Research Article

Design and Characterisation of Nitrendipine Nanocrystals for Solubility and Dissolution Enhancement

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ABSTRACT

The objective of present work was to prepare nanocrystals of Nitrendipin (NTD) to enhance its solubility and dissolution rate with aim of dose reduction and minimizing the side effects associated with its oral administration. Preparation of nanocrystals by anti-solvent precipitation method. The nanocrystals obtained were characterised mainly for particle size (PS), zeta potential (ZP), crystallinity, saturation solubility, in vitro dissolution and permeability. Results demonstrated profound effect of concentration of surfactant (polaxamer188) on both the PS and polydispersity index (PDI) values. The optimized nanocrystals formulation had particle size 335nm, PDI 0.262, practical yield 85%, and ZP in the range of 20 to -45mv. X-Ray diffraction studies (XRPD) and Differential scanning calorimetry (DSC) studies suggested nanocrystal formation and absence of crystalline peaks, indicating loss of crystallinity, additionally confirmed by scanning electron microscopy (SEM). Nanocrystals showed 30.45-fold enhancements in aqueous solubility, and 38.5-fold in phosphate buffer pH 1.2, as compared to pure NTD. In vitro release studies have demonstrated 96.186% cumulative drug release within 60 min from nanocrystals compared to 22.17% from pure NTD. Stable NTD nanocrystals formulated by anti-solvent precipitation method shows improved solubility and dissolution. It has been concluded that NTD nanocrystals were obtained with significant improvement in saturation solubility and drug losing its crystalline nature, when compared with plain drug.

Keywords: Nitrendipin, Nanocrystals, anti-solvent precipitation, Dissolution.

INTRODUCTION

It is well explained that solubility, dissolution and gastrointestinal permeability are fundamental parameters that control rate and extent of drug absorption and it's bioavailability. Hence, poor aqueous solubility is a major challenge for development of formulations, thus scientists are concerned with improving oral bioavailability of poorly soluble drugs[1-3]. NTD, a dihydropyridine calcium channel antagonist is a typical poorly water-soluble drug. For such biopharmaceutical classification system (BCS) II type compounds, the rate and degree of absorption from the gastrointestinal tract are usually controlled and limited by the dissolution process.[4,5] Plasma half-life of NTD 12-22h, bioavailability is 16-23% and partition coefficient i.e. log P value is 2.88. Numerous attempts have been made to overcome the solubility of NTD and improve its dissolution. Some of these attempts include alginate-based nanoparticles, glyceryl behenate solid lipid nanoparticles, peptide-based prodrug of lopinavir, Poly (lactic-co-glycolic acid) (PLGA) based nanoparticles[6]. However, these nanoparticles formulations suffer from poor drug loading and the involvement of the multiple complicated steps in their formulations. Even their commercial use is limited due to stability issues of the amorphous nature of the produced drug.

Nowadays, nanocrystals are considered as a formulation choice for drug showing poor solubility and dissolution rate[7]. Nanocrystals consists of stabilized submicron sized crystalline drug particles in liquid medium, usually water[8,9]. They can be produced either by precipitation technique (bottom-up approach) or by size reduction (top-down approach)[10].

Drug nanocrystals are nanoparticles being composed of 100% drug without any matrix material and mean particles size is below 1µm. The term drug nanocrystal implies a crystalline state of the discrete particles but depending on the production method, they can be partial or completely amorphous[11,12]. Nanonization of hydrophobic drug in the presence of surfactant is one of the important approaches for increasing the dissolution velocity of poorly soluble component. In the nanonization process, the coarse hydrophobic drug particles are converted small sized particle. Size reduction of drug particle is usually carried out in presence of different surfactant that imparts the physical stability to the nanocrystals vice-versa increasing the wetting property of nanosized drug particles[13].

The aim of the present investigation was to prepare poloxamer 188 stabilised NTD nanocrystals using anti-solvent precipitation method[14-17]. and investigate the influence of formulation variables on nanocrystals characteristics. Solid state characterisation, particle size (PS), zeta potential (ZP), polydispersity index, saturation solubility, *in vitro* release, and drug crystallinity parameters of freeze-dried nanocrystals were studied.

MATERIALS AND METHODS

Nitrendipine (NTD) was obtained as a gift sample from US Vitamin Ltd. Mumbai, India. Poloxamer was purchased

from Sigma chemicals, Mumbai. Polyvinyl alcohol was purchased from Alpha Chemicals, Ahmedabad. All other solvents and reagents in this work were of analytical/HPLC grade and used as provided.

Preformulation studies

The NTD was visually evaluated for physical state and color, odour and taste. Melting point of Nitrendipine was determined by open capillary tube method. Compatibility study of NTD with Poloxamer188 was determined using FTIR spectrums were recorded over the wave number 400-4000 cm^{-1}.

Formulation of Nitrendipine Nanocrystals

Formulation of NTD nanocrystals, a selection of stabilizer and its concentration is very important step. The two different stabilizers (Poloxamer 188 and Polyvinyl alcohol) was evaluated and selection of stabilizer on the basis of its hydrophilic nature. Formulate three batches to each stabilizer with varying its concentration (0.10%, 0.15%, 0.20%) and determine its particle size of those batches. At which concentration, stabilizes gives smaller particle size that concentration and that stabilizer was used for the formulation of NTD nanocrystals.[19,20]

NTD nanosuspension was prepared by the anti-solvent precipitation method. Briefly, drug NTD of different concentrations 20mg & 30mg was dissolved in a three different organic solvent (DMSO, Acetone, Acetonitrile) & 0.15% concentration of Poloxamer 188 was dissolved in water i.e. water was act as a antisolvent. The anti-solvent was cooled to below 5°C in an ice-water bath. Then, drop wise addition of organic solution into 50 ml of the pre-cooled anti-solvent at a stirring speed of 1000 rpm. Nanosuspension was prepared by adding the microliter quantity of drug solution to millilitre quantity of water quickly with continuous stirring on magnetic stirrer at 1000 rpm. Solvent was removed by stirring at 1000 rpm for next 2 hrs.

Optimization of parameters for preparation of NTD nanocrystals were, rate of addition of organic phase 0.5 ml/min, needle size 26 ½ gauges, stirring time 2 hrs., ratio of organic: aqueous phase 1:1. NTD nanosuspension was lyophilized, 5ml of nanocrystal suspension was filled in 10 ml glass vials, covered with stoppers and placed in a reeze dryer (LABCONCO)[21-22].

Saturation solubility study

Weighed amount of NTD, 10mg and the nanocrystal equivalent to 10 mg of the drug was separately introduced into 25ml Stoppard conical flasks containing 10 ml distilled water, and phosphate buffer pH 1.2. The sealed flasks were agitated by using thermostatically controlled rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. An aliquot was passed through 0.45µm membrane filter and the filtrate was suitably diluted and analysed using UV-visible double beam spectrophotometer (Jasco V-630) at the predetermined λmax (236 nm).

Characterization of NTD nanocrystals

Particle size analysis

Particle size of the nanocrystals formulation was determined by using particle size analyzer (Malvern Zetasizer Ver. 7.11 UK). Size and size distribution of the nanocrystals particles were determined through particle size analyser, after dilution with water and the diameters reported were calculated using mean particle size distribution. Measurements were performed in triplicate using 90° scattering angle at 25°C.

Zeta potential analysis

Measurement of zeta potential is also a prerequisite to know the stability of nanosuspension. Zeta potential is a measure of surface charge of particles and thus it imparts the colloidal stability due to particle-particle repulsion, as particle aggregation is less to occur for charged particles (a high zeta potential). So, the prediction of zeta potential also allows the prediction of stability of nanocrystals. Zeta potential of the nano suspended particles surface was determined by electrophoretic mobility in an apparatus such as a Malvern zetasizer (Malvern Instruments, UK) equipped with suitable software and calibrated with the supplied standard.

Process yield of freeze-drying process

The amount of product obtained after completion of process is determined by process yield. Briefly powder obtained from freeze drying was collected and product yield was obtained from following equation 1.

$$\text{Process yield} = \frac{\text{Weight of product obtained from freeze dry}}{\text{Weight of total dissolved Nitrendipine in sol}} \text{ X } 100$$

***In vitro* release study**

In vitro drug release studies were performed in USP type I dissolution apparatus I (Electrolab) according to United State Pharmacopoeia dissolution procedure. The nanocrystals of all batches were filled into hard gelatin capsules (capsule no. 03). Hard gelatin capsule put into dissolution basket was loaded with 900 ml of acidic buffer pH 1.2 with 0.5% SDS at 37 ± 0.5 °C with speed of 100 rpm. Each sample (5 ml) was withdrawn at 15, 30, 45, 60 minutes. The same volume was replaced by dissolution medium in the flask to maintain a constant volume. The samples were filtered and suitably diluted. The amount of drug dissolved determined by UV spectroscopy at λmax 236 nm.

Fourier transforms infrared spectrophotometry

FTIR spectrum shows the fundamental peaks corresponding to the chemical nature of the drug and excipients. FTIR studies were carried out in order to determine any possible interaction among drug and excipients used. IR absorption spectrum of NTD was determined by Fourier transform infrared spectrophotometer (Jasco- V-530 model). Spectra were recorded over the wave number 400-4000 cm^{-1}. Infrared

spectrums of pure drug and optimized batches were recorded. From the spectrum analysis the compatibility of ingredients in the formulations were determined.

X-Ray diffraction studies

The XRD patterns were recorded on X-ray diffracto meter (PW 1729, Philips, Netherlands). Samples were irradiated with monochromatized Cu-Ka radiation (1.542A˚) and analyzed from 50 to 500 2θ. The voltage and current used were 30 kV and 30 mA, respectively. The XRD procedure to estimate the degree of crystallinity was based upon the measurement of the total scattering and the scattering from the crystalline region of formulations & pure drug.

Differential scanning calorimetry

DSC studies were carried out using (Mettle-Toledo DSC 821 instrument). Indium and zinc standards were used to calibrate the DSC temperature and enthalpy scale. Freeze dried nanocrystals of optimized batch and pure drug were hermetically sealed in aluminium crucibles and heated at a constant rate of 10°C/min. over a temperature range of 25-300°C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 ml/min. An empty aluminium pan was used as standard reference and results were obtained in triplicates for each sample.

Scanning electron microscopy

The surface characteristics of the selected formulation were observed using a scanning electron microscope (JSM-6360; JEOL, Tokyo, Japan). The samples were gold-coated under vacuum and then examined. Acceleration during the observation was 25 kV.

Stability studies

Stability studies were carried out according to ICH guidelines Q1A (R^2). The Nanocrystal formulations were put into empty hard gelatin capsules (size 03) and subjected to stability studies at 40°C ±2°C and 75% ±5°C RH. Samples were charged in stability chambers (Aditi, Mumbai, India) with humidity and temperature control. The samples were withdrawn at specified intervals for analysis over a period of 30, 60 and 90 days. *In vitro* dissolution tests were carried conducted and percentage cumulative drug release calculated. Cumulative percentage release mean particle size, PDI, zeta potential was determined.

RESULTS AND DISCUSSION

NTD drug was found to be faint yellow in color, odourless, crystalline solid and melting point 156-160°C, which complies with E. P. limit. NTD nanocrystals were successfully prepared by the anti-solvent precipitation method. The obtained nanocrystals have been assessed for particle size analysis, zeta potential, saturation solubility and solid-state characterisation by XPRD, DSC, FTIR and SEM analysis. Compatibility studies of NTD and excipients was conducted on FTIR studies. FTIR spectrums revealed that the fundamental peaks of the NTD were retained in the physical mixture indicating absence of any chemical interaction between them.

Selection of surfactant & its concentration

Selection of best surfactant from Poloxamer188 and PVA on the basis of its hydrophilicity. Formulate the nanocrystal of by using these two different stabilizer. For each stabilizer three batches were prepared by varying its concentration (0.10%, 0.15%, 0.20%). The concentration of surfactants was optimized depending on the resultant particle size and polydisperability index of each batch. When surfactant poloxamer188 was used, particle size and PDI observed 355nm-435nm & 0.255- 0.342 as compared to PVA 520 nm - 545nm and 0.534.-0.601. Poloxamer188 was used as surfactants for stabilization of nanocrystals surface more effectively with smallest particle size and narrowest particle size distribution, observed as compared to PVA. Hence at concentration 0.15% of Poloxamer188 gives smaller particle size compare to other stabilizer and concentrations, so these concentrations were selected for formulation of nitrendipine nanocrystals.

Saturation solubility study

The optimized batch SN1 showed highest solubility in water (0.067 mg/ml), as compared with pure nitrendipine (0.0022 mg/ml) which are 30.45-fold greater than pure NTD respectively. Other formulations of nanocrystals SN2, SN3, SN4, SN5, SN6 batches were also shown to enhance the solubility in water, which was in 26 to 28-fold in range and greater than pure nitrendipine but less than formulation SN1. The solubility of formulation SN1 in acidic pH 1.2 buffer were 0.136 mg/ml. Thus, the solubility of formulations was improved 38.85-fold compared to pure nitrendipine. Saturation solubility of nanocrystals in distilled water and phosphate buffer pH 1.2 shown in table 1.

Table 1: Saturation solubility of all batches

Solvent	Pure drug	SN1	SN2	SN3	SN4	SN5	SN6
Distilled water	0.0022±0.02	0.067± 0.04	0.065±0.01	0.058±0.05	0.059±0.02	0.062±0.01	0.059±0.04
Phosphate Buffer PH 1.2	0.0035±0.05	0.136±0.03	0.129±0.04	0.119±0.02	0.116±0.01	0.121±0.04	0.127±0.02

*Indicates average triplicates ±SD (n=3)

Characterization of NTD nanocrystals

Particle size analysis (PSA)

PSA and PDI of all the formulations were measured by dynamic light scattering (DLS; Malvern Zeta Sizer, Nano-ZS90, UK). It was found that the smallest particle size was observed in formulation SN1, SN3, SN5 as compared to other formulations. SN1, SN3, SN5. formulation has average particle size 335 nm, 369nm and 424nm, while batches SN2, SN4, SN6, was average particle size 419nm to 553nm in range.

This could be explained by the decrease in surface tension by increasing the surfactant concentration, which facilitates the size reduction and stabilizes the formed nanocrystals with inhibition of aggregation. Moreover, PDI was significantly decreased by increasing the concentration of surfactant. The lowest PDI value was observed in the presence of 0.15% of Poloxamer surfactant concentration. These results were consistent with increasing the surfactant concentration leads to a significant decrease in the nanocrystals size and PDI. Particle sizes, PDI and zeta potential all formulations was shown in table 2 & Particle size distribution of batch SN1 in figure 1.

Zeta Potential Analysis

The zeta potential value of all the batches were found to be in the range of -20.5 to - 41.8 mV, optimized batch SN1 showed -41.8 mV mean zeta potential which means optimized formulation have more stable than other batches. High ZP values indicate the physical stability of the prepared nanocrystals with low probability of aggregation and crystal growth. The zeta potential of batches SN1 batch shown in figure 2.

Process Yield of Freeze-Drying Process

The process yields of freeze drying obtained with optimized parameter was in the range of 60-85%. Freeze drying yield of all formulation batches were shown in table 2.

Table 2: Mean particle size of all nanocrystal batches.

Batch Codes	Mean Particle Size (nm)	Polydispersity Index (PI)	Mean Zeta Potential (mV)	% process Yield
SN1	335	0.262	-41.8	85
SN2	443	0.476	-20.5	80
SN3	369	0.373	-29.3	60
SN4	419	0.520	-24.5	65
SN5	424	0.453	-22.5	70
SN6	553	0.606	-25.1	65

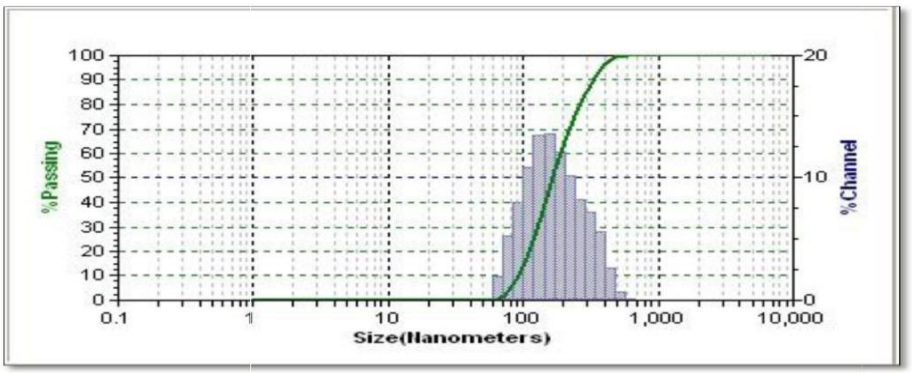

Figure 1: Particle size distribution of batch SN1

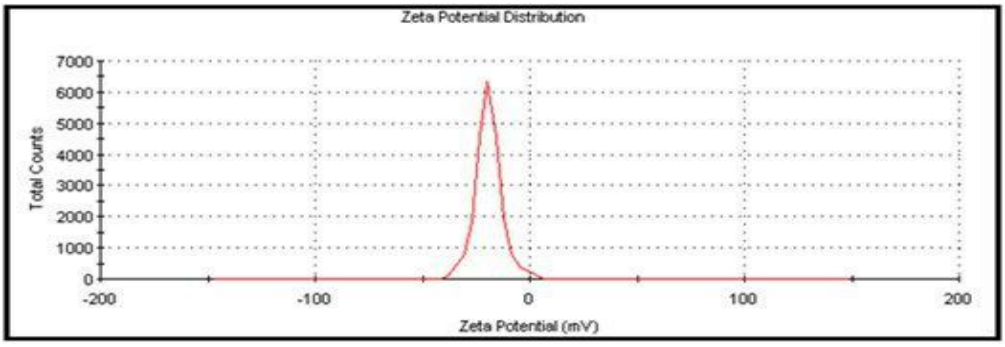

Figure 2: Zeta Potential of Batch SN1

In vitro release study

Nanocrystals formulation batch SN1 was showed significantly higher % release of drug as compared with other formulation batches. The cumulative percentage drug release of optimized batch SN1 was observed 92.20% within 3h. Moreover, the increase in the dissolution rate caused due to PS reduction can be explained by the decrease in diffusion layer thickness. The increased surface area described by the Noyes-Whitney equation and the higher surface-to-volume ratio enabled hydration over a larger surface area and, consequently, resulted in increased drug dissolution. Hence decrease in the particle size achieved will have significant effect in the drug solubility and dissolution. In vitro drug release for formulation batches SN1-SN6 were shown in figure 3.

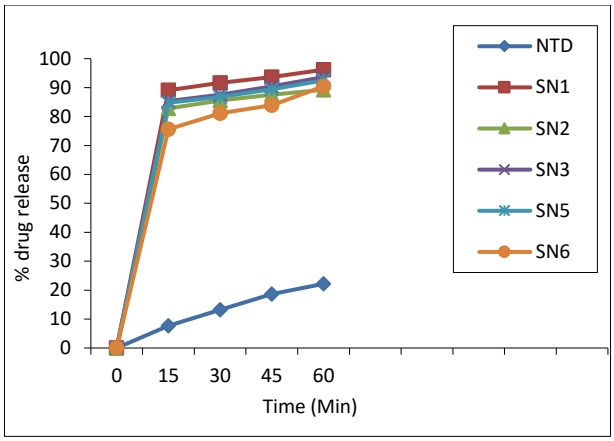

Figure 3: *In vitro* drug release for formulation batches SN1-SN6

Fourier transform infrared spectrophotometry

FTIR studies revealed that the fundamental peaks of the NTD was retained in the optimized formulation and indicating absence of any chemical interaction between NTD and excipients used. Thus, these excipients were used in the NTD nanocrystal formulation. From the FTIR studies, it can be seen that the principal peaks of the pure drug NTD were retained in the optimized batches are almost identical. The characteristics peak of the esterifies carbonyl group at 1700.95 cm^{-1}, N-H bending vibration for secondary amines at 1647.95 cm^{-1}, aryl nitro group at 1530 cm^{-1} confirm the presence of NTD. The characteristics peak of the esterifies carbonyl group at 1701.86 cm^{-1}, N-H bending vibration for secondary amines at 1648.14 cm^{-1}, aryl nitro group at 1531.11 cm^{-1} confirm the presence of NTD. It has been observed that there was no appreciable change in the position and nature of the characteristics band of drug in the formulations. It can be concluded that the drug maintains it's identity without going any chemical interaction. An overlain spectrum of pure Nitrendipine and optimized formulation is shown in figure 4.

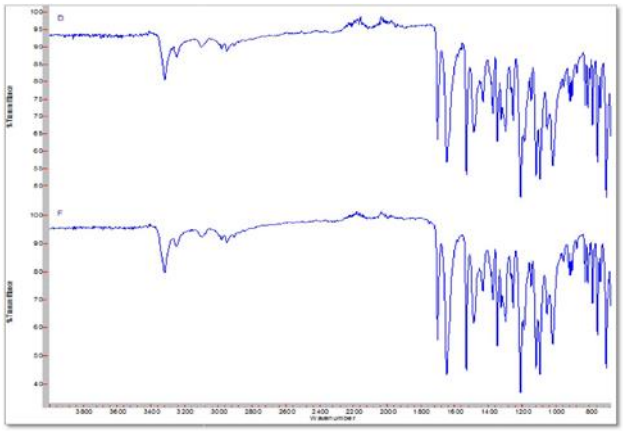

Figure 4: Overlain spectrum of pure drug(D) and optimized batch(F)

X-Ray diffraction studies

X-ray diffraction has been used to analyze potential changes in the inner structure of NTD crystals. The XRD pattern of the pure drug showed characteristic high-energy diffraction peaks at 2θ, 10.2, 15.6. 23.4, 29.8 indicating the crystalline structure of NTD. The decrease in peak intensity for nanocrystals can be attributed to the particle size reduction in formulation batches. The nanocrystals of optimized batch SN1 was characterized by less intensity of the diffraction peak, when compared with NTD pure drug, which demonstrates that the chemical structure of the drug was not changed before and after precipitation process. This clearly indicates that significant reduction in the crystallinity of the NTD nanocrystals and the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity. It was confirmed that NTD existed in amorphous state in the NTD nanocrystals because of the disappeared sharp peak of NTD in the diffraction pattern. Furthermore, maintenance of the initial crystalline state is advantageous for long-term stability. X-ray diffraction peaks of pure drug and optimised batch shown in figure 5.

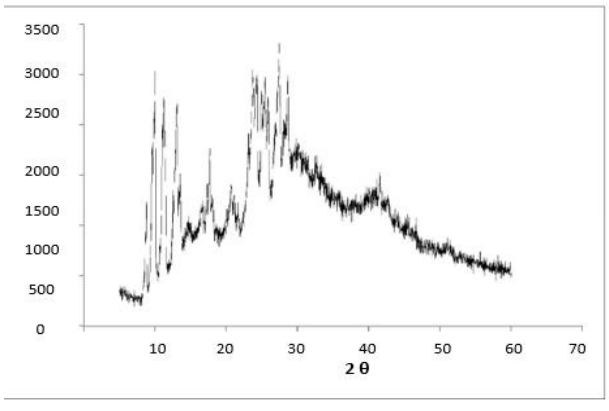

Figure 5: XRD Pattern of batch SN1

X-ray diffraction has been used to analyse potential changes in the inner structure of NTD crystals. The diffraction spectrum of pure NTD showed the drug was of crystalline nature as indicated by numerous, relative sharp and distinct peaks at a diffraction angle 2θ of 10.2, 15.1.

18.3, 21.4 and confirm the crystalline structure of drug shown in figure 8. The decrease in peak intensity for nanocrystals can be attributed to the particle size reduction in formulation batches. The nanocrystals of optimized batch SN1 was characterized by less intensity of the diffraction peak, when compared with NTD pure drug, which demonstrates that the chemical structure of the drug was not changed before and after precipitation process. This clearly indicates that significant reduction in the crystallinity of the NTD nanocrystals and the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity shown in figure 8. It was confirmed that NTD existed in amorphous state in the NTD nanocrystals because of the disappeared sharp peak of NTD in the diffraction pattern. Furthermore, maintenance of the initial crystalline state is advantageous for long-term stability.

Differential Scanning Calorimetry

DSC was performed to explore the physical changes that occurred in the drug after processing into nanocrystals. The pure drug exhibited a large and sharp endothermic peak at 159.48°C indicating the melting point. DSC thermogram of formulation SN1 showed an endothermic peak at 153.62°C ascribed to the melting of nitrendipine, indicated that the slight change in the crystalline nature. However, the endothermic peak of nitrendipine shifted about 6°C to the left due to size reduction of the crystals. It should be noted that reduction in melting temperature could increase the dissolution rate. No additional peaks were found to demonstrate the significant changes in the melting characteristics of NTD in the formulation, indicating no polymorphic changes during nano sizing process.The peaks were found to be nearly identical, with a calculated enthalpy (ΔH) of pure drug and SN1 were around 72.21 J/g, 49.56J/g, respectively. The DSC results were in support of the XRD analysis, which showed decrease in drug crystallinity. DSC results of the NTD pure drug and the formulation SN1 shown in figure 6 & 7.

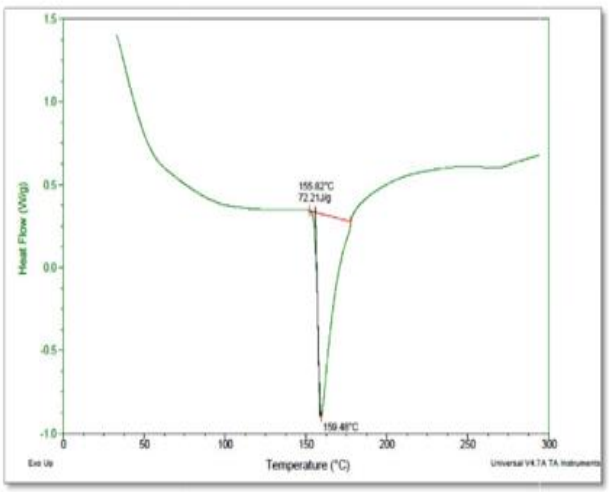

Figure 6: DSC OF Pure Drug

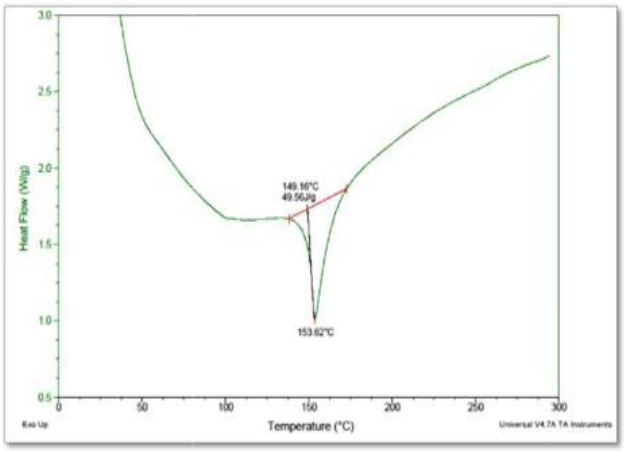

Figure 7: DSC OF Optimized Batch SN1

Scanning Electron Microscopy

Surface morphology of the formed nanocrystals was determined by using SEM and found that crystalline nature of all the formulations remains with slight change in crystallinity. It was clear that the investigated lyophilized matrix possessed a highly porous nature and rods with smooth and uniform surfaces, which led to the rapid penetration of water resulting in rapid drug dissolution. The nanocrystals were found to be flaky in shape with a narrow particle size distribution. The pure drug was irregular in shape with a broad particle size distribution. The SEM images and particle size distributions and its morphology of the NTD nanocrystals were presented in figure 8.

Figure 8: SEM Images of Optimized batch SN1

Stability Studies

The stability studies of NTD loaded nanocrystals in terms of particle size distribution, PDI, zeta potential, and % release of drug was monitored for 3 months at 2-8°C and RT 25-30°C. Cumulative percentage release of optimized batch SN1 after its stability period 30days, 60days and after 90 days was observed 94.452%, 92.543% and 90.414% respectively. There was slight decrease in percentage release but not much significant therefore it suggested that, the final formulation confirm its stability. Mean particle size was increases from 335nm to 370nm, PDI changes 0.262 to 0.372 and zeta potential changes -

41.8 to -38.2 after 90 days stability testing. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. Thus, these studies confirmed the stability of the developed formulation and its compatibility with hard gelatin capsules. Stability of NTD loaded nanocrystals shown in figure 9.

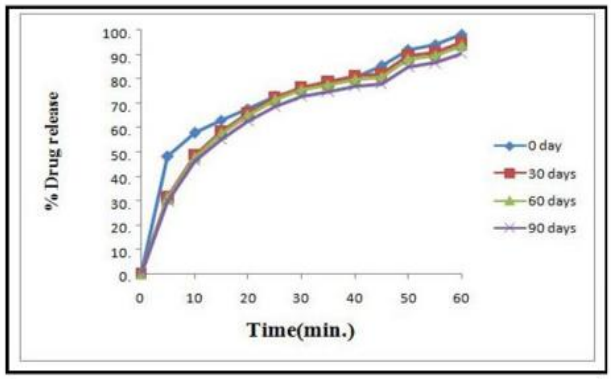

Figure 9: In-vitro stability studies of optimized batch SN1

CONCLUSIONS

A present study it can be concluded that, an antisolvent-precipitation method was successfully employed to prepared stable NTD nanocrystals. Dimethyl sulphoxide was the suitable solvents for the preparation of NTD nanocrystal. Batch SN1 showed practical yield (85%), particle size (335nm), high saturation solubility in distilled water (0.067mg/ml) and in acidic pH 1.2(0.136mg/ml) and rapid drug in vitro drug release (96.186%). The decreased particle size increases the surface area and solubility of drug manifolds and there was proportionate increase in the bioavailability of poorly soluble drug of NTD. DSC and PXRD data revealed that there was partially reduced the crystallinity of the formulated nanocrystals of NTD. Preparation of nanocrystal formulations was simple and reproducible, and thus, could be used to improve the dissolution profiles of other poorly water-soluble active drug substances.

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