7. Dissolution Enhancement of Nitrendipine by Preparing Drug Nanosuspensions Using Solvent Evaporation Technique

7.1 Introduction

Nitrendipine is a dihydropyridine calcium channel antagonist with a very low solubility for the treatment of hypertension.[1] Nitrendipine is classified as a Class II API (poorly soluble and highly permeable) by the Biopharmaceutics Classification System (BCS).[2] The absolute oral bioavailability of this drug is reported to range from about 10% to 20%, depending in part on the dosage form.[3-4]

Poorly water-soluble drugs such as Nitrendipine (NT) (~2.0 µg/ml at 37°C in water) offer challenging problems in drug formulation as poor solubility is generally associated to poor dissolution characteristics and thus to poor oral bioavailability. The basic challenge faced by the researcher for the formulation of 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.[5] There are number of formulation approaches viz., salt formation, pH adjustment, cosolvency, complexation, etc[6] used for enhancement of dissolution but none of the approach has achieved the merits of being universal. However, there are several disadvantages associated with these approaches. For example, the alteration of chemical structure by forming water-soluble derivatives often requires long processing times at a very expensive cost to derive the new chemical entities (NCEs).[7] 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.[8-9] Consequently off late nanonisation has been employed for treating the BCS Class II drugs. Nanosuspensions can prepared using various techniques namely nanoprecipitation, sonication, high speed homogenization, supercritical crystallization, milling and high pressure homogenization.[10-20] In order to enhance these characteristics, Nitrendipine nanosuspension has been prepared using

nanoprecipitation technique. The important process parameters, the concentration of nitrendipine in the organic phase, concentration of surfactant in the anti-solvent and starring speed was evaluated using 3^3 full factorial designs. It was observed that the concentration of nitrendipine in the organic phase, concentration of surfactant in the anti-solvent, and starring speed significantly affect the particle size as well as dissolution velocity. Differential Scanning Calorimetry studies confirmed that the crystallinity of the drug was maintained after the nanoprecipitation suggesting that improved dissolution of Nitrendipine nanosuspensions could be attributed to reduction in particle size.

7.2 Materials

Nitrendipine was obtained as a gift sample from Spansules Formulations, 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.

7.3 Preparation of Nanosuspensions

Nanosuspensions were prepared by the solvent evaporation technique. Nitrendipine was dissolved in a 5ml mixed solvent of PEG 200 and acetone (ratio of 1:1, v/v) at room temperature. This was poured by means of a syringe positioned with the needle directly into 20 ml water containing different amount of surfactant maintained at a temperature below 5°C and subsequently stirred at ranging agitation speed for 2 hr to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.). Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspensions at room temperature for 2 hours.

7.4 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; Concentration of drug (X_1), Concentration of Stabilizer (X_2) and Sterring speed (X_3) on dependent variables: Mean Particle Size and Drug release in 5 minute.

7.5 Characterization of Nanosuspension

7.5.1 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.

7.5.2 Differential Scanning Calorimetry (DSC)

The phase transition of Nitrendipine nanosuspension and Nitrendipine 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).

7.5.3 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 nitrendipine. Water containing 0.1M hydrochloric acid and 0.1% SDS was selected as dissolution medium[2]. 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 236 nm wavelength.

7.6 Result and Discussion

Influence of different stabilizers was investigated in nanoprecipitation technique 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.

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 7.1 and figure 7.1 it may be concluded that mean particle size varies with stabilizer and with HPMC 6cps it shows lowest size followed by then the other stabilizer. As shown in table 7.2 an appropriate amount of stabilizer is required to achieve smaller particle size. The crystal growth was protected by the adsorbed stabilizers, and the quantity of stabilizer should be enough to cover the crystal surface to provide enough steric repulsion between the crystals. Inadequate 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 which would obstruct the diffusion between the solvent and anti-solvent during precipitation.[21] The type of compound and their amount employed for stabilization has a prominent effect on particle size.[22] At the low drug concentration, the particle size was smaller with a narrow size distribution. However, at the higher drug concentration, due to greater supersaturation, a higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals. Stirring speed is obviously affecting the particle size, as increasing the stirring speed, decrease in mean particle size because of high shear force.[2]

Table 7.1 Effect of various stabilizers on particle size and size distribution

Batch Code	Stabilizer	Concentration of Drug (mg/ml)	Drug to Stabilizer ratio	Mean Particle Size
NT 1	SLS	20	1:0.2	328 ± 4.29
NT 2	HPMC 6 cps	20	1:0.2	290 ± 3.66
NT 3	PVA	20	1:0.2	337 ± 2.54
NT 4	PVPK-30	20	1:0.2	324 ± 3.25

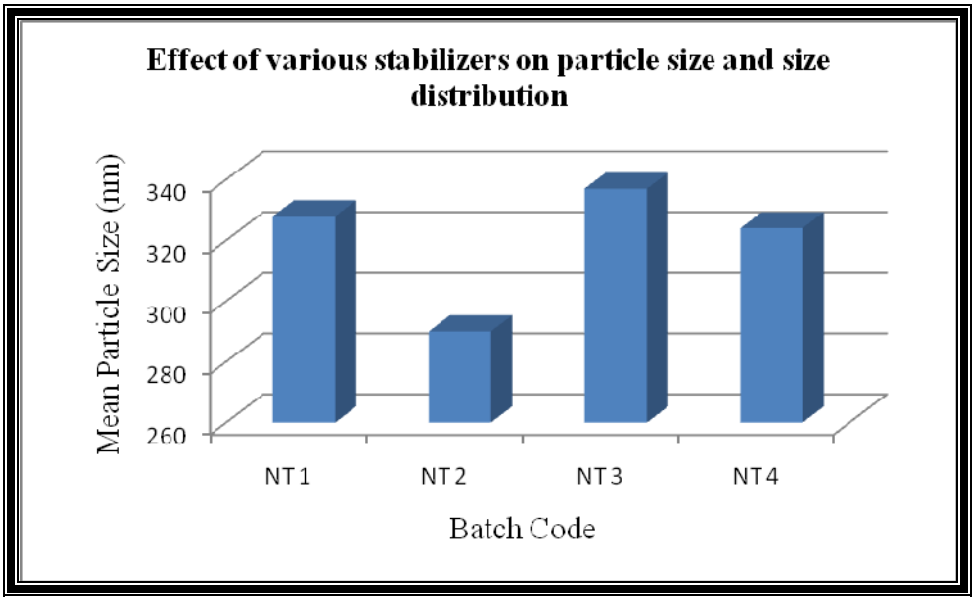

Figure 7.1. Effect of various stabilizers on particle size and size distribution

Table 7.2 For 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		
NT 5	-1	-1	-1	455 ± 4.98	82.06 ± 2.56
NT 6	-1	-1	0	363 ± 2.26	88.25 ± 3.97
NT 7	-1	-1	1	257 ± 2.43	93.02 ± 4.26
NT 8	-1	0	-1	468 ± 3.54	80.87 ± 2.30
NT 9	-1	0	0	361 ± 6.26	88.14 ± 1.43
NT 10	-1	0	1	364 ± 2.76	88.02 ± 5.22
NT 11	-1	1	-1	471 ± 2.92	80.12 ± 5.29
NT 12	-1	1	0	367 ± 3.76	87.86 ± 2.25
NT 13	-1	1	1	374 ± 2.65	86.27 ± 4.23
NT 14	0	-1	-1	562 ± 4.29	75.02 ± 3.54
NT 15	0	-1	0	522 ± 6.06	77.56 ± 2.25
NT 16	0	-1	1	504 ± 3.29	78.81 ± 1.76
NT 17	0	0	-1	461 ± 4.24	81.0 2± 6.43
NT 18	0	0	0	339 ± 5.65	89.83 ± 3.46
NT 19	0	0	1	254 ± 1.53	93.21 ± 5.22
NT 20	0	1	-1	467 ± 3.47	81.02 ± 2.59
NT 21	0	1	0	356 ± 2.63	88.98 ± 5.26
NT 22	0	1	1	268 ± 1.43	92.84 ± 3.76
NT 23	1	-1	-1	676 ± 7.32	69.14 ± 3.58
NT 24	1	-1	0	654 ± 5.28	70.83 ± 2.36
NT 25	1	-1	1	652 ± 1.28	70.88 ± 5.15
NT 26	1	0	-1	612 ± 3.29	73.56 ± 3.16
NT 27	1	0	0	590 ± 5.27	74.01 ± 2.56
NT 28	1	0	1	581 ± 3.64	74.54 ± 2.52
NT 29	1	1	-1	558 ± 5.69	76.14 ± 1.24
NT 30	1	1	0	526 ± 3.27	77.59 ± 3.59
NT 31	1	1	1	502 ± 4.47	88.86 ± 2.25

Translation of coded levels in actual units

Variable Level	Low(-1)	Medium(0)	High(+1)
Concentration of drug (mg/ml)X_1	20	40	60
Drug to Stabilizer ratio X_2	1:0.25	1:0.5	1:0.75
Stirring Speed X_3	400	800	1200

Table 7. 3 Summary of results of regression analysis

Coefficient	b_0	b_1	b_2	b_3	b_{12}	b_{23}	b_{13}	b_{123}	b_{11}	b_{22}	b_{33}	R^2
MPS	385	103.9444	-42	-54.1111	-44.4167	-6	24	-16.625	75.8333	26.3333	18.3333	0.8873
5 min	85.8044	-5.5033	3.0061	3.75	3.4016	1.1833	-0.735	1.9737	-3.69	-1.175	-1.15	0.8757

7.6.1 Factorial Equation for Mean article Size

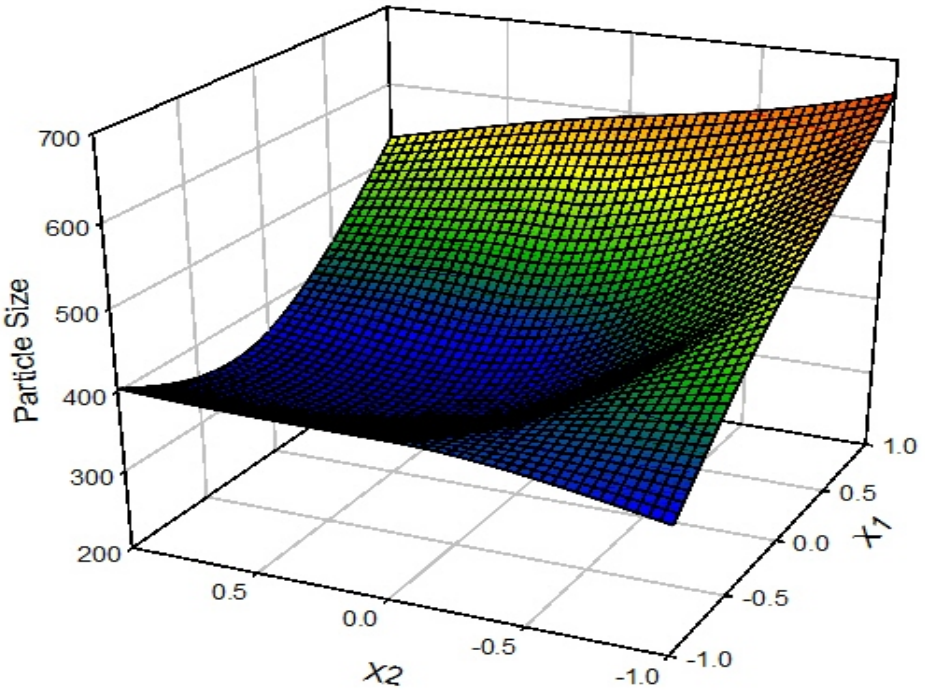

Figure 7.2 Response surface plot of effect concentration of drug (mg/ml) (X_1) and drug to stabilizer ratio (X_2) on particle size distribution

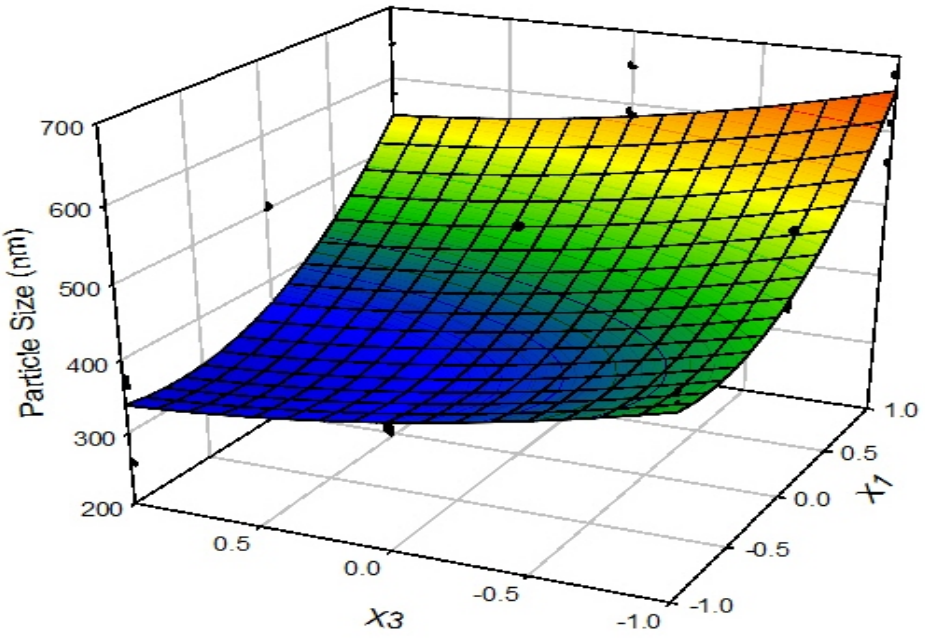

Figure 7.3 Response surface plot of effect concentration of drug (mg/ml) (X_1) and stirring speed (X_3) on particle size distribution

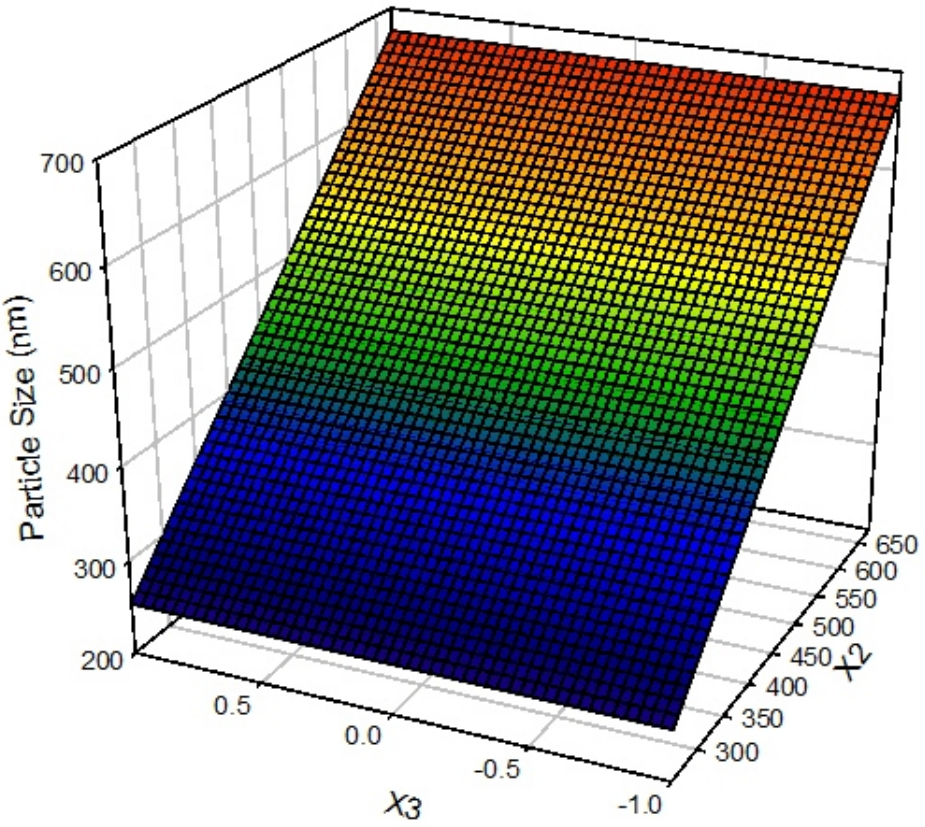

Figure 7.4 Response surface plot of effect drug to stabilizer ratio (X_2) and stirring speed (X_3) on particle size distribution

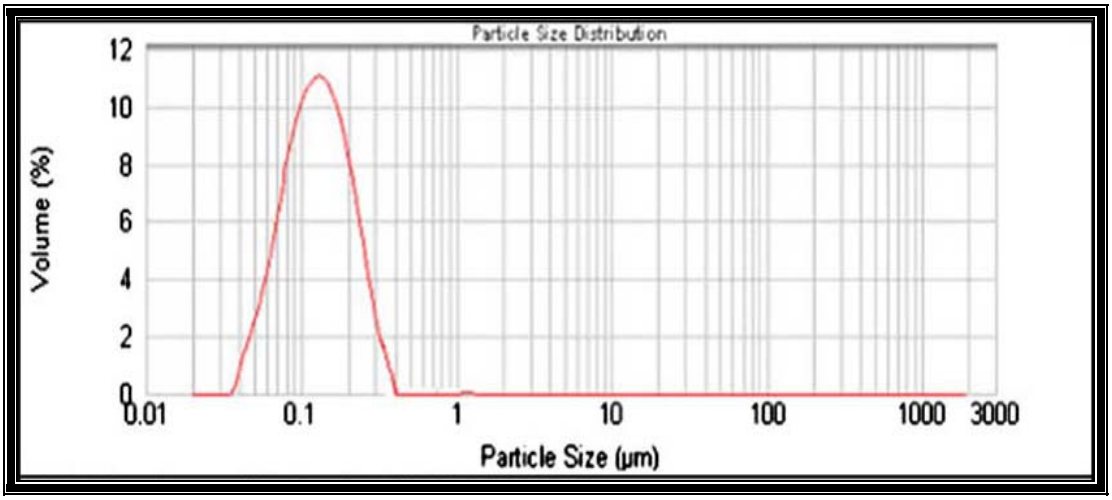

Figure 7.5 Particle size distribution batch NT 19

The mean particle size varies 254 nm to 676 nm and showed good correlation coefficient (0.8873). The particle size of different formulation was shown in table 7.2, which clearly indicates the batch NT 19 had less particle size as compare to other formulation. The batch NT 19 had a Z-average particle size of 254 nm. The particle size distribution pattern of the NT 19 is given in figure 7.5. Results of the equation indicate that the all three independent variable significantly affect the mean particle size. 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.[23]However, drug concentration was more significantly affect the mean particle size because at the higher drug concentration, due to greater supersaturation, a higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals. While as increase the steering speed result in decrease in particle size because of higher shear force. The relationship between the selected dependent and independent variables was further elucidated using response surface plots as shown in figure 7.2, 7.3 and 7.4.

$$\text{Mean article size} = 385 + 103.9444X_1 - 42X_2 - 54.1111X_3 - 44.4167X_1X_2 - 6X_2X_3 + 24X_1X_3 - 16.625X_1X_2X_3 + 75.8333X^2_1 + 26.3333X^2_2 + 18.3333X^2_3$$

7.6.2 Factorial Equation for Drug Release in 5 minute

The drug release in 5 min varies 69.14 to 93.21 % with good correlation coefficient 0.8757. Results of the equation indicate that the all three independent variable significantly affect the drug release. As the size decrease, the effective increase in particle surface area resulting increase in dissolution velocity according to the Nernst Brunner-Noyes Whitney equation

$$\text{Drug release in 5 minute} = 85.8044 - 5.5033X_1 + 3.0061X_2 + 3.75X_3 + 3.4016X_1X_2 + 1.1833X_2X_3 - 0.735X_1X_3 + 1.97375X_1X_2X_3 - 3.69X^2_1 - 1.175X^2_2 - 1.15X^2_3$$

7.6.3 Differential Scanning Calorimetry (DSC)

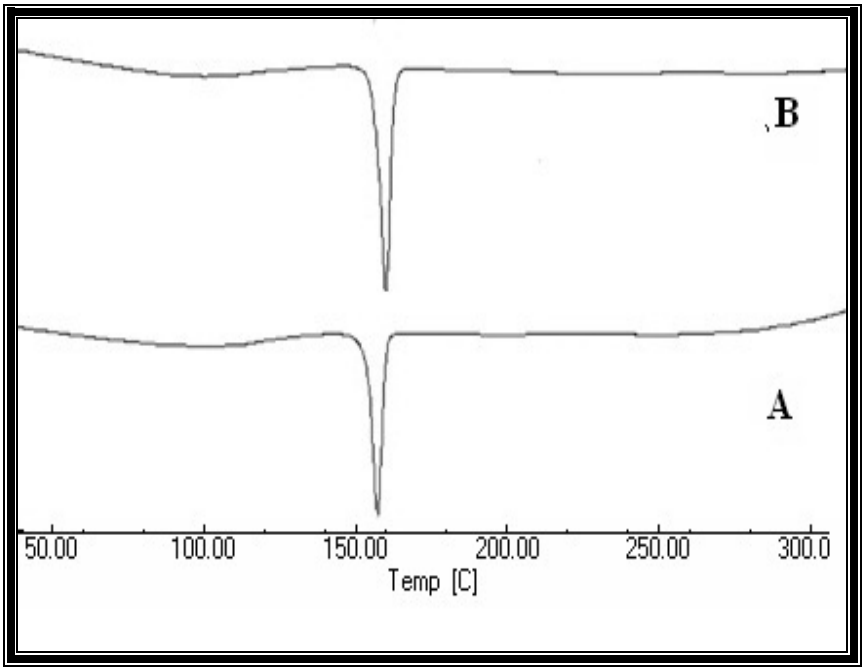

Figure 7.6 DSC study of Nitrendipine nanosuspension (158.22^{0}C) (A), Nitrendipine pure drug (160.02^{0}C) (B)

In order to verify that this dissolution rate/solubility enhancement is not due to the presence of Nitrendipine amorphous form, crystalline state evaluation of Nitrendipine nanoparticles was carried out.[24] As shown on the DSC thermograms of Nitrendipine unmilled and Nitrendipine nanoparticles are presented in Figure 7.6 From the figure it was observed that there were no major changes in the melting peaks of Nitrendipine unmilled and Nitrendipine nanoparticles. The only difference observed was a slight shift in fusion temperature (158–159 ^{0}C). These modifications were attributed to the presence of HPMC 6cps. This confirmed the crystalline state of drug with the nano formulation.

7.6.3 In *Vitro* Dissolution Profile

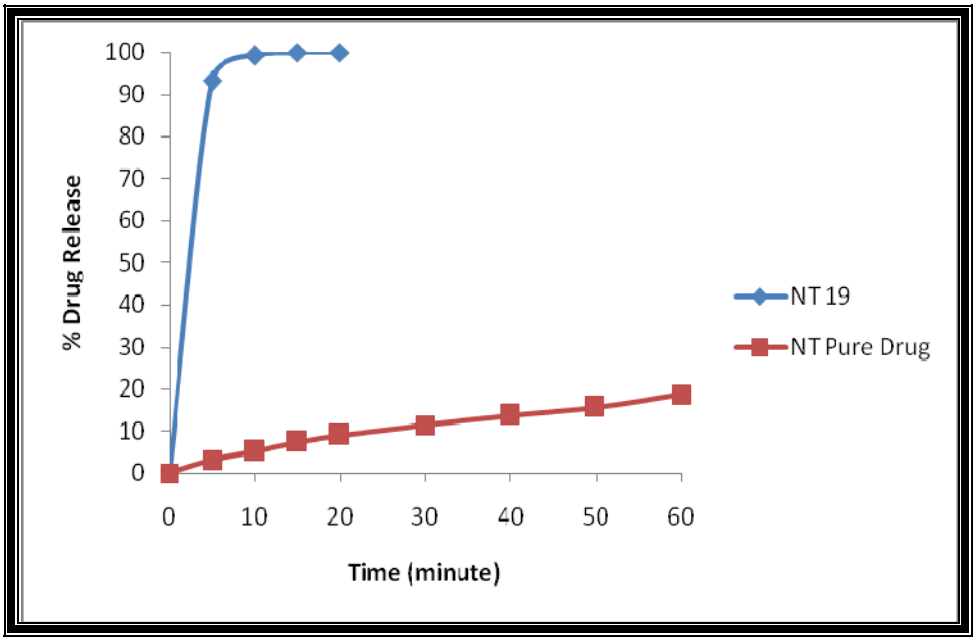

Figure 7.7 Release profiles of pure drug and optimized nanosuspension formulation in water containing 0.1M hydrochloric acid and 0.1% SDS

Dissolution studies were compared for pure drug, and optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 93.21 % within 5 min compared to amount of 18.69 % of pure drug after 1 hour in water containing 0.1M hydrochloric acid and 0.1% SDS. 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.[25]

7.7 Conclusion

Nitrendipine nanoparticles were prepared by nanoprecipitation. Nanoprecipitation technique has been described as a simple method for drug nano-sizing at laboratory scale. 3^3 full factorial design helped in identifying the significant parameters that affected the response variables. All the predetermined independent variables except drug

concentration were found to affect the dependent variables. Particle size is significantly influenced by concentration of drug, concentration of stabilizer and starring speed. Nanosized Nitrendipine dissolved significantly faster than raw drug powder. The optimized formulation maintained the crystallinity of Nitrendipine and released almost 93.21 % drug within 5 minutes.

7.8 References

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