

International Journal of Pharmaceutical Sciences and Drug Research

2017; 9(4): 149-159



Research Article

ISSN: 0975-248X
CODEN (USA): IJPSP



Optimization of Cilnidipine Nanosuspension Using a Center Composite Design

SK Nagar^{1*}, MM Soniwala²

¹School of Pharmacy, RK University, Rajkot - 360 020, Gujarat, India

²B. K. Mody Government Pharmacy College, Rajkot - 360 003, Gujarat, India

Copyright © 2017 SK Nagar *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

ABSTRACT

The purpose of this investigation was to increase the solubility and dissolution rate of Cilnidipine by the preparation of nanosuspensions with solvent antisolvent method at the laboratory scale. Drug solution of Cilnidipine in acetone this mixture was added to stabilizer solution under continuous homogenization. Central composite design was employed to study the effect of the independent variables on the dependent The relationship between the dependent and independent variables was further Elucidated using multiple liner regression analysis (MLRA) and contour plots. Various process and formulation parameters were screened like homogenization speed, solvent to antisolvent ratio, homogenization time, type of stabilizer, concentration of drug and concentration of stabilizer. Physical stability can be enhancing physical stability of this colloidal system; nanosuspensions were freeze dried using D-mannitol. Seven different stabilizers were tried. Among them Poloxamer 407, Poloxamer 188, PVA and Tween 80 yielded nanosuspension in range of 90 to 350 nm. Freeze dried nanosuspensions were filled in capsules to make a deliverable dosage form and almost 100% drug dissolved in 5 minutes. The outcome of this study reveals the immense potential of nanosuspensions for delivery of Cilnidipine by improving its solubility and dissolution rate. These results show that the preparation of Cilnidipine-loaded nanosuspensions significantly improved the *in vitro* dissolution rate, thus possibly enhancing the fast onset of therapeutic drug effect.

Keywords: Nanosuspension, Cilnidipine, Dissolution, Solubility.

DOI: 10.25004/IJPSDR.2017.090401

Int. J. Pharm. Sci. Drug Res. 2017; 9(4): 149-159

*Corresponding author: Mrs. Swati Nagar

Address: School of Pharmacy, RK University, Rajkot - 360 020, Gujarat, India

E-mail ✉: swati.nagar28@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 January, 2017; **Revised:** 05 June, 2017; **Accepted:** 14 June 2017; **Published:** 24 July 2017

INTRODUCTION

The design and formulation of a dosage form require consideration of the physical, chemical, and biological

characteristics of all the drug substances and pharmaceutical ingredients to be used in its preparation. An important property of a drug

substance is solubility, especially aqueous system solubility. The solubility dissolution behavior of a drug is a key factor to its oral bioavailability. An improvement in the solubility of poorly water-soluble drugs remains one of the most challenging tasks of drug development. The techniques that can generally overcome the problem of solubility are salt formation, micronization, use of surfactant, and use of prodrugs. However, all these techniques have certain limitations. Over the last ten years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications by increasing drug dissolution rate and solubility. For example, nanosizing techniques have been used to enhance dissolution rate by increasing drug surface area, thereby improving the oral bioavailability of poorly water-soluble drugs. Nanosuspensions are submicron colloidal dispersions of pure drug particles in an outer liquid phase. Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions either alone or with a combination of pharmaceutical excipients. The nanosuspension engineering processes currently used are precipitation, high-pressure homogenization, and pearl milling, either in water or in mixtures of water and water-miscible liquids or in non aqueous media (Solubility and dissolution directly link to the bioavailability of API. Various approaches use to increase dissolution and bioavailability of the API. Liquisolid compact is a promising approach to enhance solubility and dissolution rate. Many methods are used to increase solubility and dissolution of the API and bioavailability of the formulation in which nano drug delivery system is mainly used now a days. Nanosuspension is micron size particle of drug in a liquid layer. In the present study solvent antisolvent method is used in which drug solution of Cilnidipine in acetone this mixture was added to stabilizer solution under continuous homogenization.

Cilnidipine is antihypertensive drug having poor aqueous solubility so its dissolution and solubility in aqueous media is lower so those types of drug require enhance solubility & dissolution. The main objective of the formulation development is to enhance dissolution and solubility by using preparing nanosuspension and lyophilize it to get patient compliance.

Central composite design (CCD) is used to study effect of different variable on formulation. In design expert different type of polynomial equation, ANOVA statistic, Lack of feet, F value, P value we get. [1-3] The purpose of using design expert to optimize formulation factor such as Poloxamer concentration and stirring time on a Y1 particle size (nm), Y2 saturation solubility study (mg/ml), Y3 cumulative percentage release at 5 min (CPR 5 min). (%) were taken and a polynomial equation generated and contour plots were drawn and depending on optimize design space optimization of formula done and optimize suspension and marketed tablets are compared. [4-5]

The main aim of the formulation development to prepare nanosuspension of cilnidipine for oral administration, thereby avoiding the use to enabling to increases the saturation solubility, dissolution and oral absorption of cilnidipine. The optimized nanosuspension formulation was evaluated for in vitro dissolution profile in comparison to the pure drug and marketed formulation (Cilacar).

Table 1: Protocol of experiments for optimization stirring speed

Preliminary trial batch	Stirring speed (rpm)	Stirring Time (min)	Initial Observation	Liquid state Stability
P1	5000	10	Aggregates	--
P2	5000	20	Aggregates	--
P3	5000	30	Aggregates	--
P4	10000	10	Bluish tinge	1 Day
P5	10000	20	Bluish tinge	2 Days
P6	10000	30	Watery	2 Days
P7	15000	10	Bluish tinge	2 Days
P8	15000	20	Bluish tinge	4 Days
P9	15000	30	Watery	2 Days
P10	20000	10	Bluish tinge	>15 days
P11	20000	20	Bluish tinge	>15 days
P12	20000	30	Orange yellow	>15 days

Table 2: Protocol of experiments for optimization of stirring time and selection of stabilizers

Batch	Stabilizer concentration (0.5% w/v)	Stirring Time (min)	Initial Observation	Liquid state stability(days)
T1	PVP K30	10	Watery	1
T2	PVP K30	20	Watery	2
T3	Poloxamer 407	10	Bluish tinge	5
T4	Poloxamer 407	20	Bluish tinge	>15
T5	Poloxamer 188	10	Bluish tinge	>15 (no bluish tinge)
T6	Poloxamer 188	20	Bluish tinge (distinct)	>15
T7	HPMC E5	10	Aggregates	-
T8	HPMC E5	20	Bluish tinge	2
T9	PVA	10	Bluish tinge	5
T10	PVA	20	Bluish tinge	>15
T11	Tween 80	10	Bluish tinge	4
T12	Tween 80	20	Bluish tinge	>15
T13	SLS	10	Aggregates	--
T14	SLS	20	Watery	1 hour only

Table 3: Protocol of experiments for optimization of stirring time and selection of stabilizers

Batch	Stabilizer concentration (1% w/v)	Stirring Time (min)	Initial Observation	Liquid state stability(days)
T15	PVP K30	10	Watery	1
T16	PVP K30	20	Watery	2
T17	Poloxamer 407	10	Bluish tinge	6
T18	Poloxamer 407	20	Bluish tinge	>15
T19	Poloxamer 188	10	Bluish tinge	>15 (no bluish tinge)
T20	Poloxamer 188	20	Bluish tinge (Distinct)	>15
T21	HPMC E5	10	Aggregates	--
T22	HPMC E5	20	Bluish tinge	3
T23	PVA	10	Bluish tinge	5
T24	PVA	20	Bluish tinge	>15
T25	Tween 80	10	Bluish tinge	5
T26	Tween 80	20	Bluish tinge	>15
T27	SLS	10	Aggregates	--
T28	SLS	20	Watery	4 hours

MATERIALS AND METHODS

Materials

Cilnidipine was a gift from J. B. Chemicals & pharmaceutical limited (India). Poloxamer 407, 188 was purchased from BASF. PVP K29/32 was gift from signet chemicals.

Formulation development of Nanosuspensions

Preparation of nanosuspension was done by nanoprecipitation method with use of high Speed homogenizer. Weighed quantity of Cilnidipine was dissolved in methanol (solvent). Specified quantities of stabilizers were dissolved in 100 ml distilled water to prepare anti-solvent system. Then, organic solution of drug was added drop by drop with the use of syringe into antisolvent system/ aqueous phase with continuous homogenization condition using high speed homogenizer (Omni PDH, USA). Various process and formulation parameters were optimized. [6-7]

Optimization of process and formulation parameters

Screening of stirring speed

Weighed quantity of 100 mg of drug was mixed in 10 ml of Methanol. From the literature search, Poloxamer 188 was selected for the optimization of stirring speed. Weighed quantity of 500 mg of Poloxamer 188 was mixed in 100 ml distilled water to form antisolvent system. Then organic solution of drug was added drop by drop with the use of syringe into antisolvent system with continuous homogenization at different homogenization speeds and for different homogenization time. Different batches were prepared as shown in Table 1 and screened on the basis of their appearance and stability of liquid state Nanosuspension. [8]

Screening of stirring time and stabilizers

Accurately weighed 100 mg of drug was dissolved in 10 ml of Methanol to form organic solution of drug. Accurately weighed stabilizer was dissolved in 100 ml distilled water to form antisolvent system. Then solution of drug was added drop by drop with the use of syringe into antisolvent system with continuous homogenization at 20,000 rpm and for different stirring time. Batches were prepared as per protocol shown in Table 2 & 3 respectively and screened on the basis of their appearance and stability of liquid state nanosuspension. [8-9]

Table 4: Protocol of experiments for selection of stabilizers

Batch	Stabilizer concentration (0.5% w/v)
B1	Poloxamer 407
B2	Poloxamer 188
B3	PVA
B4	Tween 80

Selection of stabilizer

Accurately weighed 100 mg of drug was mixed in 10 ml of acetone to form organic solution of drug. Accurately weighed 500 mg of selected stabilizers from the results of Table 3 were dissolved in 100 ml distilled water to form antisolvent system. Then organic solution of drug was added drop by drop with the use of syringe into

antisolvent system with continuous homogenization at 20,000 rpm for 20 minutes. Batches were prepared as shown in Table 4. Particle size analysis was performed using Zetasizer.

Experimental Design

In full factorial design, only a limited number of factors can be investigated because an increase in the number of experiments to be carried out. The central composite design allowed us to evaluate two factors at five levels by preparing only thirteen batches. The Poloxamer 407 concentration and Stirring time play a good role in the preparation of Cilnidipine nanosuspension. Center composite design (CCD) was used to study interaction effect of variables Poloxamer 407 concentration (X_1) and Stirring time (X_2) on responses such as particle size, saturation solubility study and cumulative percentage release at 5 min ($CPR_{5 \text{ min}}$). Dependent variables selected on the basis of enhance solubility and dissolution rate. Preliminary trials, two factors used. A design consists of thirteen runs. A second- order quadratic model used and polynomial equation as follows.

$$Y_1 = B_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 + b_6X_1^3 + b_7X_2^3$$

Where, Y_1 was the dependent variables, b_0 mean 13 runs and b_1 was the estimated coefficient for factor X_1 . The main effects (X_1 and X_2) represent the result of changing one factor at a time from its low to high value.

Data analyzed by Microsoft Excel® 2010 version (Microsoft Corp. USA) for regression analysis, Analysis of variance (ANOVA). Response surface and contour plots were plotted to study responses variations against two independent variables using Design Expert® 7.1.5 (Stat- Ease, Inc. Minneapolis, USA). [10]

Criteria for optimized formula

The criterion for optimization of optimum formula was primarily based on result, i.e. Y_1 particle size (nm), Y_2 saturation solubility study (mg/ml), Y_3 cumulative percentage release at 5 min ($CPR_{5 \text{ min}}$). (%) see Table 8. The formulation corresponding to optimum responses were prepared and evaluated for % drug release. As shown in Table 9 the approximations of response values Y_1 particle size (nm), Y_2 saturation solubility study (mg/ml), Y_3 cumulative percentage release at 5 min ($CPR_{5 \text{ min}}$).

Lyophilization of optimized nanosuspensions

Transformation into solid products is required for physical stability. There are two methodologies to convert aqueous dispersions to dry powders, i.e. lyophilization and spray drying. Nanosuspensions contained in a petridish with the addition of cryoprotectant (100% w/w of drug) were frozen in deep freezer at -40°C for 2 hr for primary freezing. The petridish were then transferred to freeze dryer and the lyophilization was carried out under a vacuum at 15 mTorr -54°C for 24 hours. [11-12]

Selection of cryoprotectant

Cryoprotectant selection was carried out using OP 1. Three different cryoprotectant namely D-mannitol, Sucrose, MCC were tried for Lyophilizations. Protocol of experiments for selection of cryoprotectant is shown in Table 11. Based on visual inspection of quality and quantity of the lyophilized product, cryoprotectant was selected.

Optimization of cryoprotectant concentration

On the basis of results of batches L1, L2 and L3, cryoprotectant was selected. For optimization of the cryoprotectant concentration, three different concentrations of cryoprotectant (50%, 100% and 250% w/w of drug) were taken. Batches were taken as shown in Table 12.

Characterization of optimized lyophilized nanosuspension

Average particle size

It is based on the measurement of the Brownian motion of particles. Smaller particles show the faster Brownian motion. Nanosuspension added to the sample cell and analyzes using a soft wear.

XRD analysis

XRD study was performed to determine the change in crystalline nature of the pure drug after formulation of lyophilized nanosuspension. The voltage and the current used were 30 kV and 30 mA respectively.

DSC studies

DSC studies were performed to determine the change in crystallinity of the pure drug after formulation of lyophilized nanosuspension. The DSC thermograms of samples were recorded by weighing nearly 2 mg of sample and hermetically sealing it in an aluminium pan. The pan was pin holed to facilitate the escape of vapours.

To confirm the results of XRD studies, DSC studies were carried out. Thermograms of pure drug, optimized formula and lyophilized formula are shown in figure 10.

The pure drug shows sharp peak at 48.3°C (with an enthalpy of 89.78 J/g) corresponding to its melting point. On the other hand, F1 showed peak at 40.826°C with enthalpy of 23.204 J/g and F2 showed peak at 41.737°C with enthalpy 23.957 J/g. This reduction in enthalpy and melting point can be due to decreased crystallinity of pure drug which is supported by results of XRD analysis. [13-14]

Drug content

Lyophilized nanosuspensions equivalent to 10 mg of drug was taken in 100 ml volumetric flask and diluted up to 100 ml with methanol. The absorbance of resulting solution was measured at 240.0 nm and drug content was calculated. [15]

Table 5: Results of stirring time and stabilizers

Batch	Stabilizer concentration (0.5% w/v)	Particle size nm	PDI	Centrifugation Study	Drug Content %
B1	Poloxamer 407	92.12	0.101	No settling	98.98 %
B2	Poloxamer 188	140.0	0.138	No settling	99.12 %
B3	PVA	350.4	0.062	No settling	99.45%
B4	Tween 80	317.5	0.091	No settling	99.78%

Table 6: Factors and their different levels for Central composite design for preparation liquisolid tablets

Independent Variables	Levels				
	Lowest (-α)	Low (-1)	Medium (0)	High (+1)	Highest (+α)
Poloxamer 407 concentration (%) (X ₁)	0.36	0.4	0.5	0.6	0.64
Stirring time (X ₂) Min	18.59	19	20	21	21.41
Transformed values	-1.414	-1	0	+1	+1.414
Y ₁ particle size (nm)					
Y ₂ saturation solubility study (mg/ml)					
Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)					
Dependent variables					

Table 7: Experimental matrix and results

RUN	Independent Variables		Responses		
	X ₁ (Poloxamer 407 concentration %)	X ₂ (Stirring time)	Y ₁ particle size (nm)	Y ₂ saturation solubility study (mg/ml)	Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)
NS ₁	0.00	1.414	99.00	45.00	95.00
NS ₂	-1.414	0.00	195.00	30.00	70.12
NS ₃	0.00	0.00	92.12	50.00	99.00
NS ₄	0.00	0.00	94.52	49.12	100.00
NS ₅	0.00	0.00	91.45	51.00	98.74
NS ₆	0.00	0.00	93.00	50.00	99.00
NS ₇	-1.00	1.00	145.00	39.00	79.00
NS ₈	1.00	-1.00	120.00	40.00	72.00
NS ₉	0.00	0.00	92.45	50.22	101.00
NS ₁₀	1.00	1.00	105	42.00	85.00
NS ₁₁	1.414	0.00	115	46.00	84.00
NS ₁₂	0.00	-1.414	160	32.00	86.00
NS ₁₃	-1.00	-1.00	160	45.00	76.00

Saturation solubility determination

Saturation solubility of lyophilized nanosuspensions was carried out in phosphate buffers 6.8 known excess

of lyophilized nanosuspension (200 mg) were added to 10 ml distilled water. The sample was rotated at 20 rpm in an orbital shaker at 25± 0.5°C for 24 hours. The

stirred samples were taken in test tubes and centrifuged at 10,000 rpm for 15 minutes. Supernants was then filtered (0.45µm, Gelman, Mumbai) and analyzed spectrophotometrically at 243 nm. Triplicate determination was performed.

In vitro Dissolution

Dissolution studies of nanosuspensions were performed in triplicate using USP Type II dissolution apparatus. nanosuspensions equivalent to 10 mg of cilnidipine were taken and put in dissolution vessels containing 900 ml of 1% SLS in maintained at 37 ± 0.5°C and stirred at 75 rpm. Samples were withdrawn using 0.22µ nylon merck filter at 1 to 10. Samples were suitably diluted and concentration of cilnidipine was determined spectrophotometrically at 243 nm. For lyophilized nanosuspension equivalent to 10 mg of cilnidipine were taken and placed in dissolution vessel. [16-17]

Table 9: Regression analysis of central composite design batches

	Coefficient	Y ₁ particle size (nm)	Y ₂ saturation solubility study (mg/ml)	Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)
Model	β ₀	+92.71	+50.07	+99.55
	β ₁ (X ₁)	-24.14	+3.83	+2.70
	β ₂ (X ₂)	-14.53	+3.05	+3.59
	β ₁₂ (X ₁ X ₂)	+0.00	-0.50	+2.50
	β ₃ (X ₁ ²)	+28.71	-5.85	-12.69
	β ₄ (X ₂ ²)	+15.86	-5.60	-5.97
Cubic	r ²	0.9475	0.9242	0.9286
	Adjusted r ²	0.9100	0.8701	0.8776
	PRESS	5150.64	337.34	766.49

Table 10: Results of optimized batches

S. No.	Responses	Experimental Values	Predicted Values	%Relative Error*
CPB ₁	Y ₁ particle size (nm)	92	92.33	0.35
	Y ₂ saturation solubility study (mg/ml)	49.50	49.79	0.58
	Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)	99	98.37	0.63
CPB ₂	Y ₁ particle size (nm)	92.00	92.0399	0.04
	Y ₂ saturation solubility study (mg/ml)	48.50	48.98	0.97
	Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)	99	98.50	0.50
CPB ₃	Y ₁ particle size (nm)	92.00	92.70	0.76
	Y ₂ saturation solubility study (mg/ml)	50.00	50.08	0.15
	Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)	100	99.55	0.45

* Relative Error (%) = (predicted value - Experimental value)/predicted value×100 %.

Table 11: Selection of cryoprotectant

Batch	Cryoprotectant	Appearance of freeze dried product
L1	D-mannitol	Fluffy powder
L2	Sucrose	Waxy film
L3	MCC	Brittle film

Table 12: Selection of cryoprotectant

Batch Code	Optimize batch	Cryoprotectant Conc (%)	Appearance
L4	OP1	50	Film
L5	OP1	100	Fluffy powder
L6	OP1	250	Fluffy powder
L7	OP2	50	Film
L8	OP2	100	Fluffy powder
L9	OP2	250	Fluffy powder
L10	OP3	50	Film
L11	OP3	100	Film
L12	OP3	250	Film

RESULTS AND DISCUSSION

Optimization of process and formulation parameters Screening of stirring speed

Development of dosage form

Lyophilized nanosuspension equivalent to 10 mg of drug was filled in capsule shells and they were evaluated for parameters.

Disintegration time

Place 1 capsule in each of the six tubes of the basket and, place a disc. Operate the apparatus, using purified water as media maintained at 37 ± 2°C. The disintegration time was noted when there was no residue on the screen of apparatus. [18-20]

Table 8: Dependent variables with constraints in Central Composite Design

Response variables	Constraints
Y ₁ particle size (nm)	91 ≤ Y ₁ ≤ 95
Y ₂ saturation solubility study (mg/ml)	45 ≤ Y ₂ ≤ 51
Y ₃ cumulative percentage release at 5 min (CPR _{5min}). (%)	95 ≤ Y ₂ ≤ 101

At 5000 rpm, nanosuspension was not formed after stirring for 10, 20 or 30 minutes. Homogenization at 10,000 rpm and for 10, 20 and 30 minutes produced nanosuspensions initially but they possessed very low liquid state stability i.e. 2 days only. At 15,000 rpm, after stirring for 10 minutes, nanosuspension got formed with bluish tinge but it was stable for only 2 days. At 15,000 rpm after stirring for 20 minutes, there was distinct bluish tinge but nanosuspension was stable for 4 days only. At 15,000 rpm after stirring for 30 minutes, similar results were obtained in terms of liquid state stability. At 20,000 rpm, stirring for 10, 20 and 30 minutes produced nanosuspensions with stability of more than 15 days.

Conclusion-The dispersion effectiveness was heavily dependent on shear applied and the time the particles spent in the shear zone. A processing time of a few minutes was sufficient to produce the desired nanosuspension. From the above results, it was

concluded that homogenization at 20,000 rpm is vital for preparation of stable nanosuspension.

Table 13: Particle size of nanosuspension

Batch	Z. Avg (d.nm)	Dia. (nm)	PDI
NS ₁	99.00	100.12	0.120
NS ₂	195.00	200.15	0.138
NS ₃	92.12	90.12	0.101
NS ₄	94.52	95.45	0.105
NS ₅	91.45	92.00	0.109
NS ₆	93.00	95.00	0.108
NS ₇	145.00	160.12	0.138
NS ₈	120.00	125.45	0.128
NS ₉	92.45	93.52	0.101
NS ₁₀	105.00	110.00	0.108
NS ₁₁	115.00	116.12	0.184
NS ₁₂	160.00	165.45	0.085
NS ₁₃	160.00	168.23	0.086
CPB ₁	92.33	93.23	0.102
CPB ₂	92.03	92.12	0.108
CPB ₃	92.70	92.15	0.102

Table 14: Drug content of nanosuspension

Batch	Drug content (%)
NS ₁	98.98±0.12
NS ₂	99.16±0.13
NS ₃	99.45±0.56
NS ₄	99.12±0.01
NS ₅	102.01±0.04
NS ₆	101.01±0.1
NS ₇	100.02±0.4
NS ₈	99.12±0.09
NS ₉	100.14±0.2
NS ₁₀	98.23±0.5
NS ₁₁	100.24±0.2
NS ₁₂	98.56±0.1
NS ₁₃	99.67±0.13
CPB ₁	101.01±0.1
CPB ₂	100.02±0.4
CPB ₃	99.12±0.09
L ₅	101.01±0.1
L ₈	100.02±0.4
L ₁₁	101.01±0.1

Table 15: Saturation solubility of nanosuspension

BATCH	Saturation solubility determination (mg/ml)
L ₅	10.00
L ₈	9.52
L ₁₁	9.81
Pure drug	2.0

Table 16: Disintegration time of lyophilized batches

Batch	Disintegration time (min)
C ₁	2 min 30 sec
C ₂	1 min 59 sec
C ₃	2 min 10 sec

Screening of stirring time and stabilizers

From the above results it was concluded that homogenization at 20000 rpm and for 20 minutes with stabilizer concentration (0.5% w/v) would produce nanosuspension of good quality in terms of particle size and liquid state stability. To check whether increasing the stabilizer concentration (1% w/v) would yield nanosuspensions after homogenization at 20,000 rpm and for 10 minutes, further experiments were carried out. The results are shown in Table 3. [9, 21] From the above results it was concluded that, homogenization at

20,000 rpm and for 20 minutes is crucial for preparation of nanosuspension. Moreover, nanosuspensions were not obtained with PVP K30, SLS and HPMC E5 while Poloxamer 407, Poloxamer 188, PVA and Tween 80 nanosuspensions.

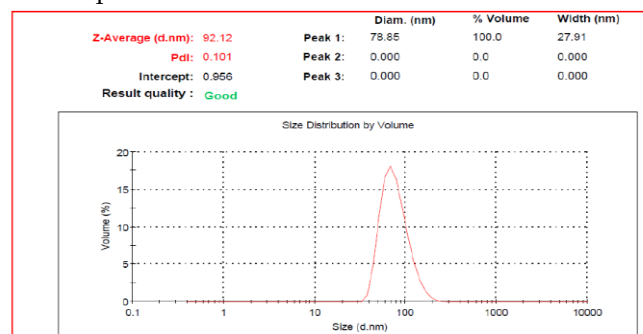


Fig. 1: Particle size analysis of B1 (stabilizer Poloxamer 407)

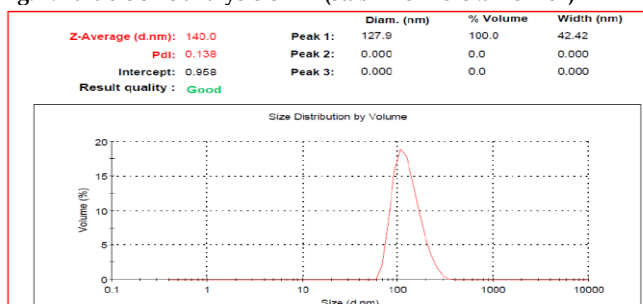


Fig. 2: Particle size analysis of B2 (stabilizer Poloxamer 188)

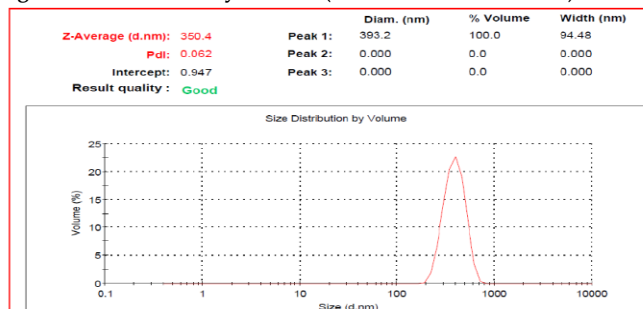


Fig. 3: Particle size analysis of B3 (stabilizer PVA)

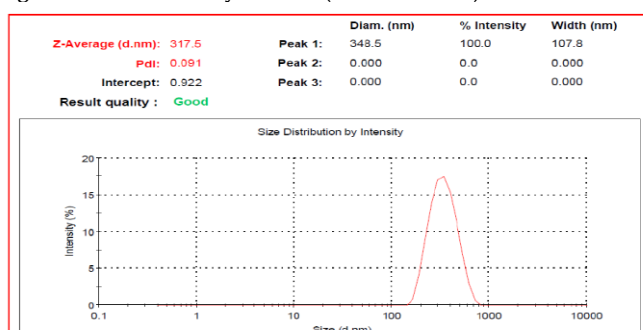


Fig. 4: Particle size analysis of B4 (stabilizer Tween 80)

Selection of stabilizer

Drug content was found out by suitably diluting nanosuspension (equivalent to 10 mg of drug) with methanol and measuring its absorbance at 240 nm. From the absorbance drug content was determined. Centrifugation study was performed on the prepared batches. Centrifugation was carried out at 10,000 rpm for 10 minutes and observed for settling. [22-23]

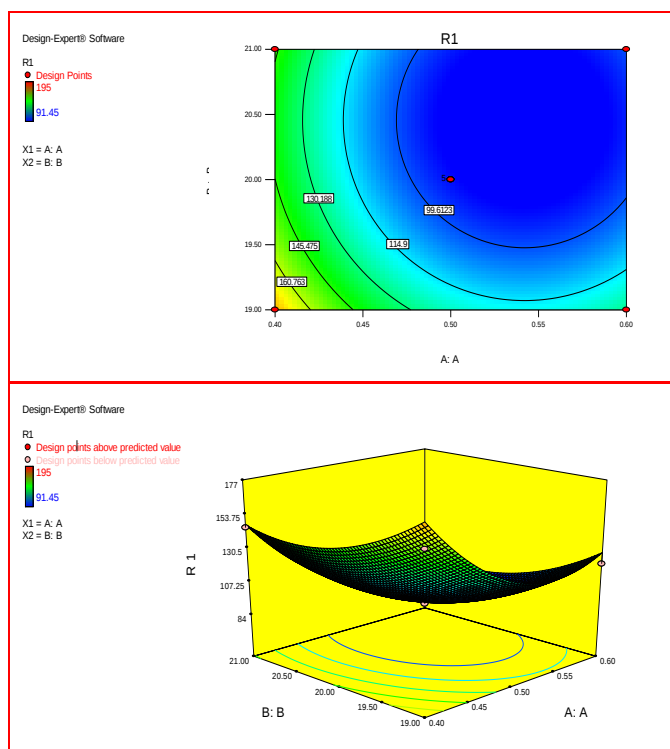


Fig. 5: Response surface plot and contour plot showing the effect of X_1 and X_2 on particle size

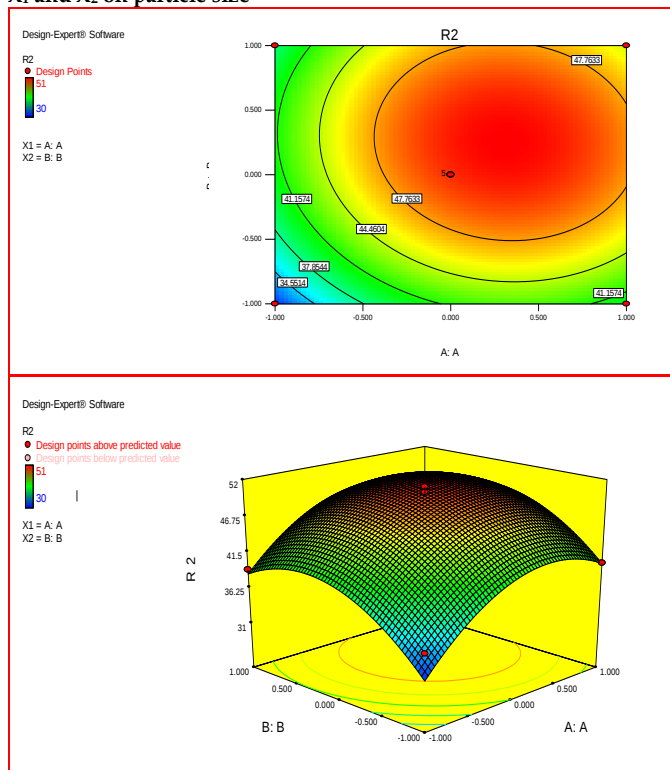


Fig. 6: Response surface plot and contour plot showing the effect of X_1 and X_2 on saturation solubility

Nanosuspensions of Cilnidipine were obtained successfully with Poloxamer 188 & 407, PVA and Tween 80 while PVP K30, SLS and HPMC E5 were unable to produce Nanosuspensions

Both the Poloxamers; Poloxamer 188 and Poloxamer 407 have the same overall molecular structure. On the other hand, Cilnidipine and that of Tween 80 were substantial for formation of smaller particles and stabilization of nanosuspension. As reported by Sepassi

et al., Tween 80 is a molecule of small size (Molecular weight 1310 g/mol) so it forms a thin adsorption layer on the drug nanoparticles and offers a less effective steric stabilization than higher molecular weight polymers. This may be the reason for relatively larger particles of Cilnidipine with Tween 80 (i.e. Mean particle diameter 317.5 nm) than that of with Poloxamers. Vinyl groups of PVA (Polyvinyl alcohol), due to their hydrophobic nature tend to adsorb onto the hydrophobic part of Cilnidipine nanoparticles while -OH extend themselves outside into the aqueous environment and thus providing stabilization to the nanoparticles and preventing agglomeration. -OH bonds of PVA makes hydrogen bonding with water molecules (antisolvent system) and thus viscosity of it increases. [24] SLS is an anionic surfactant which provides electrostatic stabilization. Cilnidipine is a very non-polar molecule (log P 8.6).

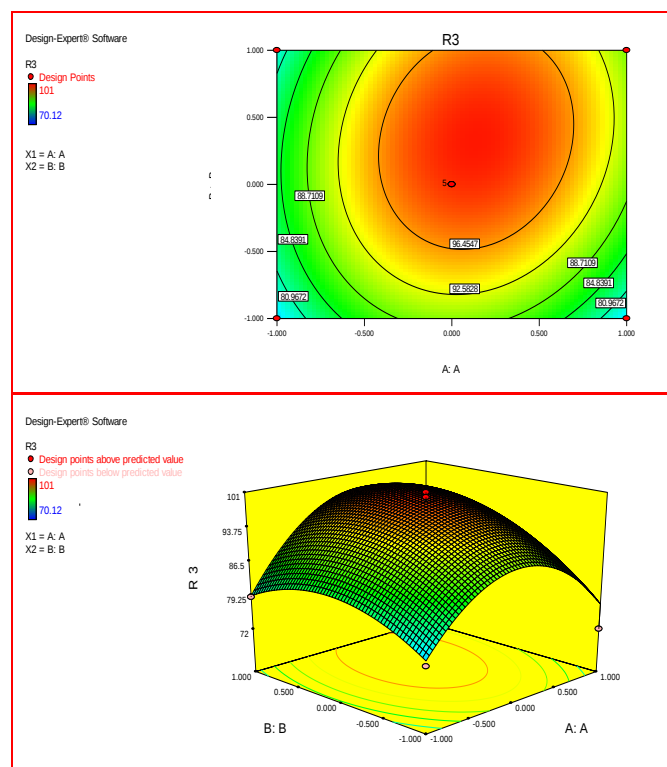


Fig. 7: Response surface plot and contour plot showing the effect of X_1 and X_2 on cumulative percentage release at 5 min (CPR_{5min}). (%)

Among all the stabilizers tried, PVP K30 possesses the highest molecular weight (50,000 g/mol). So due to its higher molecular weight, PVP K30 may exert more kinetic restriction in process of adsorption on the surface of drug nanoparticles and slower diffusion resulting in their inability to produce nanosuspension. The reason for inability of HPMC E5 to produce Cilnidipine nanosuspension remained unknown. As Tween 80 interferes with the drug in assay, it was dropped and not considered for further studies. Centrifugation studies at 10,000 rpm for 10 minutes did not show any settling of particles. This might be due to Brownian motion exhibited by colloidal systems like nanosuspensions which opposes the settling force. [25-26]

Experimental Design

Contour plots and response surface analysis

Three-dimensional response surface plots and Two-dimensional contour plots are presented in Figure 5, 6, 7.

Effect of particle size on nanosuspension

For dependent variable Y_1 if, X_1 from $-\alpha$ to $+\alpha$ level increased and keeping X_2 at lower level particle size decreases from 195 to 114 nm. If keeping X_1 constant and X_2 level increased from $-\alpha$ to $+\alpha$ angle of repose will decrease up to 160 to 99 nm. A lowest particle size of 91.45 was observed with Poloxamer concentration 0.50 and stirring time 20 min (Batch 5) which suggests good particle size. [27-28]

Influence of formulation composition factor on saturation solubility study

For dependent variable Y_2 if, X_1 from $-\alpha$ to $+\alpha$ level increased and keeping X_2 at lower level particle size increases from 30 to 41 mg/ml. If keeping X_1 constant and X_2 level increased from $-\alpha$ to $+\alpha$ angle of repose will increase up to 32 to 48 mg/ml. A highest saturation solubility of 51 mg/ml was observed with Poloxamer concentration 0.50 and stirring time 20 min (Batch 5) which suggests good saturation solubility.

Influence of formulation composition factor on cumulative percentage release at 5 min (CPR_{5 min}). (%)

For dependent variable Y_3 if, X_1 from $-\alpha$ to $+\alpha$ level increased and keeping X_2 at lower level particle size increases from 70 to 84 %. If keeping X_1 constant and X_2 level increased from $-\alpha$ to $+\alpha$ angle of repose will increase up to 86 to 95%. A highest cumulative percentage release at 5 min of 100% was observed with Poloxamer concentration 0.50 and stirring time 0 min (Batch 5) which suggests cumulative percentage release at 5 min.

Optimization of formula and Validation of CCD

After preparing the polynomial equations of the dependent and independent variables, the in situ formulations were optimized for the responses Y_1 , Y_2 and Y_3 . The desirable ranges of these responses were described in Table 10 and Figure 8. Therefore, to verify the evolved models, the optimum formulation was prepared according to the above values of the factors and subjected to the analysis of responses. As shown in Table 10 and Figure 8. It was demonstrating that the observed value of a new batch was quite near to predicted value.

The overlay plot of optimized batch is given in Table 10 Figure 8. The predicted batch shows significant reproducibility within the percentage deviation. From the result shows that the predictive value close to the experimental value so design is significant.

Lyophilization of optimized nanosuspensions

As shown in Table 11, D-Mannitol served the purpose of cryoprotection very well while sucrose gave waxy film and microcrystalline cellulose gave brittle film. This may be due to recrystallization of mannitol around drug nanoparticles during water removal process. D-Mannitol would be the most suitable cryoprotectant. [29]

Optimization of cryoprotectant concentration

D-Mannitol 50% w/w of drug could not provide sufficient cryoprotection while fluffy powder with good yield was obtained with 100 and 250% D-Mannitol. At low concentration D-Mannitol could not provide adequate cryoprotection. Unavailability of molecularly dispersed D-Mannitol, in case of 50% w/w of drug can be the reason for film formation. From the above results, it was concluded that d-Mannitol 100% w/w of drug would be sufficient for cryoprotectant effect. [30-31]

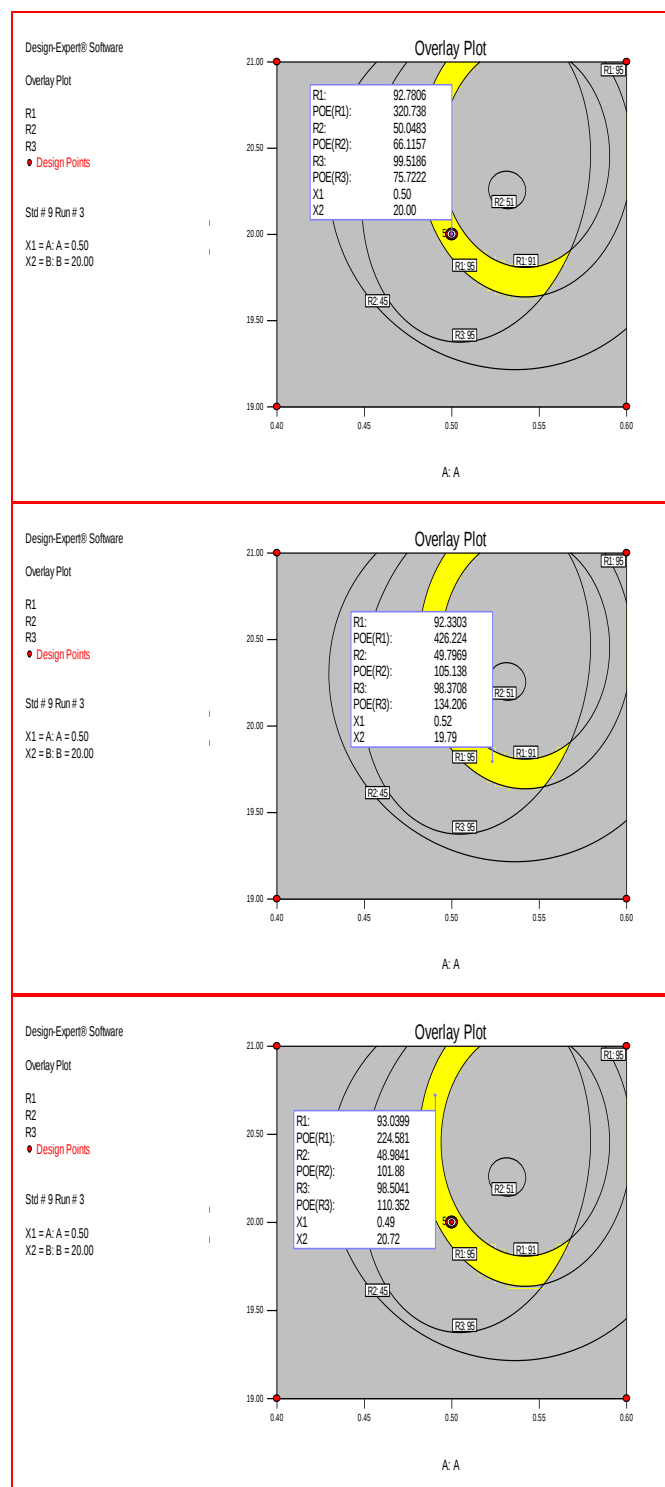


Fig. 8: Overlay plot showing combined effects of factors X_1 and X_2 on Y_1 , Y_2 , Y_3

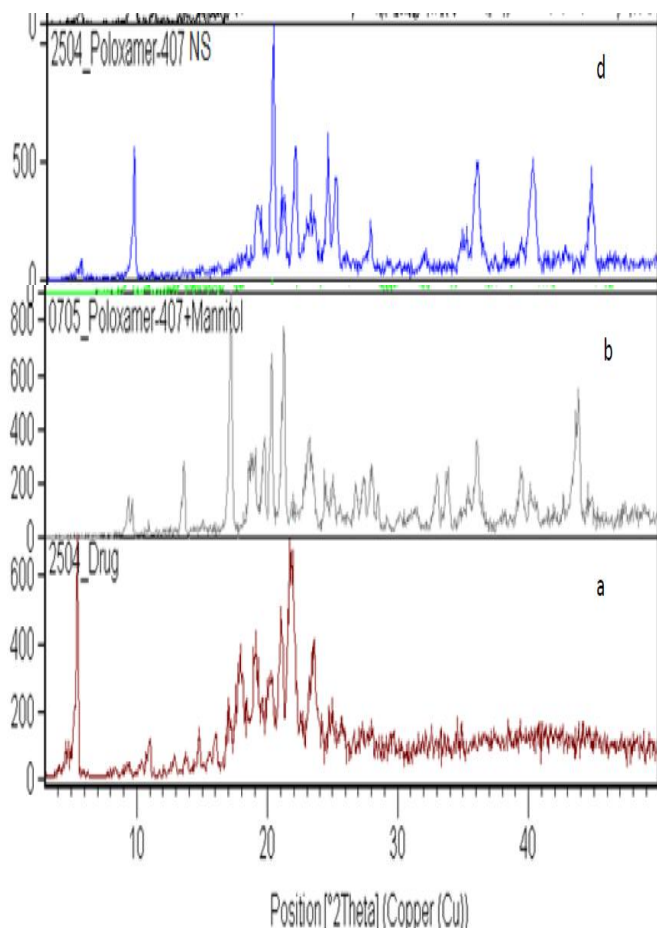


Fig. 9: XRD analysis of pure drug, optimized nanosuspension, optimized lyophilized suspension

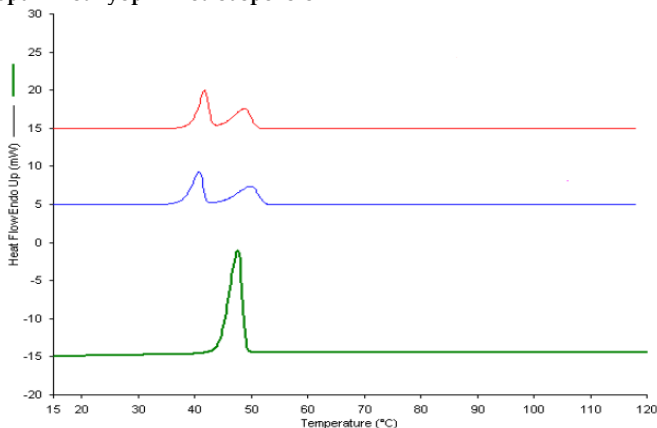


Fig. 10: DSC thermogram of Pure drug, optimized nanosuspension, optimized lyophilized

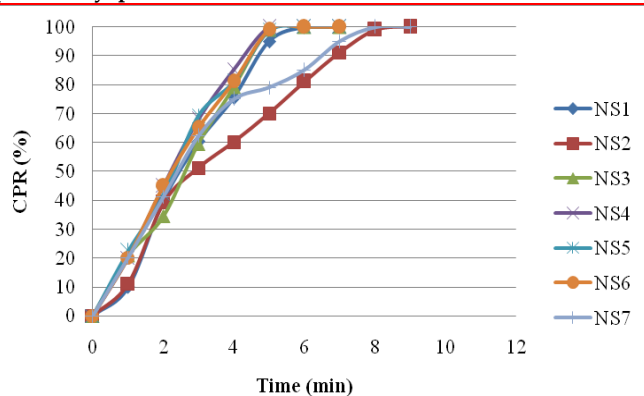


Fig. 11: Dissolution profile of nanosuspension NS₁ to NS₇

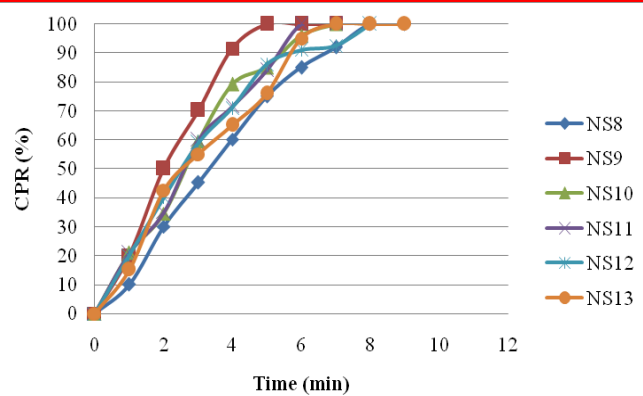


Fig. 12: Dissolution profile of nanosuspension NS₈ to NS₁₃

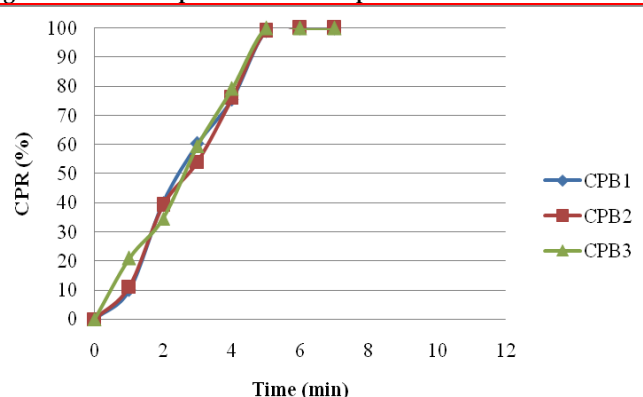


Fig. 13: Dissolution profile of nanosuspension CPB₁ to CPB₃

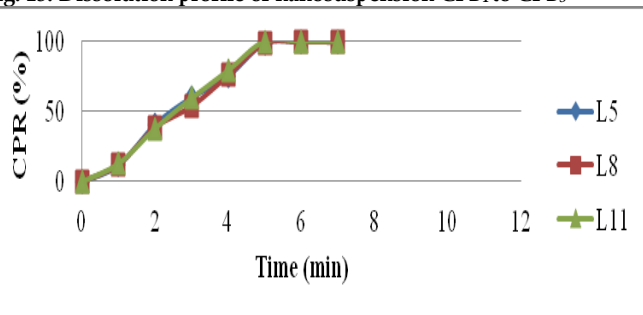


Fig. 14: Dissolution profile of nanosuspension L₅, L₈ & L₁₁

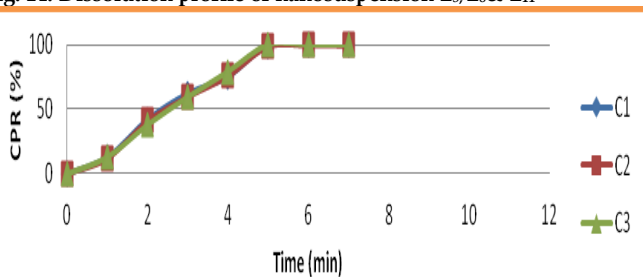


Fig. 15: Dissolution profile of capsule

Characterization of optimized lyophilized nanosuspension

Average particle size

Particle size analysis results of hydrated nanosuspension are shown in Table 13. From the PDI and zeta potential value optimize batch having a zeta potential lower than 93.0 nm and PDI value of 0.108 to 0.102. So from data conclude that particle size of nano range found from the nanosuspension preparation mentioned in Figure 1 to 3.

XRD analysis

XRD study of lyophilized blank batches (without drug, only with mannitol and Poloxamer 407) was also performed to ascertain the change in crystalline nature of drug as mentioned in Figure 9. From the data obtained from XRD analysis, decrease in peak height was observed.

DSC studies

To confirm the results of XRD studies, DSC studies were carried out. Thermograms of pure drug, optimized formula and lyophilized formula are shown in Figure 10.

The pure drug shows sharp peak at 48.3°C (with an enthalpy of 89.78 J/g) corresponding to its melting point. On the other hand, F1 showed peak at 40.826°C with enthalpy of 23.204 J/g and F2 showed peak at 41.737°C with enthalpy 23.957 J/g. This reduction in enthalpy and melting point can be due to decreased crystallinity of pure drug which is supported by results of XRD analysis. [13-14]

Drug content

Drug content of the lyophilized suspension were found in the range of 95 to 105 which is acceptable. Data mentioned in Table 14.

Saturation solubility determination

From the result of solubility conclude that Batch L5 having a highest solubility of 10 mg/ml compare to pure drug 2.00 mg/ml. All data mentioned in Table 15.

In vitro Dissolution

From the dissolution studies it is evident that dissolution velocity of L5 was dramatically increased in case of freeze dried nanosuspensions. The % drug released in 5 minutes was 77.75% and 72.53% in case of F1 and F2 as compared to drug (19.1). Almost 100% drug dissolved from freeze dried nanosuspensions in 20 minutes while only 36.075% of pure drug got dissolved in 60 minutes. So it is clear that by freeze dried nanosuspension increased rate and extent of dissolution. This may be due to increased saturation solubility, decrease in particle size to nano size and subsequent increase in surface area of particles and decreased crystallinity of particles.

Development of dosage form

Lyophilized nanosuspension equivalent to 10 mg of drug was filled in capsule shells and they were evaluated for parameters.

Disintegration time

Place 1 capsule in each of the six tubes of the basket and, place a disc. Operate the apparatus, using purified water as media maintained at $37 \pm 2^\circ\text{C}$. The disintegration time was noted when there was no residue on the screen of apparatus. [18-20]

Solvent-antisolvent precipitation technique using high speed homogenizer was selected for preparation of nanosuspension. Various process and formulation parameters were optimized. Process parameters namely stirring speed and stirring time were varied and their influence on appearance and liquid state stability was examined. It was concluded that stirring

at 20,000 rpm for 20 minutes is essential for the preparation of nanosuspension. Formulation parameters namely type of stabilizer, solvent to antisolvent ratio, concentration of drug and concentration of stabilizer were optimized. Seven different stabilizers were selected namely PVP K30, Poloxamer 407, Poloxamer 188, HPMC E5, PVA, Tween 80 and SLS. Among them Poloxamer 407, Poloxamer 188, PVA and Tween 80 yielded nanosuspension in particle size range of 90-350 nm. Other stabilizers could not produce nanosuspensions due to poor stabilization of newly created surfaces of drug nanoparticles. It was found that increasing solvent to antisolvent ratio decreased particle size due to better super saturation of solvent system. It was observed that increase in drug concentration increased particle size. It was found that enhancing stabilizer concentration up to certain level decreased particle size significantly due to adequate adsorption of stabilizer molecules on the surfaces of drug nano particles.

REFERENCES

1. Dhiman S, Thakur GS. Nanosuspension: A recent approach for nano drug delivery system. *Int J Curr Pharm Res.* 2011; 34:96-101.
2. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. *Advanced drug delivery reviews.* 2001 Mar 23; 47(1):3-19.
3. Jyothi BJ, Sreelakshmi K. Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. *Journal of young pharmacists.* 2011 Jan 1; 3(1):4-8.
4. Müller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. *Eur J Pharm Biopharm.* 2000; 50(1): 61-77.
5. Chingunpituk J. Nanosuspension Technology for Drug Delivery. *Walailak J Sci & Tech* 2007; 4(2): 139-153.
6. Patel M, Shah A, Patel NM, Patel MR, Patel KR. Nanosuspension: A novel approach for drug delivery system. *JPSBR.* 2011 Jul; 1(1):1-0.
7. Patravale VB, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. *Journal of pharmacy and pharmacology.* 2004 Jul 1;56(7):827-40.
8. Prabhakar Ch. A Review on Nanosuspension in Drug Delivery. *Int J Pharm & Bio Sci.* 2011; 2(1): 549-58.
9. De PK, Chakraborty S, Das S. Nanosuspensions: Potent vehicles for drug delivery and bioavailability enhancement of lipophilic drugs. *Journal of Pharmacy Research Vol.* 2012 Mar;5(3):1548-54.
10. Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Müller RH, Ehlers S. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine *Mycobacterium avium* infection. *Journal of Antimicrobial Chemotherapy.* 2000 Jan;45(1):77-83.
11. Jacobs C, Kayser O, Müller RH. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. *International journal of pharmaceutics.* 2000 Mar 10;196(2):161-4.
12. Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G. Eudragit RS100® nanosuspensions for the ophthalmic controlled delivery of ibuprofen. *European Journal of Pharmaceutical Sciences.* 2002 Jul 31;16(1):53-61.
13. Chen Y, Liu J, Yang X, Zhao X, Xu H. Oleanolic acid nanosuspensions: preparation, in- vitro characterization and enhanced hepatoprotective effect. *Journal of pharmacy and pharmacology.* 2005 Feb 1;57(2):259-64.
14. Zhang JY, Shen ZG, Zhong J, Hu TT, Chen JF, Ma ZQ, Yun J. Preparation of amorphous cefuroxime axetil nanoparticles by