables on the response parameters can also be predicted from the respective response surface plots.

Evaluation parameters

Optimized metaxalone nanosuspension was evaluated for various parameters such as particle size, zeta potential, saturation solubility, DSC, XRPD and dissolution profile. [11]

Determination of particle size (PS)

Mean particle size of nanosuspension was obtained using Malvern Nanoseries Nano-ZS, which follows principle of LASER light diffraction. Nanosuspension was added (after suitable dilution) to the sample cell and put into the sample holder unit and measurement (n=3) was carried out with the help of software of same instrument.

Determination of saturation solubility (SS)

5 ml of nanosuspension was transferred in 10 ml centrifugation tube and centrifuged for 30 minutes, using Sigma centrifuge at 25000rpm. Supernatant was analyzed for metaxalone content by spectrophotometrically using UV-visible spectrophotometer at 280 nm after suitable dilution with 2% SLS aqueous media and 2% SLS aqueous media was used as blank. Concentration of metaxalone was calculated from absorbance using equation derived from the standard curve. The saturation solubility of bulk drug was also measured in the same way.

Stability studies

Stability studies for Metaxalone bulk powder and optimized nanosuspension formulation were conducted as per ICH guidelines by using stability chambers at two different storage conditions; room temperature (25°C & 45% RH) and accelerated condition (45°C & 75% RH) for 3 months.

DSC and XRPD study

Differential scanning calorimetric (DSC) analysis was performed to check any physical incompatibility between drug and used excipients. X-ray powder diffraction (XRPD) study was performed to check presence of polymorphic forms of drug in nanosuspension formulation.

RESULTS AND DISCUSSION

Optimization of key parameters using factorial design

Experimental results for preliminary parameters such as selection of surfactant, ratio of beads, concentration of drug and concentration of beads are shown in tables

2 to 5 below, bold highlighted parameters in each table was selected and kept constant for further experimentation.

Contour Plots

Two dimensional contour plots were constructed for both particle size and saturation solubility as shown in Figure 2 and 3. Figure 2 showed contour plot for PS at prefixed values of 200, 250, 300, 350 and 400nm. The contour plot was found to be non-linear. Thus the relationship between independent variables for PS is not linear.

Table 1: Factors and levels of independent variables in 3^2 full factorial design for formulation of Metaxalone nanosuspension

Independent variables	Levels		
	Low (-1)	Medium (0)	High (+1)
Poloxamer 407 concentration in % w/v (X_1)	0.5%	0.75%	1.0%
Stirring time in hours (X_2)	10	14	18

Table 2: Effect of surfactant on preparation of nanosuspension

Batch No.	Type of surfactant	Conc. of surf. (w/v)	Conc. of drug (w/v)	Larger: smaller beads ratio	Conc. of beads (w/v)	Stirring time	Mean particle size	Avg. PDI
P1	Poloxamer 407						210.1nm	0.198
P2	PVP K-30	1 %	0.5%	0 : 100	100%	14 h	236.5 nm	0.298
P3	Polysorbate 80						320.5 nm	0.188

Table 3: Effect of larger: smaller beads ratio on nanosuspension

Batch no.	Conc. of Poloxamer 407 (w/v)	Conc. of drug (w/v)	Larger: smaller beads Ratio	Conc. of beads (w/v)	Stirring time	Mean particle size(nm)	Avg. PDI
P4			100 : 0			361.9	0.347
P5			75:25			350.0	0.451
P6	1%	0.5%	50:50	100%	14 h	339.6	0.429
P7			25:75			295.2	0.389
P8			0:100			243.9	0.312

Table 4: Effect of concentration of drug on nanosuspension

Batch No.	Conc. of Poloxamer 407 (w/v)	Conc. of drug (w/v)	Conc. of beads (w/v)	Stirring time	Mean particle size (nm)	Avg. PDI
P9		0.5%			243.9	0.312
P10	1%	0.75%	100%	14 h	270.5	0.320
P11		1.0%			320.0	0.312

Table 5: Effect of concentration of beads on nanosuspension

Batch No.	Conc. of Poloxamer 407 (w/v)	Conc. of beads (w/v)	Stirring time	Mean particle size (nm)	Avg. PDI
P12		80%		259.1	0.365
P13	1%w/v	100%	14 h	243.9	0.312
P14		120%		223.5	0.234

Table 6: Check point analysis

Batches	X1	X2	Predicted PS	Experimental PS	Predicted SS	Experimental SS
C1	0.400	10.00	414.59	425.0	1000.2	1050.0
C2	0.682	11.88	354.36	362.0	1589.5	1700.0
C3	0.894	16.11	228.67	235.0	2611.4	2550.0

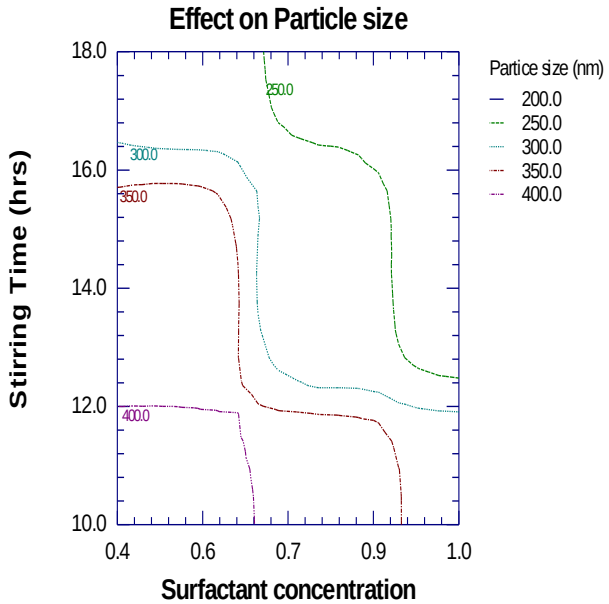

Fig. 2: Contour plot for the effect on particle size

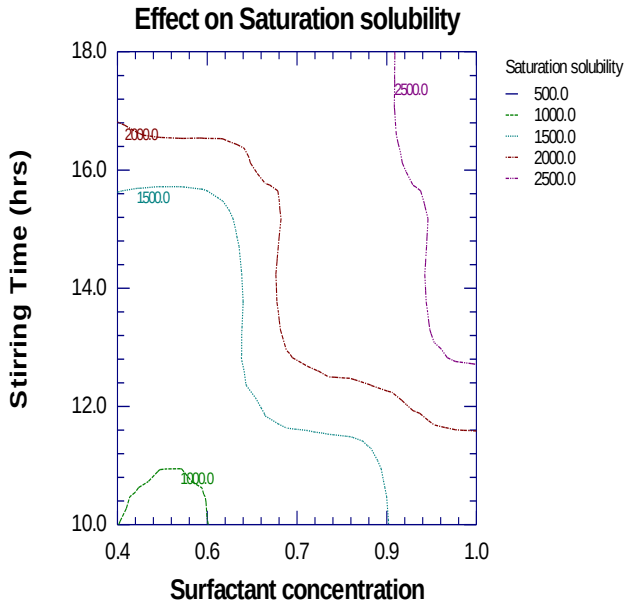

Fig. 3: Contour plot for the effect on saturation solubility

Figure 3 showed contour plot for SS at prefixed values of 500, 1000, 1500, 2000 and 2500µg/ml. The contour plot was found to be non-linear. Hence, the relationship between independent variables for SS is not linear.

Checkpoint Analysis

Three check points C1 to C3 were prepared to validate the evolved equators (Table 6). The computed PS and SS values from the contours are shown in the same table and at these three checkpoints nanosuspensions were prepared experimentally using the same procedure keeping the other process variables as constants. When both experimentally obtained and theoretically computed PS and SS values were compared using student 't' test, the difference was found to be nonsignificant ($p>0.05$) in both cases. This confirms the utility of established contour plots and reduced polynomial equation for both PS and SS in the preparation of Metaxalone nanosuspension.

Response Surface Plots

Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables. Figure 4 showed the response surface plot obtained as a function of Poloxamer 407 concentration and stirring time for PS. A decrease in PS with increase in the Poloxamer 407 concentration and stirring time was observed. Figure 5 showed the response surface plot obtained as a function of 407 concentration and stirring time for SS. Rise in SS was observed with increase in Poloxamer concentration and stirring time.

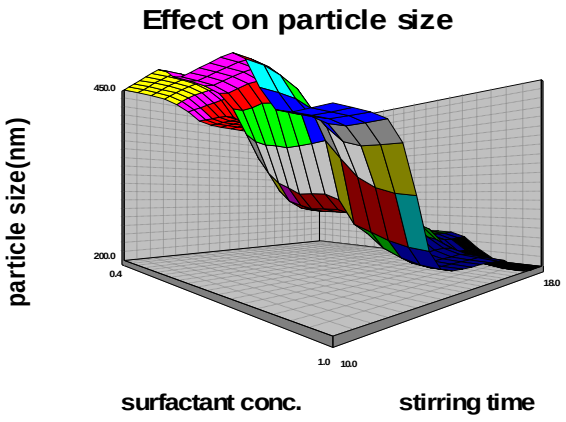

Fig. 4: 3D surface plot for the effect on particle size

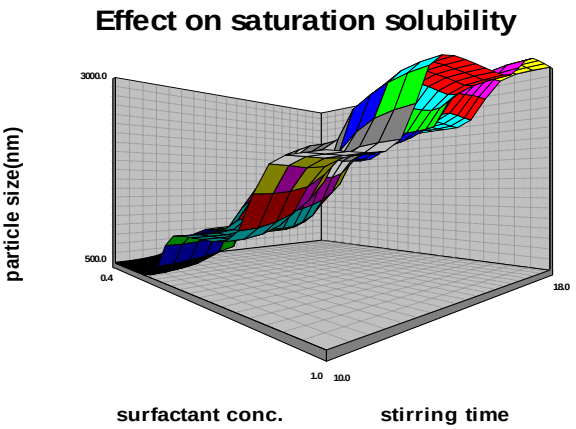

Fig. 5: 3D surface plot for the effect on saturation solubility

Based on optimization of preliminary parameters and by applying 3^2 factorial design below mentioned formulation was finalized as optimized metaxalone nanosuspension formulation.

Table 7: Optimized formulation of Metaxalone nanosuspension

Type of surfactant	Poloxamer 407
Ratio of beads	100% small
Concentration of drug	0.5% w/v
Concentration of beads	120% w/v
Concentration of Poloxamer 407	1.0% w/v
Stirring time	18 hours

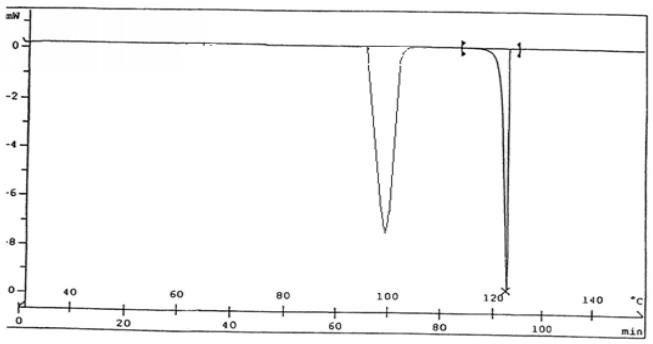

Fig. 6: DSC thermogram of metaxalone nanosuspension

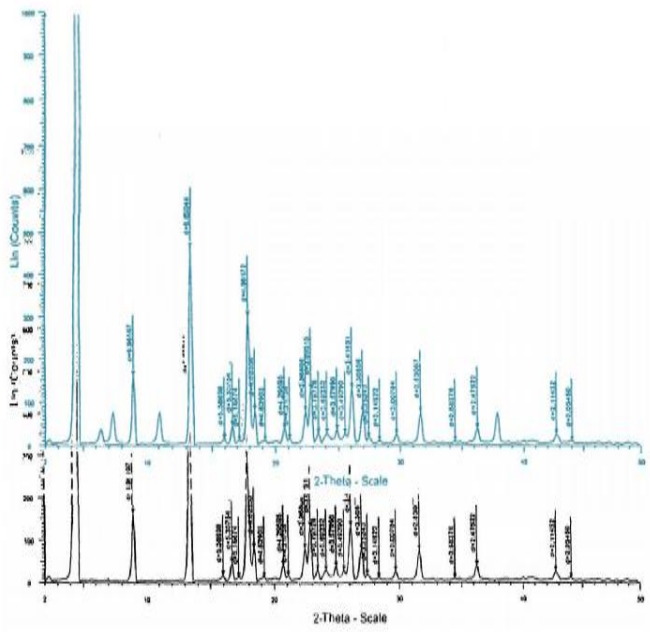

Fig. 7: XRPD overlay of metaxalone powder and metaxalone nanosuspension (bottom to top)

Evaluation of optimized formulation

Particle size and Zeta potential

Optimized metaxalone nanosuspension showed 215.3 ± 5.65 nm particle size, -32.2 ± 0.59mV zeta potential and 7.5 ± 1.2 mg/ml saturation solubility. [12]

DSC and XRPD study

DSC thermogram shown in figure 6 indicated no interaction between metaxalone and excipients during nanosuspension formulation. Similarly, from XRPD overlay of metaxalone bulk powder and metaxalone nanosuspension represented in figure 7, it can be concluded that input polymorphic form of metaxalone remains after nanosuspension formulation as well and there is no polymorphic conversion.

Dissolution study

Based on dissolution results mentioned in table 8, optimized metaxalone nanosuspension showed around 99% drug release within 30 minutes whereas dissolution percentage from metaxalone bulk powder and SKELAXIN® tablet were around 52% and 46% respectively within 30 minutes.

Stability study

Based upon 3 months stability results as mentioned in tables 9 and 10, it was concluded that optimized nanosuspension was stable.

Table 8: Cumulative % drug release from Metaxolone powder, SKELAXIN® tablet and Optimized Nanosuspension.

S. No	Time (Min)	Cumulative % drug release		
		Metaxalone powder	SKELAXIN® tablet	Optimized Nanosuspension
1	0	0	0	0
2	2	8 ± 1.03	4 ± 2.05	55.2 ± 3.65
3	4	17 ± 1.96	10 ± 1.6	65.32 ± 5.69
4	6	26 ± 2.68	22 ± 1.58	76.2 ± 3.65
5	10	33 ± 2.95	30.1 ± 2.5	85.5 ± 2.36
6	15	43 ± 4.96	41 ± 2.6	95.38 ± 3.65
7	30	52 ± 5.67	46.3 ± 3.25	99.2 ± 2.65
8	45	64 ± 4.69	63.2 ± 3.5	99.35 ± 1.65
9	60	72.2 ± 5.69	73 ± 3.68	99.69 ± 0.95
10	80	80.1 ± 1.9	82.2 ± 2.5	Almost same
11	100	85.3 ± 1.52	85.5 ± 2.3	
12	150	85.4 ± 1.4	86 ± 2.5	

Table 9: Particle size analysis upon stability

Stability condition	Formulation	Average of mean particle size (nm ± S.D.)			
		Initial	30 days	60 days	90 days
Room temperature (25°C; 45% RH)	Metaxalone powder	15150 ± 578	15350 ± 758	15380 ± 600	15385 ± 615
	Optimized Nanosuspension	215.3 ± 5.65	275.6 ± 17.6	285 ± 15.8	301.3 ± 25.9
Accelerated condition (45°C; 75% RH)	Metaxalone powder	15150 ± 578	15950 ± 652	16080 ± 620	16120 ± 613
	Optimized Nanosuspension	215.3 ± 5.65	280.5 ± 10.2	289.5 ± 25.6	312.6 ± 33.1

Table 10: Drug content determination of upon stability

Stability condition	Formulation	%Assay (± S.D.)			
		Initial	30days	60days	90days
Room temperature (25°C; 45% RH)	Metaxalone powder	99.9 ± 0.1	99.9 ± 0.3	99.5 ± 0.3	98.6 ± 0.4
	Optimized Nanosuspension	99.73 ± 0.36	99.01 ± 0.36	98.25 ± 0.65	98.96 ± 0.25
Accelerated condition (45°C; 75% RH)	Metaxalone powder	99.9 ± 0.1	98.8 ± 0.2	96.5 ± 0.5	92.2 ± 0.8
	Optimized Nanosuspension	99.73 ± 0.36	98.5 ± 0.5	90.9 ± 1.9	89.2 ± 2.5

The present work had demonstrated the use of 3^2 factorial design, derived reduced polynomial equation, two dimensional contour plots and response surface plots in optimizing formulation variables in the preparation of Metaxalone nanosuspension by media milling technique. By using the factorial design, particle size of 215.3 nm and maximum saturation solubility of 2805µg/ml was achieved with less number of experiments and could predict the PS and SS for various combinations of the formulation variables using the contour plots and response surface plots. These methodologies could therefore be employed successfully to any process which involves the effects and interactions of many experimental variables. Thus desirable goals can be achieved by systematic formulation approach in shortest possible time with reduced number of experiments and thereby reducing the cost of development of the formulations.

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