6. Dissolution Enhancement of Nimodipine by Preparing Drug Nanosuspensions Using Media Milling Technique

6.1 Introduction

According to the food and drug administration nanoparticulate drug is not a generic drug rather are considered as "newdrug". It is not bioequivalent to its microcrystalline or solubilized form, administered at the same dosage and therefore a nanoparticulate drug can be patented.[1] It also offers fewer side effects, lower doses and faster onset of action. Nanosuspensions are liquid dispersion consisting of solid drug nanoparticles which are stabilized by polymer and or surfactant.[2] Nanosizing has been proven to be an effective tool for an active moiety considered as "brick dust candidate". Extensive review has been done on the various approaches for development and characterization of nanosuspensions,[3-6] nevertheless briefly it has been classified in to two basic approaches i.e. bottom up technology and top down technology.

Nimodipine (NM) is chemically described as 3-(2-methoxyethel)5-propane-2-yl2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, used in the treatment of various cardio vascular disorders such as angina pectoris, cardiac arrhythmia and hypertension. The major therapeutic indication of Nimodipine is for the prevention and treatment of delayed ischaemic neurological disorders, which often occur in patients with subarachnoid hemorrhages.[7] NM is a poorly water-soluble drug, which is one of the reasons that it has a low bioavailability and limited clinical efficacy.[8] For "low solubility/high permeability" drugs, dissolution plays an important role in their absorption.[9]

Poorly water-soluble drugs such as Nimodipine offer challenging problems in drug formulation as poor solubility is generally associated to poor dissolution characteristics and thus to poor oral bioavailability. The drug nanoparticles are generally suspended in an aqueous media and are termed as nanosuspensions. Nanosuspensions can prepared using various techniques namely nanoprecipitation, sonication, high speed homogenization, milling and high pressure homogenization.[10-15] The aim of this study was, to employ the nanosuspension technique to produce Nimodipine nanoparticles for oral administration. The dissolution is the rate-limiting factor for absorption of Nimodipine. In order to enhance these characteristics, preparation of Nimodipine

nanosuspension has been achieved using media milling technique. We investigated the nanoparticle formation of Nimodipine via considering the effects of drug-to-stabilizer ratio, stabilizer-to-stabilizer ratio and amount of beads (zirconium oxide) on the mean particle size, and dissolution properties of Nimodipine

6.2 Materials

Nimodipine was obtained as a gift sample from Lincoln Pharmaceutical Ltd., India. Different grtades of Hydroxy propyl methyl cellulose and Hydroxy propyl cellulose was obtained from Ruitai Pharmaceutical Co, China. Polyvinylpyrrolidone (PVPK-30), Polyvinyl Alcohol (PVA) polysorbate 20, PEG6000 and sodium lauryl sulphate were supplied by Loba Chemie. Pvt. Ltd., Mumbai. All the reagents used were of AR grade and double distilled water was used throughout the study.

6.3 Preparation of Nanosuspensions

Nimodipine powder (2 %w/v) was dispersed in an 10ml aqueous solution containing varying ratio of different surfactant/s in 20 ml vial The resulting coarse pre-dispersion was comminuted using zirconium oxide beads (milling media) on a magnetic stirrer (1 MLH, Remi Laboratory Instrument). Various parameters like the effect of stirring time and ratio of different size of zirconium oxide beads were optimized by keeping the drug::surfactant::milling media volume (1:1:50) (As Batch NM 5) as constant initially, then the optimized conditions of stirring time and ratio of different size of zirconium oxide beads were used throughout the study to optimize stabilizer, drug to stabilizer ratio and volume of milling media using 3^3 factorial designs to achieve minimum particle size. The stirring was continued for specific time period at 800 rpm for the preparation of optimized nanosuspension formulation. Whole process is performed in dark area since Nimodipine degrades in presence of light.

6.4 Factorial Design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+ b_{12}X_1X_2+ b_{13}X_1X_3+b_{23}X_2X_3+ b_{123} X_1X_2X_3+ b_{11}X^2_1+ b_{22}X^2_2+ b_{33}X^2_3$$

where, Y is the dependent variable, b_0 is the arithmetic mean response of the 27 runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2), (X_1X_3), (X_2X_3) and ($X_1X_2X_3$) show how the response changes when two or more factors are simultaneously changed. The polynomial terms (X^2_1), (X^2_2) and (X^2_3) are included to investigate nonlinearity. On the basis of the preliminary trials a 3^3 full factorial design was employed to study the effect of independent variables; drug to stabilizer ratio (X_1), Stabilizer to stabilizer ratio (X_2) and % V/V of Milling Media (X_3) on dependent variables: Mean Particle Size and Drug release in 5 minute.

6.5 Characterization of Nanosuspension

6.5.1 Particle Size and Size Distribution

The mean particle diameter and size distribution of the prepared nanosuspension was measured using a Masterasizer 2000 (Malvern Instruments, UK).Particle size detection range for Malvern SM 2000 is 0.02 to 2000 μm. The average particle size was measured after performing the experiment in triplicates.

6.5.2 Differential Scanning Calorimetry (DSC)

The phase transition of Nimodipine nanosuspension and Nimodipine pure drug was analyzed by differential scanning calorimetry (DSC- Shimadzu 60, Shimadzu Co., Kyoto, Japan). In DSC analysis, the samples were weighed (5 mg), hermetically sealed in flat bottom aluminum pans, and heated over a temperature range of 50 to 300°C at a constant increasing rate of 10°C/min in an atmosphere of nitrogen (50 mL/min).

6.5.3 In *vitro* Dissolution

In *vitro* dissolution studies were performed using USP dissolution test apparatus-II (paddle assembly). Dissolution was carried out on an equivalent of 10 mg of Nimodipine. 4.5 acetate buffer was used as the dissolution medium.[16] The volume and temperature of the dissolution medium were 900 ml, and 37.0±0.2◦C, respectively. Samples (5ml) were

withdrawn at regular intervals of 5 min for 60 min and replaced with fresh dissolution medium. Samples were filtered through 0.2μ whatman filter paper and assayed spectrophotometrically on SHIMADZU UV-VISIBLE spectrophotometer at 317 nm wavelength.

6.6 Result and Discussion

6.6.1 Effect of various stabilizers on particle size and size distribution

For the efficient size reduction of the drug particles, water soluble polymers and surfactants have been used as additives to inhibit the particles agglomeration and improve the physicochemical characteristic of the drug. Influence of different stabilizers was investigated in media milling method with a fixed concentration of the drug. The type of compound and their amount employed for stabilization has a prominent effect on particle size. Small particles, which spontaneously aggregate to decrease the surface energy, were stabilized by a layer of surfactant or/and protective polymer. Eight stabilizers were tested for their stabilization potential. Important function of stabilizer is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. As data shown in table 6.1 and figure 6.1 it may be concluded that mean particle size varies with stabilizer and with PVA it shows lowest size followed by then the other stabilizer. As data shown in table 4 we can conclude that types of stabilizer and concentration of stabilizer also affect the particle size of the formulation.

Table.6.1 Effect of various stabilizers on particle size and size distribution

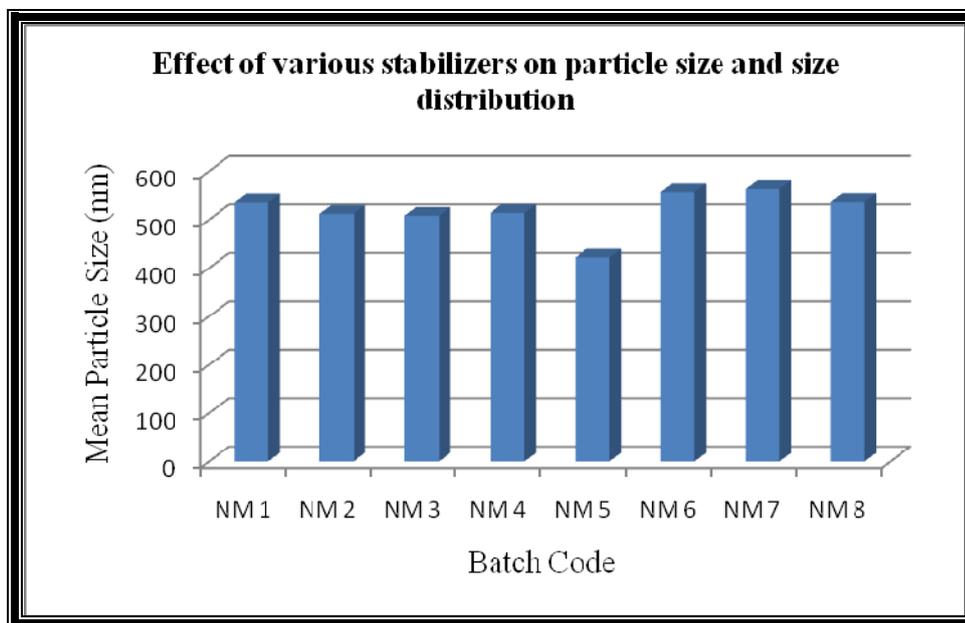| Batch Code	Stabilizer	Drug to Stabilizer Ratio	Mean Particle Size (nm)
NM 1	SLS	1:1	535 ± 3.24
NM 2	Polysorbate 80	1:1	512 ± 2.43
NM 3	HPMC 6 cps	1:1	508 ± 2.84
NM 4	PEG 6000	1:1	514 ± 5.28
NM 5	PVA	1:1	422 ± 5.27
NM 6	HPC	1:1	556 ± 2.54
NM 7	PVPK-30	1:1	562 ± 3.84
NM 8	HPMC15csps	1:1	536 ± 2.48

Figure 6.1. **Effect of various stabilizers on particle size and size distribution**

6.6.2 Effect of stirring time on particle size and size distribution

As shown in table 6.2 and figure 6.2, effect of stirring time on particle size was optimized by keeping 50:50 ratio of different diameter (0.1 mm and 0.5 mm) of zirconium oxide beads and keeping the drug: surfactant: milling media volume (1::1::50) constant. Lowest 422 nm mean particle size was achieved after 24 hrs stirring of 50:50 ratios of zirconium oxide beads. Further stirring up to 28 hours lead to increased particle size due to increased surface free energy. Also another interesting result observed during the milling process there was a noteworthy fast reduction of the mean particle size during the initial few hours. Subsequently the rate of the particle size reduction was slowed down. This may be probably due to the fact that, mostly deagglomeration of drug particles took place initially, followed by the breakage of the crystals due to cleavage and fracture. The later process usually requires more mechanical stress.[17]

Table. 6.2 Effect of stirring time on particle size and size distribution (Batch NM 5)

Time (Hr)	Mean Particle Size (nm)
Initial	4024 ± 2.29
2	3459 ± 5.94
4	2856 ± 3.54
6	2345 ± 2.38
8	1902 ± 4.24
10	1223 ± 4.24
12	997 ± 2.83
24	422 ± 5.27
26	530 ± 2.64
28	648 ± 3.76
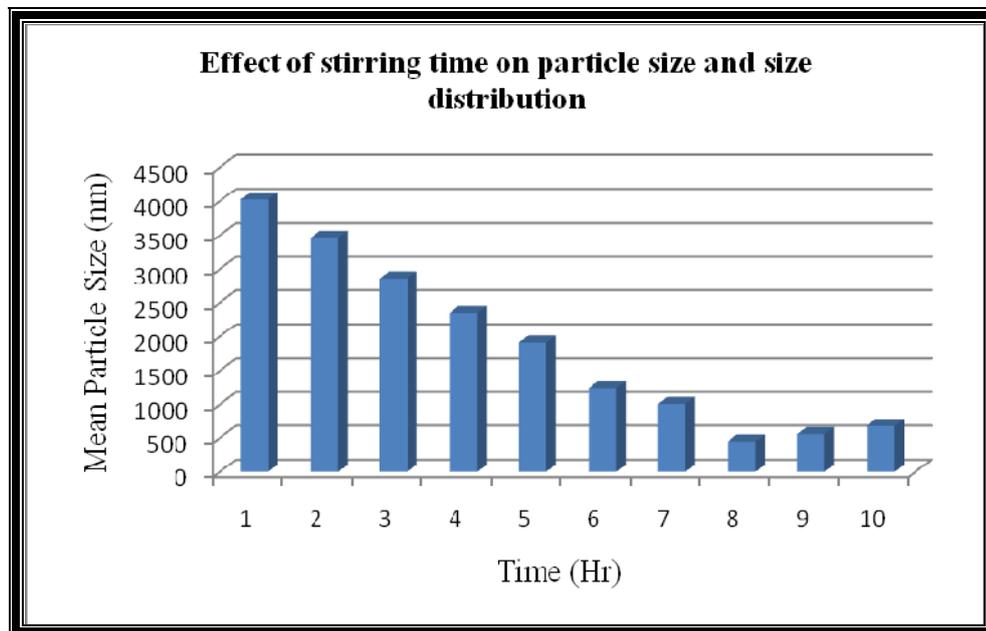
Figure 6.2 Effect of stirring time on particle size and size distribution (Batch NM 5)

6.6.3 Effect of ratio of beads on particle size and size distribution

The efficiency of the milling depends on the immensity of grinding energy and the size of the milling media is an important factor to control the efficiency of the process. In order to improve the efficiency of the milling process, a study was conducted to optimize the size of the milling media. As data shown in table 6.3 lowest particle size of 421 nm was observed at 50:50 resulting ratio of different size of zirconium oxide beads. When the ratios of different size of zirconium oxide beads were different than 50:50, resulting nanosuspensions had higher particle size. It may be possible because of at that ratio beads were closely packed and lead to reduced void space between various size beads. At different ratios other than this, the void spaces were found to be higher and attrition between drug particles and beads were at maximum.[18]

Table. 6.3 Effect of ratio of beads on particle size and size distribution

Batch Code	Small Size (0.1mm)	Big Size (0.5 mm)	Mean Particle Size (nm)
NM 9	0	100	1238 ± 2.75
NM 10	25	75	843 ± 4.29
NM11	50	50	421 ± 3.26
NM 12	75	25	623 ± 2.28
NM 13	100	0	1074 ± 5.21

Table 6.4 3^3 full factorial design lay out

Batch Code	Variable level in coded form			Mean Particle Size	Drug release in 5 min
	X_1	X_2	X_3		
NM 14	-1	-1	-1	689 ± 2.23	65.24 ± 3.24
NM 15	-1	-1	0	685 ± 5.29	66.52 ± 2.43
NM 16	-1	-1	1	686 ± 4	66.02 ± 2.55
NM 17	-1	0	-1	636 ± 4.45	68.26 ± 5.64
NM 18	-1	0	0	630 ± 3.28	69.18 ± 6.56
NM 19	-1	0	1	633 ± 2.24	68.04 ± 8.24
NM 20	-1	1	-1	582 ± 2.36	72.53 ± 4.27
NM 21	-1	1	0	576 ± 6.37	72.94 ± 2.25
NM 22	-1	1	1	581 ± 2.32	72.84 ± 3.49
NM 23	0	-1	-1	528 ± 7.29	76.07 ± 2.64
NM 24	0	-1	0	526 ± 5	76.92 ± 6.57
NM 25	0	-1	1	527 ± 2.54	76.03 ± 8.87
NM 26	0	0	-1	478 ± 3.27	80.14 ± 4.25
NM 27	0	0	0	473 ± 3.24	81.97 ± 1.89
NM 28	0	0	1	475 ± 6.37	80.12 ± 7.25
NM 29	0	1	-1	429 ± 2.36	84.14 ± 2.66
NM 30	0	1	0	424 ± 6.24	84.96 ± 5.59
NM 31	0	1	1	429 ± 2.47	84.45 ± 5.96
NM 32	1	-1	-1	379 ± 4.24	88.14 ± 2.84
NM 33	1	-1	0	376 ± 2.78	89.23 ± 5.79
NM 34	1	-1	1	378 ± 4.24	88.25 ± 8.22
NM 35	1	0	-1	324 ± 2.83	92.56 ± 1.98
NM 36	1	0	0	319 ± 3.96	93.23 ± 7.26
NM 37	1	0	1	323 ± 2.59	99.12 ± 5.24
NM 38	1	1	-1	283 ± 4.94	95.14 ± 4.65
NM 39	1	1	0	279 ± 2.29	96.97 ± 6.25
NM 40	1	1	1	284 ± 5.65	95.86 ± 2.36

Translation of coded levels in actual units

Variable Level	Low(-1)	Medium(0)	High(+1)
Drug to Stabilizer Ratio (X_1)	1:0.5	1:1	1:1.5
Stabilizer to Stabilizer (%)X_2	0.25:0.75	0.5:0.5	0.75:0.25
% V/V of Milling Media (X_3)	40	50	60

Table 6.5 Summary of results of regression analysis

	b_0	b_1	b_2	b_2	b_{12}	b_{23}	b_{13}	b_{123}	b_{11}	b_{22}	b_{33}	R^2
MPS	471.8519	-152.944	-50.3889	-0.66667	2.833333	0.416667	0.5	3.43E-14	3.611111	3.277778	3.777778	0.999828
5 min	81.41778	12.05167	3.745	0.472778	0.151667	0.040833	0.543333	0.135	0.581667	-0.72167	-0.605	0.987142

6.6.4 Factorial equation for Mean article Size

The mean particle size varies 279 nm to 689 nm and showed good correlation coefficient (0.9998). The particle size of different formulation was shown in table 6.4, which clearly indicates the batch NM 39 had less particle size as compare to other formulation. The batch NM 39 had a Z-average particle size of 279 nm. The particle size distribution pattern of the NM 39 is given in figure 6.6. Results of the equation indicate that the X_1 (Drug-to-Stabilizer ratio) and X_2 (Stabilizer to Stabilizer ratio) significantly affects the mean particle size ($p < 0.05$). As increase the concentration of stabilizer, it decreases the mean particle size, while increase in the media volume led to slight increase in the mean particle diameter. The stabilizer concentration is also an important parameter influencing crystal size. An appropriate stabilizer and its concentration were used for each drug concentration to achieve smaller particle size. This can be explained by complete adsorption of stabilizer on the crystal surface. Crystal was protected by the adsorbed stabilizers, and the amount of stabilizer should be sufficient for full coverage on the crystal surface to provide enough steric repulsion between the crystals. Insufficient surface coverage of stabilizer could result in rapid crystal growth and agglomeration, while high concentration of stabilizer could result in enhanced viscosity of the solution.[19] The relationship between the selected dependent and independent variables was further elucidated using response surface plots as shown in figure 6.3, 6.4 and 6.5.

$$\text{Mean article size} = 471.8518 - 152.9444X_1 - 50.3888X_2 - 0.6666X_3 + 2.8333X_1X_2 + 0.4166X_2X_3 + 0.5X_1X_3 + 3.4336E\text{-}14X_1X_2X_3 + 3.6111X^2_1 + 3.2777X^2_2 + 3.7777X^2_3$$
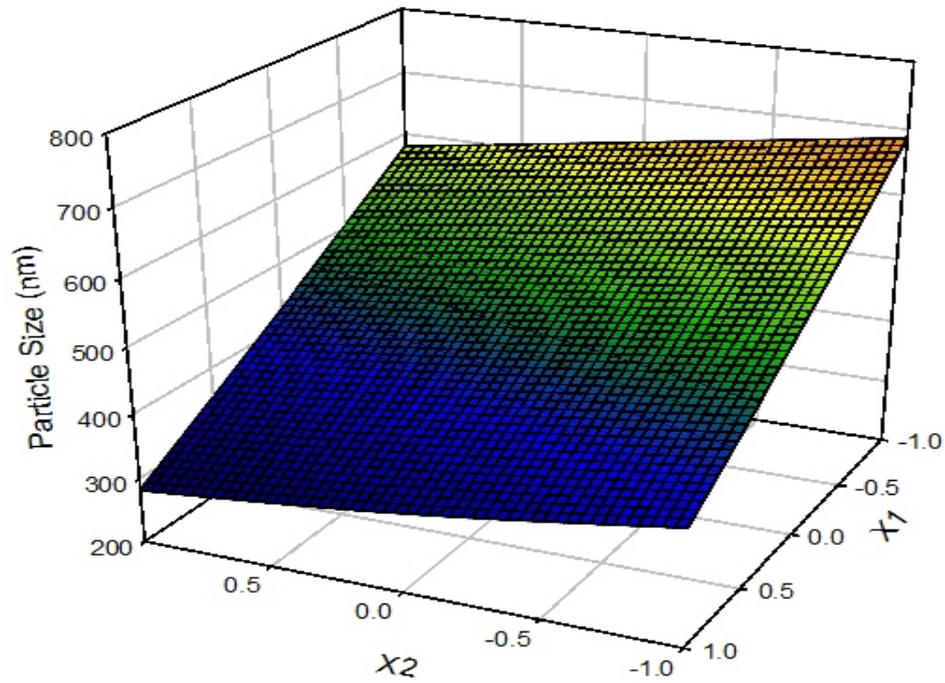
Figure 6.3 Response surface plot of effect drug to stabilizer ratio (X_1) and Stabilizer to Stabilizer (%) (X_2) on particle size distribution
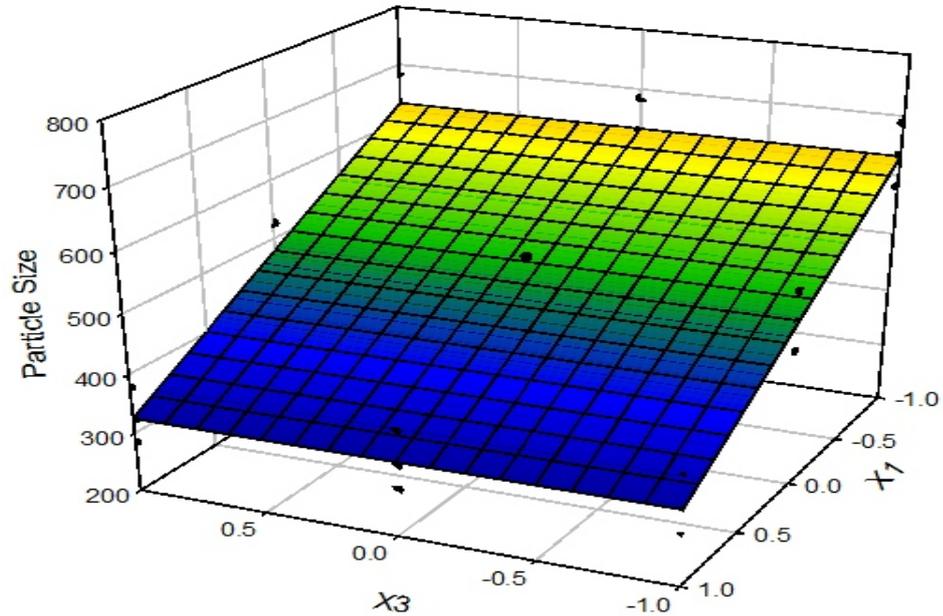

Figure 6.4 Response surface plot of effect drug to stabilizer ratio (X_1) and % V/V of Milling Media (X_3) on particle size distribution
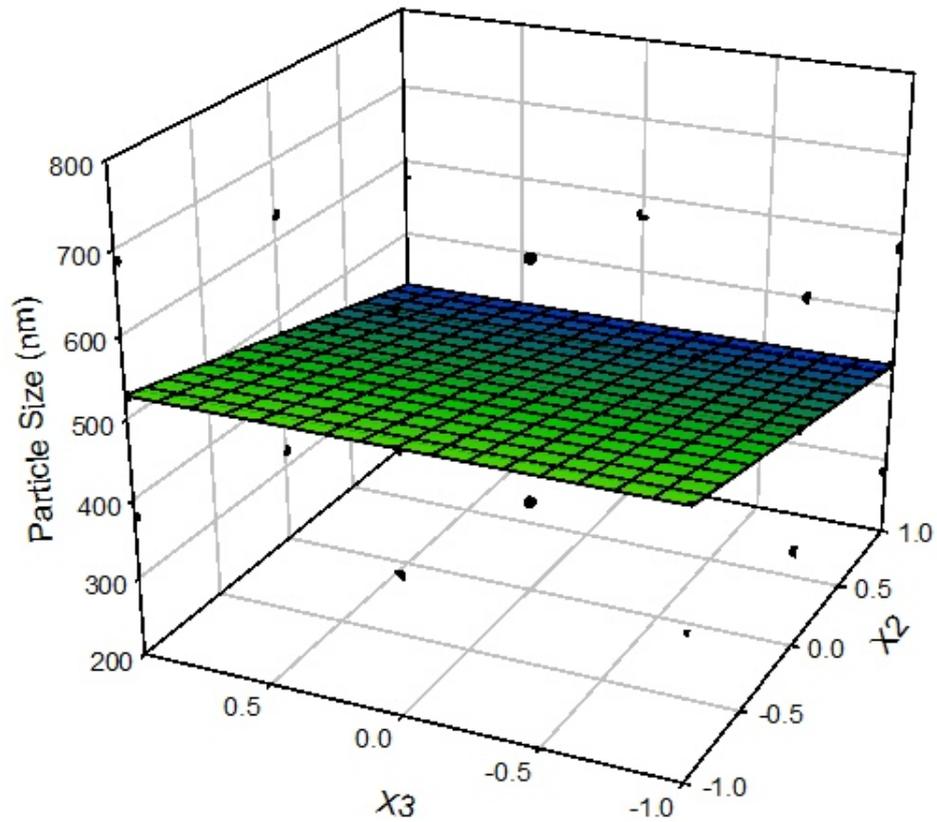
Figure 6.5 Response surface plot of effect stabilizer to stabilizer (%) (X_2) and % V/V of milling media (X_3) on particle size distribution
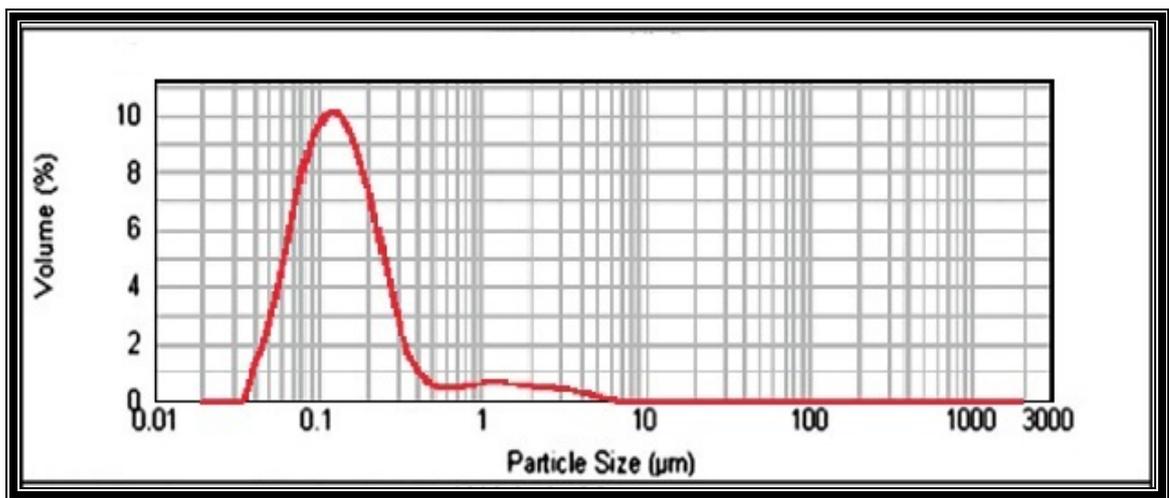

Figure 6.6 Particle size distribution batch NM 39

6.6.5 Factorial equation for Drug Release in 5 minute

The drug release in 5 min varies 65.24 to 96.97 % with good correlation coefficient (0.9871). Results of the equation indicate that the X_1 (Drug-to-Stabilizer ratio) and X_2 (Stabilizer to Stabilizer ratio) significantly affects the mean particle size (p<0.05). As previously seen that the independent variable affect the mean particle size, hence indirectly affect the drug release by increasing the surface area. As the size decrease, the effective increase in particle surface area resulting increase in dissolution velocity according to the Nernst Brunner-Noyes Whitney equation.

Drug release in 5 minute $= 81.4177 + 12.051X_1 + 3.745X_2 + 0.4727X_3 + 0.1516X_1X_2 + 0.0408X_2X_3 + 0.5433X_1X_3 + 0.135X_1X_2X_3 + 0.5816X^2_1 - 0.7216X^2_2 - 0.306X^2_3$

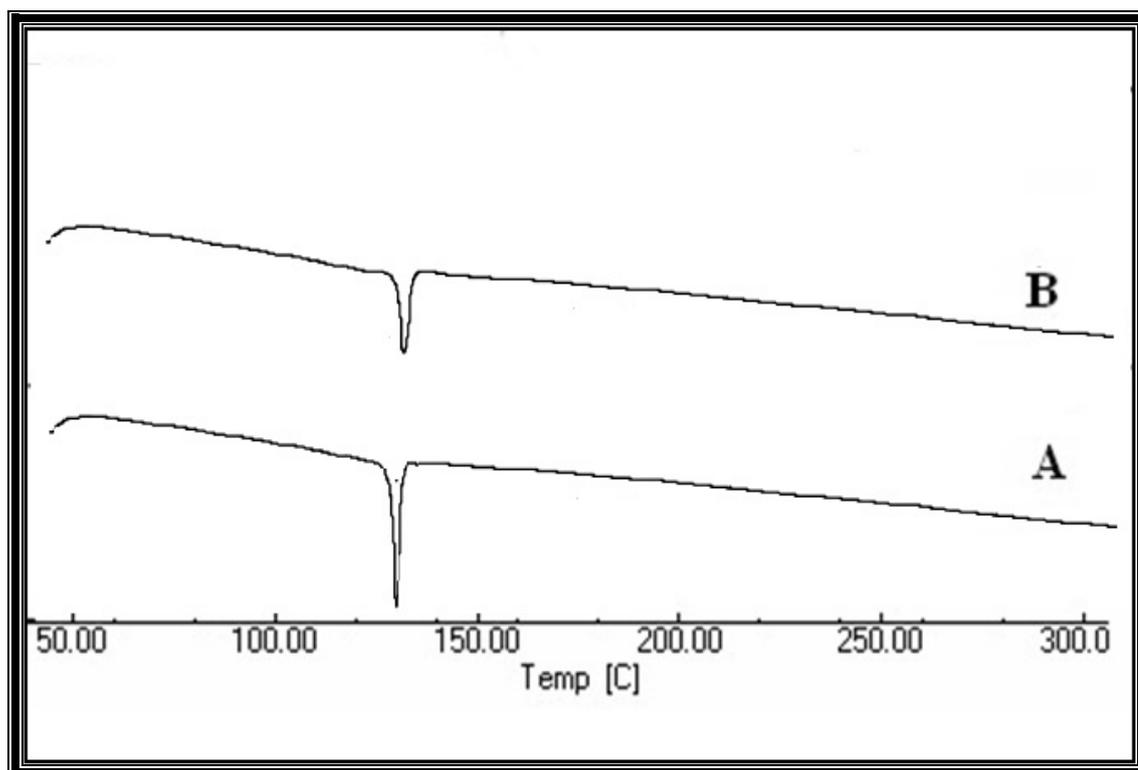**6.6.6 Differential Scanning Calorimetry (DSC)**

Figure. 6.7 DSC tharmogram of pure Nimodipine (A) and Otimized nanosuspension (B)

In order to verify that this dissolution rate/solubility enhancement is not due to the presence of Nimodipine amorphous form, crystalline state evaluation of Nimodipine nanoparticles was carried out.[20] As shown on the DSC thermograms of Nimodipine unmilled and Nimodipine nanoparticles are presented in Figure 6.7. From the figure it was observed that there were no major changes in the melting peaks of Nimodipine unmilled and Nimodipine nanoparticles. The only difference observed was a slight shift in fusion temperature. These modifications were attributed to the presence of stabilizers. This confirmed the crystalline state of drug with the nano formulation.

6.6.7 In vitro dissolution profile

Dissolution studies were compared for pure drug, and optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 96.97 % within 5 min compared to amount of 3.25 % of pure drug in 4.5 acetate buffer. The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for increase in dissolution rate. This enhanced dissolution rate can be attributed to the higher surface area of nanocrystals available for dissolution and the decreased diffusion layer thickness.[21]
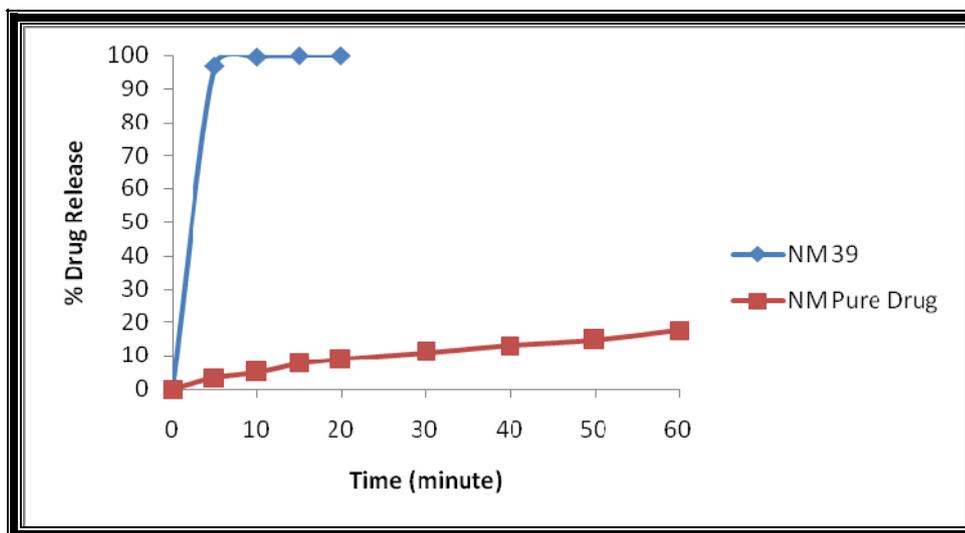

Figure 6.8 Release profiles of pure drug and optimized nanosuspension

6.7 Conclusion

NM nanosuspension was prepared and the optimized formulation showed better dissolution as compared to pure drug. Media milling technique has been described as a simple method for nanosizing of Nimodipine at laboratory scale. Particle size is influenced by milling time, modifying the drug to stabilizer ratio and amount and size of zirconium beads. Nanosized Nimodipine dissolved significantly faster than raw drug powder. The improved formulation could offer improved drug delivery strategy which might allow concomitant use of Nimodipine.

6.8 Reference

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