5. Dissolution Enhancement of Nifedipine by Preparing Drug Nanosuspensions Using Media Milling Technique

5.1 Introduction

Approximately 40% or more of the new chemical entities (NCE) generated during drug discovery are poorly soluble in water.[1] The basic challenge faced by the researcher for the formulation of such poorly soluble drugs is the low oral bioavailability and erratic absorption of the drugs from the gastrointestinal tract due to their low saturation solubility and dissolution velocity. The low saturation solubility results in a low concentration gradient between the gut and blood vessel and leads to a limited transport of drug.[2] For poorly soluble drugs as seen in BCS Class II, the dissolution of the drugs in the gastrointestinal fluid media is the rate limiting step for the absorption of the drugs.[3] Hence for efficient absorption of drugs from the gastrointestinal tract for improving their therapeutic efficacy, there is an imminent need for studies in designing novel strategies for their dissolution enhancement.

There are number of formulation approaches viz., salt formation, pH adjustment, cosolvency, complexation, etc., used for enhancement of dissolution but none of the approach has achieved the merits of being universal. Micronization of poorly soluble drugs has been applied for many years to improve dissolution velocity of poorly soluble drugs but reducing the drug to micron size does not increase the saturation solubility of the drug, and at such a low saturation solubility, as generally observed in BCS Class II drug, the increment in the dissolution characteristics does not help to a great extent.[4-5] Consequently off late nanonisation has been employed for treating the BCS Class II drugs. When the drug is being reduced to nanosized level there is an obvious increase in its saturation solubility assisted by improvement in the dissolution characteristics which could be attributed to the effective increase in particle surface area according to the Nernst Brunner-Noyes Whitney equation.[6] 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, supercritical crystallization, milling and high pressure homogenization.[7-17] The drug nanoparticles are generally suspended in an aqueous media and are termed as nanosuspensions.

Nifedipine (NF) a highly potent calcium-channel blocker belonging to the group of 1,4-dihydropyridines widely used in the treatment of vascular diseases such as hypertension, angina pectoris and anti-atherosclerotic activity was used as for reparation of nanoparticle.[18-19] Nifedipine has low aqueous solubility (~20 μg/ml) and could be classified as a BCS class II drug.[20-21] For poorly soluble drugs, the dissolution of the drugs in the gastrointestinal fluid media is the rate limiting step for the absorption of the drugs. So, nanonisation has been employed for treating Nifedipine. In this work the production of nanosuspensions was intended for oral use of Nifedipine using a media milling technique at laboratory scale. The aim of this investigation was to develop and optimize Nifedipine nanosuspension which could offer improved dissolution as compared present formulation approaches.

5.2 Materials

Nifedipine was obtained as a gift sample from Lincoln Pharmaceutical Ltd., India. Hydroxy propyl methyl cellulose (HPMC 6cps) was obtained from Ruitai Pharmaceutical Co, China. Polyvinylpyrrolidone (PVPK-30), Polyvinyl Alcohol (PVA), Polysorbate 20 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.

5.3 Preparation of Nanosuspensions

Nifedipine 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 (1:0.5:50) (Batch NF 3) 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 concentration of HPMC 6cps and volume of milling media using 3^2 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 Nifedipine degrades in presence of light.

5.4 Factorial Design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses: $Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_1^2+b_{22}X_2^2$

where, Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The optimized conditions of stirring time and ratio of different size of zirconium oxide beads were used throughout the study to optimize concentration of HPMC 6cs and volume of milling media using 3^2 factorial designs to achieve minimum particle size.

5.5 Characterization of Nanosuspension

5.5.1 Particle Size and Size Distribution

The mean particle size 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. Nanosuspension was added to the sample dispersion unit, and stirred at 2000 rpm with magnet in order to reduce the interparticulate aggregation, and laser obscuration range was maintained between 10-20 %. The average particle size was measured after performing the experiment in triplicates.

5.5.2 Differential Scanning Calorimetry (DSC)

The phase transition of Nifedipine nanosuspension and Nifedipine 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).

5.5.3 In *vitro* Dissolution Profile

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 Nifedipine. Deionized water (0.05% polysorbate 20) was used as the dissolution medium.[22] The volume, pH and temperature of the dissolution medium were 900 ml, 7.0 and 37.0±0.2^{0}C, respectively. Triplicate reading were taken for each measurement. 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 235 nm wavelength.

5.6 Result and Discussion

5.6.1 Effect of various stabilizers and their concentration 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.[9] Influence of different stabilizers with different concentration 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.[23] Small particles, which spontaneously aggregate to decrease the surface energy, were stabilized by a layer of surfactant or/and protective polymer. Four stabilizers (SLS, HPMC 6cps, PVA and PVPK-30) 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 5.1 and figure 5.1 it may be concluded that mean particle size varies with stabilizer as well as changing in the concentration of stabilizer, and formulation stabilized with HPMC 6cps (1:0.5) shows highest particle size reduction as compared to other assorted polymers.

Table 5.1. Effect of various stabilizers and their concentration on particle size and size distribution

Batch Code	Stabilizer	Drug to Stabilizer Ratio	Mean Particle Size (nm)
NF 1	SLS	1:0.5	445 ± 2.24
NF 2	SLS	1:1	480 ± 1.41
NF 3	HPMC 6 cps	1:0.5	355 ± 4.26
NF 4	HPMC 6 cps	1:1	378 ± 2.35
NF 5	PVA	1:0.5	392 ± 1.63
NF 6	PVA	1:1	367 ± 3.36
NF 7	PVPK-30	1:0.5	412 ± 1.12
NF 8	PVPK-30	1:1	380 ± 2.58

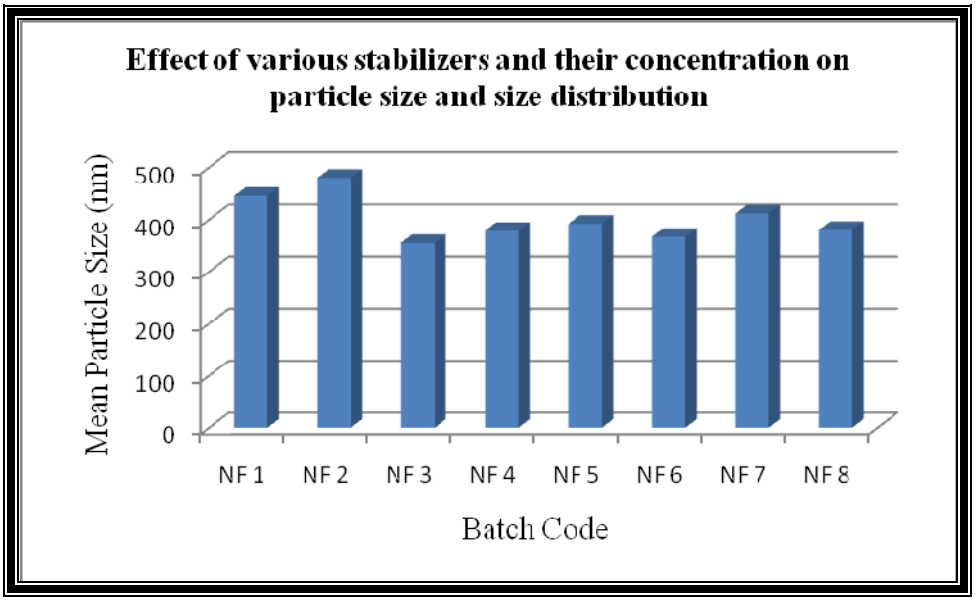

Figure 5.1. **Effect of various stabilizers and their concentration on particle size and size distribution**

5.6.2 Effect of stirring time on particle size and size distribution

As shown in table 5.2 and figure 5.2, 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::0.5::50) constant. Highest particle size reduction was seen with 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.[23]

Table 5.2. Effect of stirring time on particle size and size distribution (Batch NF 3)

Time (Hr)	Mean Particle Size (nm)
Initial	4026 ± 4.21
2	3487 ± 1.89
4	2994 ± 1.57
6	2326 ± 3.62
8	1865 ± 4.25
10	1209 ± 3.25
12	1005 ± 3.12
24	355 ± 2.41
26	525 ± 1.64
28	602 ± 2.87

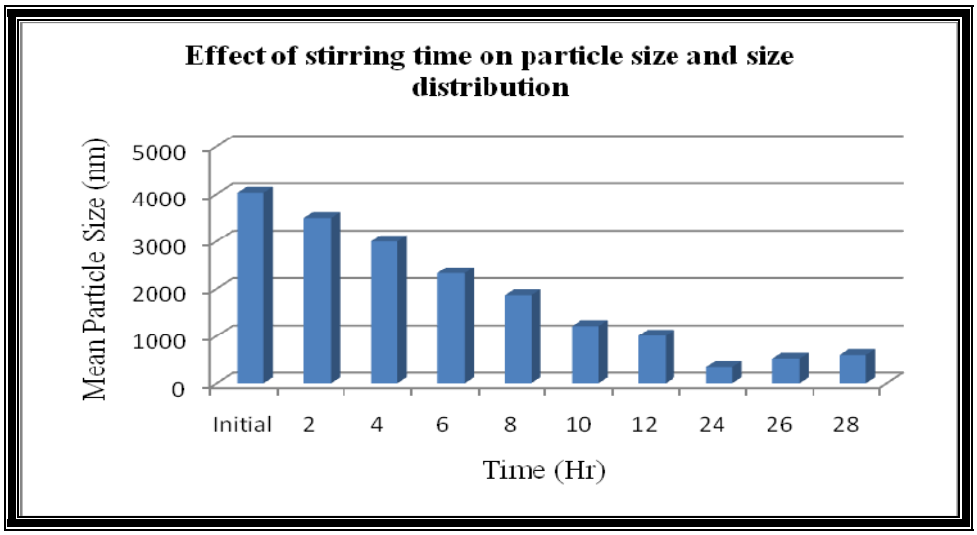

Figure 5.2 Effect of stirring time on particle size and size distribution (Batch NF 3)

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

The efficiency of the milling depends on the intensity 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 5.3 highest particle size reduction was achieved at 362 nm using 50:50 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.[24]

Table 5.3. 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)
NF 9	0	100	1352 ± 2.36
NF 10	25	75	623 ± 2.14
NF 11	50	50	362 ± 4.23
NF 12	75	25	741 ± 1.53
NF 13	100	0	1128 ± 2.48

Table 5.4. 3^2 Full factorial design layout

Batch Code	Drug to Stabilizer Ratio (X_1)	% V/V of Milling Media (X_2)	Mean Particle Size (nm)	% Drug release in 20 min
NF 14	-1	-1	338 ± 4	78.23 ± 1.03
NF 15	-1	0	283 ± 6	89.14 ± 1.16
NF 16	-1	1	342 ± 11	76.65 ± 2.03
NF 17	0	-1	372 ± 6	71.69 ± 0.47
NF 18	0	0	358 ± 5.56	74.84 ± 0.31
NF 19	0	1	386 ± 5.29	69.45 ± 3.07
NF 20	1	-1	382 ± 10.39	70.12 ± 2.60
NF 21	1	0	368 ± 11.26	72.43 ± 2.19
NF 22	1	1	402 ± 7	66.12 ± 3.52

Translation of Coded Levels in Actual Units			
Variables Level	**Low (-1)**	**Medium (0)**	**Higher (+1)**
Drug to Stabilizer Ratio (X_1)	1:0.3	1:0.6	1:0.9
% V/V of Milling Media (X_2)	40	50	60

Table 5.5. Summary of results of regression analysis

Coefficient	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
Mean Particle Size	349.3333	31.5	6.3333	4	-19.5	34	0.9459
% Drug release in 10 min	35.0044	-5.39	-0.8666	-0.735	3.3933	-6.5466	0.8652
% Drug release in 20 min	76.5	-5.8916	-1.3033	-0.605	3.455	-6.76	0.9318

5.6.4 Factorial equation for particle size and size distribution

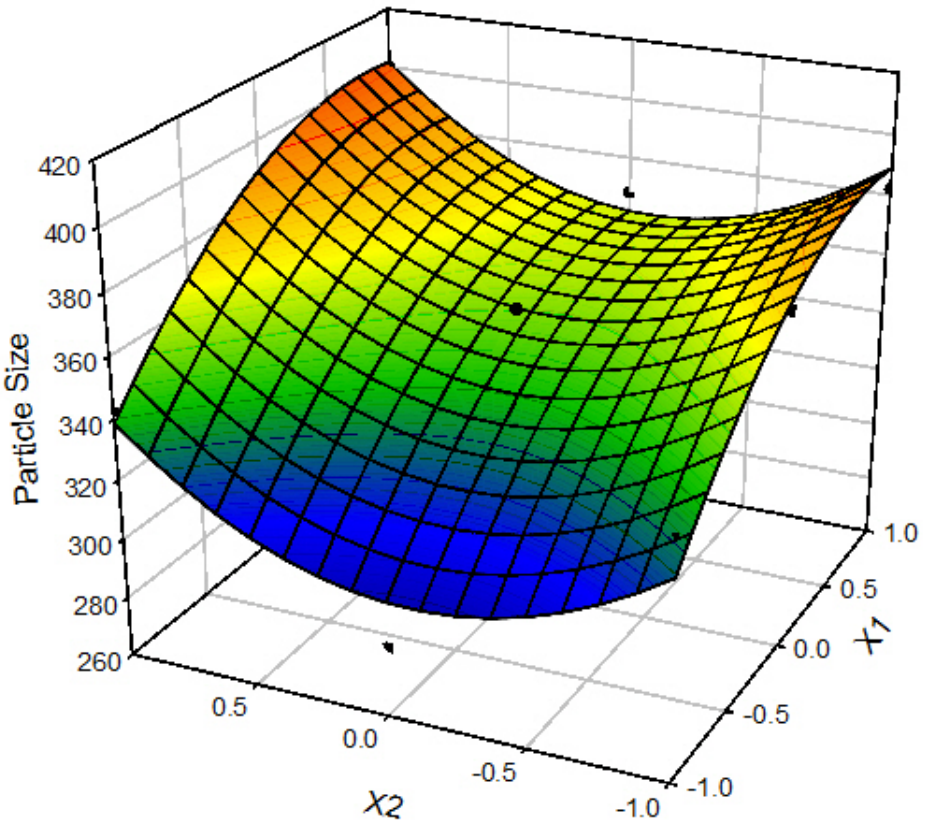

Figure 5.3 Response surface plot of effect drug to stabilizer ratio (X_1) and % V/V of milling media (X_2) on particle size distribution

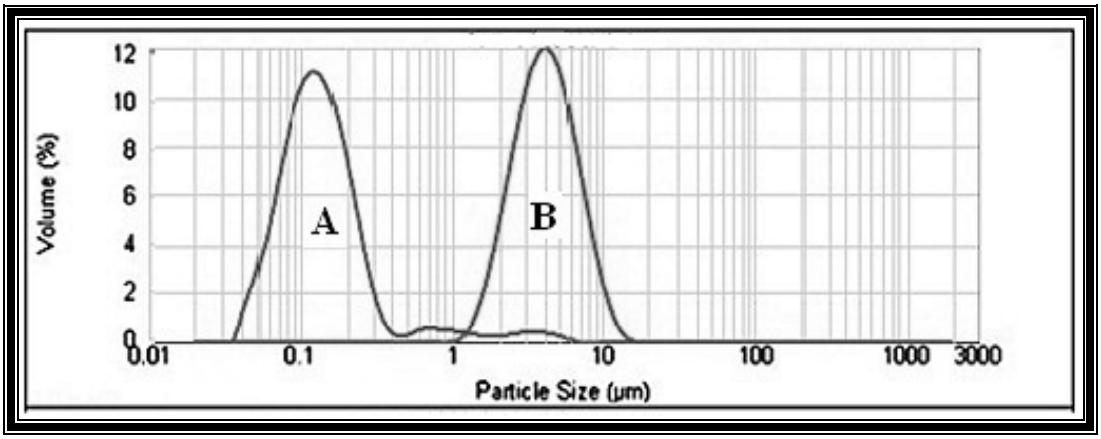

Figure 5.4. Particle size graph of batch optimized nanosusension NF 15 (A) and pure drug (B)

The mean particle size varies 283 nm to 402 nm and showed good correlation coefficient (0.9459). The particle size of different formulation was shown in table 5.4, which clearly indicates the batch NF 15 with particle size 283 nm had highest particle size reduction compared to other formulation. The batch NF 15 had a Z-average particle size of 283 nm. The particle size distribution pattern of the NF 15 is given in figure 5.4. Results of the equation indicate that the X_1 (polymer-to-drug ratio) significantly affects the mean particle size ($p<0.05$). As increase the concentration of stabilizer, it increases the mean particle size, while increase in the media volume led to slight increase in the mean particle diameter. The relationship between the selected dependent and independent variables was further elucidated using response surface plots as shown in figure 5.3. The stabilizer concentration is also an important parameter influencing crystal size. An appropriate stabilizer concentration was 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.[25]

$$\text{Mean Particle Size} = 349.3333 + 31.5X_1 + 6.3333X_2 + 4\,X_1X_2 - 19.5\,X_1^2 + 34\,X_2^2$$

5.6.5 Factorial equation drug release

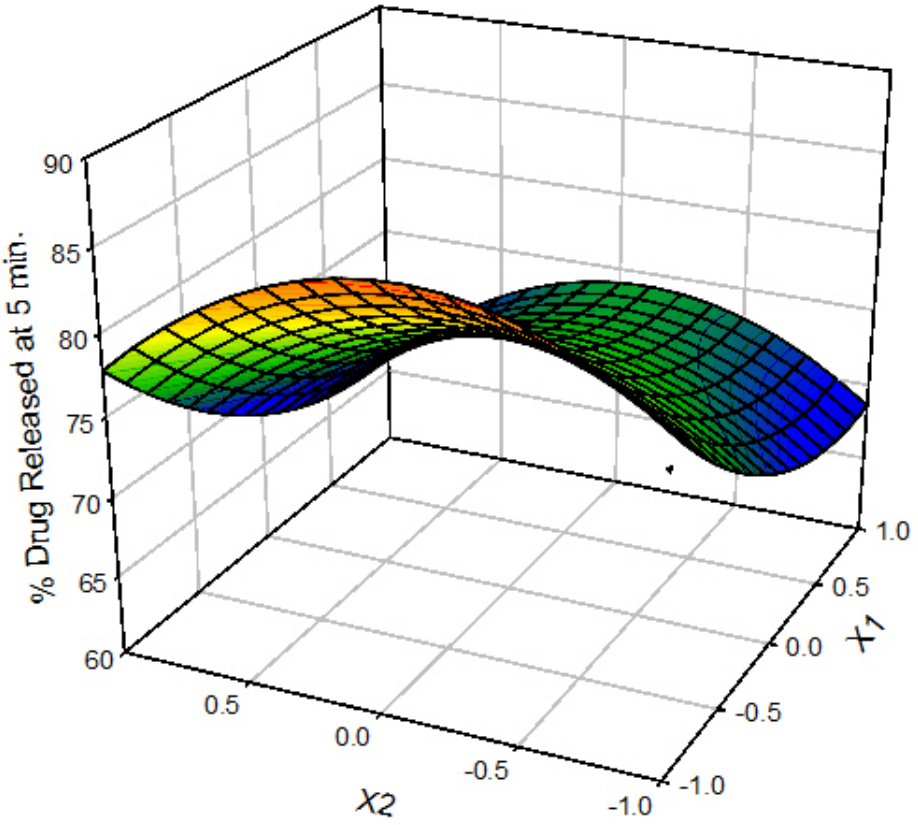

Figure 5.5 Response surface plot of effect of drug to stabilizer ratio (X_1) and % V/V of milling media (X_2) on drug release

From the in vitro dissolution studies it was observed that, the drug release at 20 minutes varies from 66.12 to 89.14 % with good correlation coefficient 0.9318. Results of the equation indicate that X_1 (polymer-to-drug ratio) significantly affects the drug release ($p<0.05$). As previously seen that the independent variable affect the mean particle size, hence indirectly affect the drug release by increasing the surface area. The relationship between the selected dependent and independent variables was further elucidated using response surface plots as shown in figure 5.5. As increase the concentration of stabilizer, it decreases the amount of drug release, while increase in the media volume led to slight decrease in drug release.

Drug release in 20 min = 76.5 - 5.89167 X_1 - 1.30333 X_2 -0.605 X_1X_2 + 3.455 X_1^2 - 6.76 X_2^2

5.6.6 Differential scanning calorimetry (DSC)

In order to verify that this dissolution rate/solubility enhancement is not due to the presence of Nifedipine amorphous form, crystalline state evaluation of Nifedipine nanoparticles was carried out.[26] As shown on the DSC thermograms of Nifedipine unmilled and Nifedipine nanoparticles are presented in Figure 5.6. From the figure it was observed that there were no major changes in the melting peaks of Nifedipine unmilled and Nifedipine nanoparticles. The only difference observed was a slight shift in fusion temperature (174–173 °C). These modifications were attributed to the presence of HPMC 6cps. This confirmed the crystalline state of drug with the nano formulation.

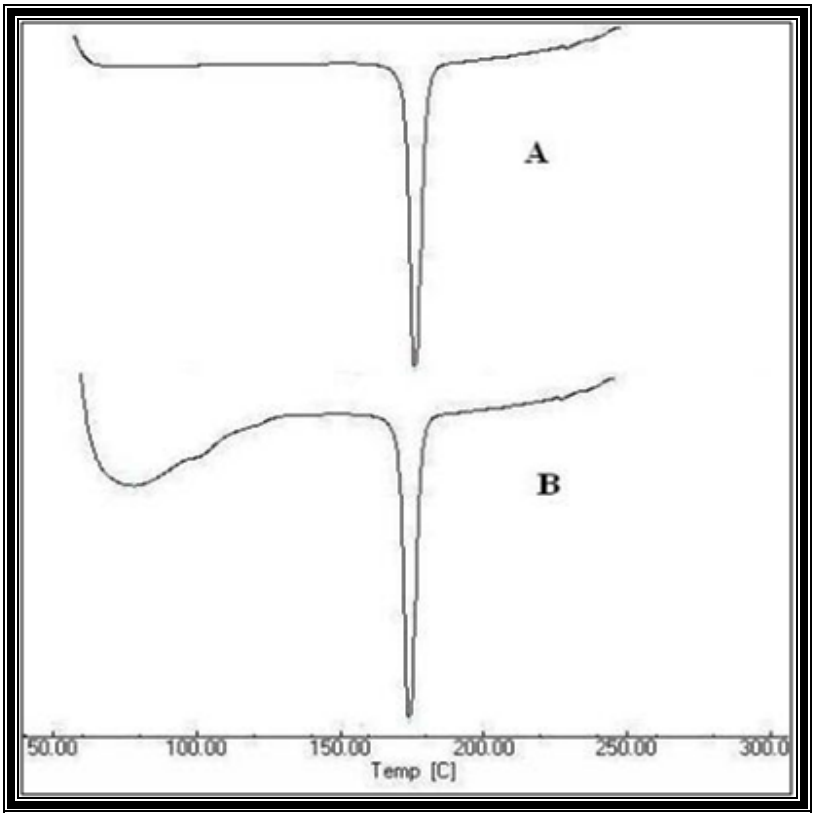

Figure 5.6 DSC tharmogram of pure Nifedipine (A) and optimized nanosuspension (B)

5.6.7 In *vitro* dissolution profile

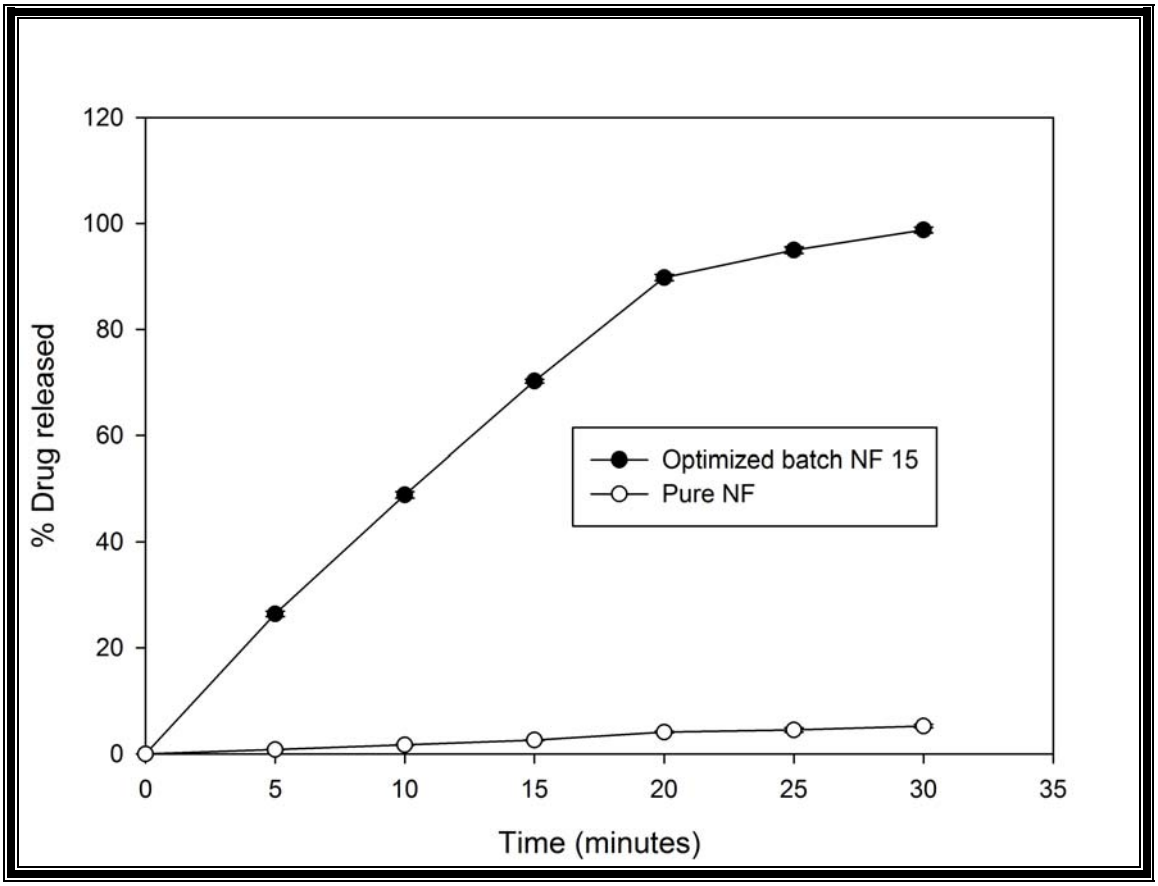

Figure 5.7 Release profile of pure drug, and optimized nanosuspension formulation in deionized water (0.05% polysorbate 20)

Dissolution studies were compared for pure drug, and optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 89.14 ± 3.12 % within 20 min compared to amount of 1.76 ± 1.28 % of pure drug in Deionized water (0.05% polysorbate 20). 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.[27]

5.7 Conclusion

Nifedipine 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 Nifedipine at laboratory scale. All the predetermined independent variables were found to affect the dependent variables from the resultant nanosuspension. The factorial design at three levels employed helped in understanding the process of nanosuspension formation. Particle size is influenced by milling time, modifying the drug to stabilizer ratio and amount and size of zirconium beads. Nanosized Nifedipine dissolved significantly faster than raw drug powder.

5.8 References

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