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Research Article

Development of Nanonized Nitrendipine and Its Transformation into Nanoparticulate Oral Fast Dissolving Drug Delivery System

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Abstract. The present research focuses on the development of a nanoparticulate (nanocrystals-loaded) rapidly dissolving (orodispersible) tablet with improved solubility and bioavailability. The nanosuspension (NS) was prepared by antisolvent sonoprecipitation technique and the optimized NS was lyophilized to obtain nanocrystals (NCs), which were evaluated for various parameters. The nitrendipine (NIT) nanoparticulate orodispersible tablet (N-ODT) was prepared by direct compression method. The optimized N-ODT was evaluated for pre and post compression characteristics, *in vivo* pharmacokinetic and stability profile. The optimized NS showed a particle size of 505.74 ± 15.48 nm with a polydispersity index (PDI) of 0.083 ± 0.006. The % NIT content in the NCs was found to be 78.4 ± 2.3%. The saturation solubility of NIT was increased remarkably (26.14 times) in comparison to plain NIT, post NCs development. The DSC and p-XRD analysis of NCs revealed the perseverance of the integrity and crystallinity of NIT on lyophilization. The results of micromeritic studies revealed the good flow-ability and compressibility of NCs blend. All the post-compression properties of N-ODT were observed within the standard intended limit. The dispersion, wetting, and disintegration time of the optimized batch of N-ODT was found to be 39 ± 1.13 s, 44.66 ± 1.52 s, and 33.91 ± 0.94 s respectively. The *in vitro* dissolution study displayed 100.28 ± 2.64% and 100.61 ± 3.3% of NIT released from NCs (in 8 min) and N-ODT (in 6 min) respectively, while conventional NIT tablet took 30 min to release 99.94 ± 1.57% of NIT. The *in vivo* pharmacokinetic study in rabbits demonstrated significantly ($p < 0.05$) higher bioavailability of NIT on release from N-ODT than the conventional NIT tablet. Thus, N-ODT could be a promising tool for improving the solubility and bioavailability of NIT and to treat cardiovascular diseases effectively.

KEY WORDS: nitrendipine; nanocrystals; orodispersible tablet; design of experiment; pharmacokinetics.

INTRODUCTION

Cardiovascular diseases (CVD) are one of the important causes of illness and death globally. As per the scientist's estimation, the mortality and death rate due to CVD would be more in the future. Among the different CVD, hypertension is one of the major diseases affecting the people (1).

The oral route (OR) among the different routes is of significant importance due to its various noteworthy features (ease of administration, patient's compliance, slightest sterility constraints and supple design in dosage forms). However in OR, drug solubility is playing an imperative job in producing a pharmacological response by achieving a desired plasma concentration (above minimum effective concentration; MEC). Drugs with poor water solubility are often required in large doses to achieve therapeutic plasma concentrations after oral administration and create a huge problem in formulation development (2).

Nitrendipine (NIT) is a dihydropyridine calcium channel blocker (CCB) used to treat hypertension and angina pectoris. It acts primarily through coronary and systemic arteries dilation and enhanced delivery of oxygen to the myocardial tissue (3). It is a poorly water-soluble (BCS class II) drug with a solubility of about 2.0 μg/mL in water at 37°C (4). Moreover, it undergoes extensive first-pass hepatic metabolism and has a poor oral bioavailability (only 10–20%) in humans (5). Therefore, there is an unmet need to develop novel formulations that can enhance solubility and dissolution rate and can minimize the presystemic metabolism of NIT to tackle the poor bioavailability related issues of NIT successfully.

Various approaches used to improve the solubility of drugs include micronization, nanonization, complexation,

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solid dispersion etc. (6,7). Among these approaches, nanosuspension/nanocrystals (NS/NCs) have received great attention for the delivery of poorly water-soluble drug entities. The NCs are capable of enhancing solubility and dissolution rate of weakly soluble drugs by providing increased drug particle surface area coming in contact with the dissolution medium and thereby enhancing oral bioavailability (8–12). Moreover, NCs can be administered by different kinds of the route such as intravenous, oral, ocular, dermal, and pulmonary (13–15).

Fast dissolving drug delivery systems (FDDDS) are the dosage forms meant to self-disperse quickly within seconds, on contact with the hydrating environment of oral cavity in the form of saliva (16–18). These have been developed to produce the quick onset of action of a drug by attaining immediate plasma drug concentration mediated by tongue's rich vascular network and to minimize first-pass hepatic metabolism by bypassing the liver due to favored pre-gastric absorption of the drug. Among the many fast dissolving solid dosage forms, orodispersible tablet (ODT) has gained more attention due to its simplicity of administration, high patient conformity particularly for patients having swallowing difficulty such as pediatric, geriatric and psychic categories of patients. Moreover, the disintegration of ODT takes place quickly in the oral cavity itself, thereby producing fast onset of action of drugs (19,20).

Therefore, the primary objective of the present research was the development and characterization of nanoparticulate solid oral dosage form of a practically water insoluble (5), antihypertensive agent - NIT by converting the optimized batch of formulated drug loaded nanosuspensions into fast dissolving tablet dosage form making use of lyophilization technique, so as to render improved solubility, dissolution rate, bioavailability and rapid onset of action of the drug. Various methods used to obtain the desired purpose are antisolvent sonoprecipitation technique to prepare NS with 3^2 optimal response surface design employed to optimize it using Stat Ease Design Expert 10 software and data obtained was analyzed statistically using Analysis of Variance (ANOVA). Prepared NS was evaluated by different methods including particle size and size distribution determination, saturation solubility and *in vitro* drug release determination along with stability testing of NCs obtained by lyophilization of the optimized NS. The NCs were further transformed through direct compression technique to orodispersible tablet, which was optimized by 2^3 full factorial design (FFD) using the same software and ANOVA applied to analyze statistically. Then, the optimized N-ODT was evaluated by various procedures like determination of hardness, friability, weight-variation, disintegration characteristics, *in vitro* drug release, *in vivo* pharmacokinetic profiling (in comparison with the conventional tablet dosage form of NIT) along with the stability testing, as the salient ones.

MATERIALS AND METHODS

Materials

NIT was gifted by Concept Pharmaceuticals Ltd., Aurangabad (M.S.) India. Polyethylene glycol-200 (PEG-200) was obtained from Govt. College of Pharmacy, Karad (M.S.) India. Hydroxy propyl methyl cellulose-E6 (HPMC), croscarmellose sodium (CCS), microcrystalline cellulose PH 102 (MCC PH 102), lactose anhydrous, mannitol, magnesium stearate, sodium hydroxide, and potassium dihydrogen phosphate were purchased from Vijay Scientific Supplies, Aurangabad. Acetone and octanol were purchased from Dodal Enterprises, Aurangabad. The analytical grade materials and reagents were employed for the study.

Methods

Preparation of Nanosuspensions

An antisolvent sonoprecipitation technique was employed for the preparation of NS (5,21). Firstly HPMC-E6 was dissolved in 20 mL of distilled water (antisolvent solution) by maintaining a temperature below 5°C using an ice bath. Then, 480 mg of NIT was dissolved in an eight mL of acetone (drug solution). Both the solutions were streamed through a 0.45 μm filter. Then, 4 mL of drug solution was introduced rapidly by placing a needle (24 G) vertically over the antisolvent solution maintained under stirring at 900 rpm to develop a suspension. Finally, the above suspension was immediately shifted to a Nessler's tube (2.5 cm diameter) and subjected to probe ultrasonication (Probe ultrasonicator; Sonics & Materials Inc., USA, vibra cell) to obtain NS. To minimize the crystal growth rate and accelerate the nucleation rate, the whole process was carried out at a temperature below 5°C.

Optimization of NS

The formulation of NS was optimized by using 3^2 optimal response surface design (22). The two factors were concentration of HPMC-E6 and time length of probe ultrasonication. The impact of these factors was studied on the dependent variable. Initially, four different stabilizers (PVP-K30, Tween-80, PVA and HPMC-E6) were employed to formulate NIT nanosuspensions and a suitable one was selected based on particle size and used further (Table I). Then, overall 9 runs were spawned with optimized stabilizer (HPMC-E6) using software and the design matrix as shown in Table II.

Obtained optimized nanosuspension was rapidly frozen in liquid nitrogen and lyophilized under vacuum (pressure < 10 Pa) for 15 hour to obtain fluffy dry powder (nanocrystals) soluble in water, which was then compressed to nanoparticulate ODT.

Evaluation of NS

Particle size. The particle size and size distribution of formulated NCs after reconstitution was determined by Zetasizer (Beckman Coulter Delsa Nano C Particle Size Analyzer). The instrument operates on the proposition of photon correlation spectroscopy (PCS), which is an extensively used technique for the analysis of hydrodynamic size of the particles in solution (5).

Differential Scanning Calorimetry Analysis. The differential scanning calorimetry (DSC) analysis of plain NIT,

Table I. Effect of Different Stabilizers Used for the Preparation of NIT Nanosuspension on Particle Size

Trial formulation no.	Stabilizer	Concentration of NIT (mg/mL)	Drug to stabilizer ratio	Time for probe ultrasonication (min)	Mean particle size (nm)
TF 1	PVP-K30	50	2:3	25	2850.46 ± 23.78
TF 2	Tween-80	50	2:3	25	1388.23 ± 12.62
TF 3	**HPMC-E6**	**50**	**2:3**	**25**	**764.2 ± 17.25**
TF 4	PVA	50	2:3	25	1068.1 ± 33.008

physical mixture of NIT with different polymers, NCs and physical mixture of NCs with different polymers (1:1% w/w) was carried out using differential scanning calorimeter (TA-60WS Thermal Analyzer, Shimadzu, Japan). Thermograms were recorded by heating the samples over the range between 30°C and 300°C at 10°C/min scan rate in a dry nitrogen atmosphere. An Al_2O_3 pan was used as the reference material (5).

Fourier Transform Infrared Spectroscopic Analysis. The Fourier transform infrared (FTIR) analysis of plain NIT, physical mixture of NIT with different polymers and physical mixture of NCs with different polymers was performed to evaluate drug-excipients compatibility using an FTIR spectrophotometer (Shimadzu Corporation) over the wavenumber 4000 to 500 cm^{-1}. Sample in small quantities was placed between KBr pellets, which was further positioned into the sample holder and scattered uniformly to acquire the infrared spectrum (23).

Powder X-ray Diffraction Analysis. The crystallinity of NIT and NCs was assessed using X-ray Diffractometer (VirTis Advantage Plus at Wockhardt Lmt., Aurangabad, M.S. India) using Cu-Kα radiation (λ = 1.54 A° and voltage V = 40 kV, 50 mA). Using a zero background sample holder, the samples were scanned between 5° and 60° diffraction angle (2θ) with an increment of 0.02° at 1 s/step (24,25).

Saturation Solubility. The shake-flask method was used to determine the saturation solubility of NCs. A surplus amount of sample was added in a 10 mL of double distilled (DD) water and kept on an orbital shaker (HMG India, Mumbai) maintained at 300 rpm, 37°C temperature for 72 h. On completion of the set time period, the sample was centrifuged at 10,000 rpm for 10 min (Remi Electrotechnik Instruments). This was followed by filtration of supernatant using a membrane filter (0.45 μm) to separate out any un-dissolved drug. Finally, the filtrate was diluted with DD water and subjected to analysis using a UV-visible spectrophotometer (Pharmaspec-1700, Shimadzu Corporation, Tokyo, Japan) at λ_{max} = 239 nm and the NIT content was determined (16).

Drug Content Determination. The % NIT content in the NCs was determined by dissolving NCs (5 mg) in 5 mL of DD water. The supernatant obtained following centrifugation at 5000 rpm for 10 min was suitably diluted and analyzed for the drug content by UV-visible spectrophotometer at 239 nm using DD water as a blank. The analysis was performed in triplicate (26).

Stability Study of NCs. The stability study of NCs was executed in screw-capped glass vials at accelerated stability conditions of 40 ± 2°C/75 ± 5% RH for a period of 6 months. The physical stability of NCs was estimated by deducing parameters like particle size and PDI at predetermined time intervals in triplicate (27).

Preparation of Nanocrystals-Loaded ODTs

A direct compression method was espoused for the preparation of NIT NCs-loaded ODTs (27,28). Briefly, accurately weighed quantities of optimized NIT NCs (lyophilized powder) and MCC PH102 (directly compress-

Table II. Formulation and Optimization of NIT Nanosuspensions Using HPMC-E6 as Stabilizer

Formulations	Ingredients			Response: Particle size (nm)
	Concentration of NIT (mg) in 4 mL of acetone	Concentration of HPMC-E6 (% w/v) in 20 mL of Water	Time length of probe ultrasonication (min)	
F1	240	1	25	852.66 ± 20.40
F2	240	1	30	804.18 ± 25.94
F3	240	1	35	983.72 ± 31.91
F4	240	2	25	769.34 ± 25.14
F5	**240**	**2**	**30**	**505.74 ± 15.48**
F6	240	2	35	748.82 ± 10.57
F7	240	3	25	720.05 ± 27.95
F8	240	3	30	808.39 ± 13.52
F9	240	3	35	945.57 ± 22.22

ible diluent) were passed through sieve no. 60 and mixed homogeneously for 15 min on an ointment tile with the help of a spatula (blend 1). Then, the weighed quantities of lactose, mannitol and CCS (superdisintegrant) were passed through sieve no. 40 and mixed homogeneously for 10 min (blend 2). Blend 2 was mixed with blend 1 homogeneously for further 20 min and then magnesium stearate was added and mixed for 2 min. Finally, the obtained powder blend was compressed using an 8 mm size flat-faced punch on a single punch rotary compression machine (BPRESS-I, CIP Machinery Pvt. Ltd., Ahmedabad).

Optimization of N-ODTs

Nanocrystals-loaded ODTs (N-ODTs) were optimized using 2^3 full factorial design (FFD). The three factors were the concentration of CCS, concentration of MCC PH 102 and compression force at two different levels. The impact of these factors was analyzed on the dispersion time, a dependent variable. The overall 8 runs were spawned as shown in Table III.

Evaluation of Optimized N-ODTs

Pre-compression Evaluation

The flow-ability of a powder or blend is of vital significance in the production of dosage forms. It influences getting an even feed as well as consistent filling of tablet dies, so as to avoid high dose discrepancy. The prepared NIT-NCs tablet blend was studied for angle of repose (deduced by fixed funnel method), Hausner's ratio and Carr's index to determine its flow property and compressibility (29,30).

Post-compression Evaluation

Thickness. A digital thickness gauge (VJ Instruments, India) was used to determine the thickness of ten randomly selected tablets. The thickness was expressed as (mean ± SD) in mm (31).

Diameter. The diameter of the tablet was deduced by a digital thickness gauge choosing ten tablets randomly and was expressed as (mean ± SD) in mm (32).

Hardness. Six tablets were tested for the hardness using Pfizer hardness tester (Shreeji Chemicals Instrument Division, Mumbai) and it was expressed in kg/cm^2 (33). A range of 3–5 kg/cm^2 is considered an acceptable one for ODT (34).

Friability. The friability of tablets was studied by using Roche Friabilator (Secor Laboratory Instruments). Briefly, 20 tablets were weighed and placed into the Friability test apparatus, which was operated for 100 revolutions at a speed of 25 rpm. After completion of 100 revolutions tablets were dusted and weighed again. The % loss in weight expressing friability was calculated by using the following formula:

$$\% \text{ Loss in weight} = \text{Initial Wt.} - \text{Final Wt.} / \text{Initial Wt.} \times 100$$

Weight Variation. Twenty tablets were weighed individually and collectively both using digital weighing balance (AUX220, Shimadzu Corporation, Tokyo, Japan) and the mean weight was calculated. The individual weight of each tablet was compared with the calculated average weight and % deviation was reported (31).

Drug Content Uniformity. Five crushed tablets were transferred separately into a 100 mL volumetric flask and volume was adjusted by PBS pH 6.8. The content in the volumetric flasks was sonicated to achieve complete dissolution and then the solution was filtered through a membrane filter (0.45 μm). Finally, 1 mL of the filtered solution was diluted up to 25 mL with the same buffer solution and the absorbance was measured making use of a UV-visible spectrophotometer at 239 nm against PBS 6.8 as a blank (32).

Dispersion Time. Dispersion time of N-ODT was determined by placing three tablets individually into 10 mL of PBS (pH 6.8) accommodated in a Petri dish of internal diameter (ID) 10 cm. The buffer solution was maintained at 37 ± 0.5°C temperature. The time consumed by each tablet for complete

Table III. Formulation and Optimization of N-ODTs

Formulations	Ingredients (mg)						Compression force (ton)	Response: dispersion time (sec)
	NIT NCs	MCC (PH 102)	CCS	Lactose (anhydrous)	Mannitol	Magnesium stearate		
F1	30	133.0	11.97	124.03	40	11	2	52
F2	30	133.0	19.00	117.00	40	11	2	60
F3	**30**	**140.47**	**11.97**	**116.56**	**40**	**11**	**2**	**39**
F4	30	140.47	19.00	109.53	40	11	2	46
F5	30	133.0	11.97	124.03	40	11	4	72
F6	30	133.0	19.00	117.00	40	11	4	78
F7	30	140.47	11.97	116.56	40	11	4	89
F8	30	140.47	19.00	109.53	40	11	4	103

dispersion was recorded and the result was expressed as the average of dispersion time values with ± standard deviation. This test can also be termed as modified disintegration test (35,36).

Disintegration Time. The disintegration test was carried out employing a disintegration test apparatus (V-Scientific, India) operating at 28–32 cycles per minute on six tablets (as specified in I.P.-1996) using PBS (pH 6.8) maintained at 37 ± 2°C. The time required for the complete disintegration, without any palatable mass remaining in the apparatus for each tablet was measured in seconds. The test was executed 3 times (36).

Wetting Time. It is a significant parameter, which provides information regarding the disintegration traits of the tablet. Briefly, a tissue paper with dual folds was positioned over a petri dish (ID-10 cm) containing 10 mL of distilled water. Then, a tablet was placed on the tissue paper surface and the time taken by the tablet for getting fully wet was measured. The test was conducted in triplicate (36,37).

In Vitro Dissolution Study. The *in vitro* dissolution test of NIT from NCs and N-ODT was performed by USP dissolution test apparatus-II (paddle type) (DISSO 2000, LABINDIA Analytical Instruments, Mumbai, India) using PBS (pH 6.8) as the dissolution medium and was compared with conventional NIT tablet. A weighed quantity of NCs and single N-ODT (equivalent to 10 mg of NIT) were separately dispersed in 900 mL of PBS, stirred at 50 rpm and maintained at 37 ± 0.5°C temperature. After predetermined time intervals of 2 min for 20 min, a 5 mL sample was withdrawn and at the same time an equal volume of fresh medium was transferred back to the apparatus. The test samples withdrawn were analyzed, after centrifugation and suitable dilutions with dissolution medium at 239 nm using a UV-visible spectrophotometer. All measurements were performed in triplicate (38,39).

In Vivo Pharmacokinetic Study. The animal handling, caring, and the protocol for the study were approved by the animal ethical committee (Savitribai Phule, Pune University). Six male white rabbits (N = 6, n = 3 for each group or each formulation) weighing 2.3 ± 0.2 kg were used for the study. The rabbits were fasted for the duration of a night (12 h) before administration of the formulations, but had free means to approach water. The animals were split into 2 groups (A and B) randomly, with 3 animals in each group. Group A rabbits were anesthetized with intravenous injection of phenobarbital at a dose of 25 mg/kg so as to secure the existence of the N-ODT in the oral cavity, avoiding passing down the gastrointestinal tract. They were then set on a table, held in a horizontal position by the lower jaw using a rabbit holder. Careful placing of the N-ODT formulation (equivalent to 10 mg of NIT) on the tongues of group "A" rabbits was done. Conventional tablet of NIT (equivalent to 10 mg of NIT) as a control was administered orally through oral gavage, by dispersing in 2 mL of distilled water to rabbits of group "B."

Retrieval of blood samples (0.5 mL each) by marginal ear vein puncture at different time intervals (0-predose, 15, 30, 45, 60, 120, 180, 360, 720, and 1440 min) post-dosing for pharmacokinetic analysis was performed. Blood samples were collected in centrifuge tubes carrying sodium citrate as an anticoagulant (3.4% w/v). The plasma was separated out by the centrifugation of samples at 3500 rpm for 10 min at room temperature (40). Finally, the NIT was estimated by HPLC (JASCO LC-NET II/ADC, Jasco Corporation, Japan) and C18Hs (5 μm, 250 × 4.6 mm) column. The mobile phase used was a mixture of methanol, acetonitrile and water (50:25:25 v/v/v) with a flow rate of 1.2 mL/min and the UV detector was set at 239 nm. The samples to be analyzed were re-dissolved separately in 30 μL of the mobile phase and 20 μL samples were injected into HPLC (5). The standard pharmacokinetic (PK) parameters (mean ± SD) of NIT were procured from plasma drug concentration versus time profile applying a non-compartmental model with Kinetica® 5.0 PK/PD Analysis, Demo Version 5.0 software (Thermo SCIENTIFIC, USA). The study was conducted in triplicate (41).

Stability Study of N-ODT. The study for determining the stability of the optimized batch of N-ODT was executed by charging the samples in high-density polyethylene (HDPE) bottles for a period of 6 months at accelerated stability conditions (40 ± 2°C/75 ± 5% RH). At predetermined time intervals, the physical stability of N-ODTs was evaluated in terms of physical appearance, hardness, disintegration time, drug content uniformity and dissolution profile (27).

RESULTS AND DISCUSSIONS

Optimization of NS

Effect of Formulation Variables on Particle Size

Contour plot (Fig. 1a) and 3D surface response plot (Fig. 1b) were used to reveal the effect of stabilizer concentration (A) and time length of probe ultrasonication (B) on the particle size of NS. Both variables demonstrated a mixed (positive and negative) effect on particle size. The increase in the A and B caused a decrease in particle size of NS (negative effect) up to a certain level and above that level the increase in both A and B caused an increase in the particle size (positive effect). Thus, a curvilinear 3D surface response plot was obtained. Among the **9** batches prepared, the NS 5 displayed minimum particle size (505.74 ± 15.48 nm) in comparison to other batches. Therefore, batch NS 5 was selected as an optimized batch of the NS formulation. The assessment of results through ANOVA depicted F-value to be 49.49 and p value to be 0.0001, which indicates linear model to be significant. The best-fitted model for particle size (R^2 = 0.9612) was found to be a linear model. The polynomial equation was generated on the basis of the best-fitted model, wherein the (+) sign indicates the increase in the response and the (-) sign indicates a decrease in the response.

The final equation in terms of coded factors for particle size is:

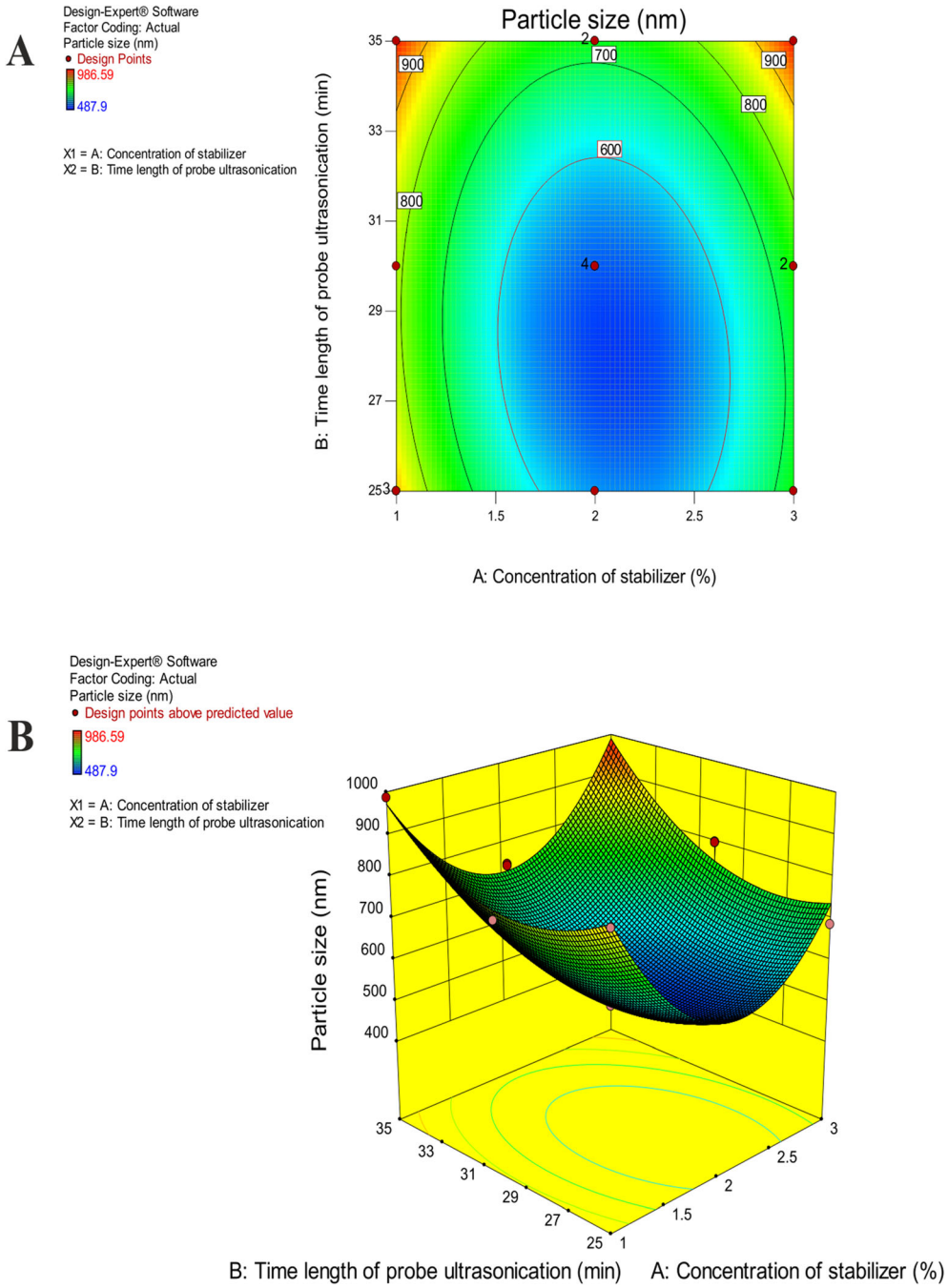

Fig. 1. **a** Contour plot and **b** Response surface plot for independent variables of NS with particle size as a response

$$\text{Particle size} = +533.44 - 31.55*A + 84.5*B + 40.41*AB + 251.69*A^2$$

DSC Analysis

The thermal analysis by DSC was used to check the compatibility between the drug and formulation excipients. The DSC thermograms of plain NIT, NIT in a physical mixture with different excipients, NCs, and NCs with tablet excipients are shown in Fig. 2. The DSC thermogram of plain NIT (Fig. 2a) showed a peak corresponding to NIT at 160.8°C. Besides, the DSC thermograms of NIT with HPMC-E6 (Fig. 2b) and NIT with CCS (Fig. 2c) separately displayed sharp endothermic peaks at 159.9°C and 161.15°C respectively, which are in correspondence to the peak of NIT. Thus, the obtained results demonstrated the perseverance of drug integrity in combination with different excipients and thereby revealing the compatibility of NIT with formulation excipients. The DSC thermogram of NCs (Fig. 2d) displayed a sharp

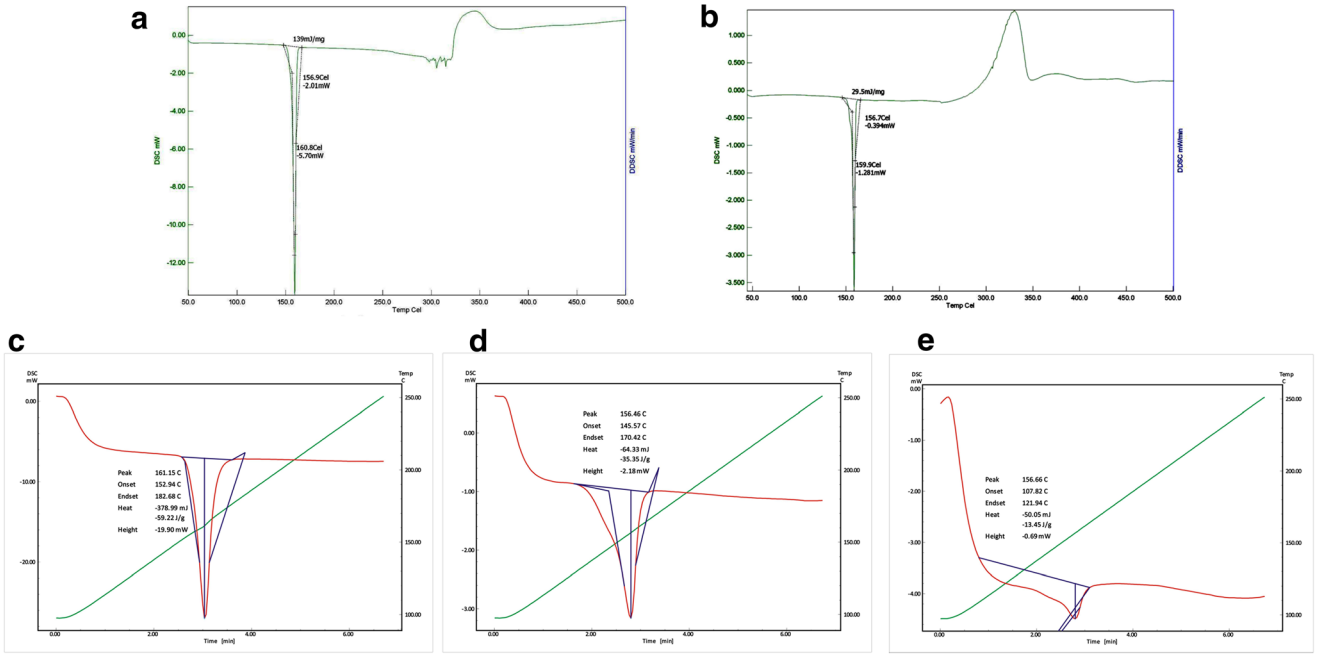

Fig. 2. DSC thermograms of **a** plain NIT, **b** physical mixture of NIT and HPMC-E6, **c** physical mixture of NIT and CCS, **d** NCs, and **e** physical mixture of NCs with MCC and CCS

endothermic peak at 156.46°C, which is attributed to the peak of NIT. This indicates that NIT integrity has not been affected by lyophilization and also it is present in crystalline form. However, the size reduction of drug crystals caused the drifting of the endothermic peak for about 4°C to the left. No glass transition region of the drug was seen in the thermogram of the lyophilized nanocrystals. This also suggests that NIT still remains in a crystalline state.

Moreover, a DSC thermogram of NCs in combination with MCC and CCS (Fig. 2e) showed an endothermic peak at 156.66°C which is very close to the peak value of 156.46°C depicted in the DSC thermogram of NCs (Fig. 2d). This obtained result revealed no interaction between NCs and tablet excipients and therefore the formulation of N-ODT is favored. Besides, we observed the broadening and shortening of the peak in the thermogram of NCs with MCC and CCS. This can be attributed to the change in the crystallinity on mixing of NCs with tablet excipients (27).

FTIR Analysis

The FTIR analysis was also used to assess the drug and formulation excipients compatibility. The FTIR spectra of plain NIT and NIT with a physical mixture of excipients and NCs with tablet excipients are shown in Fig. 3.

In the FTIR spectra of plain NIT (Fig. 3a), the characteristic peaks at 1701.22, 1215.15 cm^{-1}, and around 1545 cm^{-1} are attributed to the esterified carbonyl group and N-H bending vibration for secondary amines respectively. The peaks at a wavenumber around 3085 cm^{-1} and around 750 and 700 cm^{-1} are peaks corresponding to C-H stretching vibration of an aromatic ring and monosubstituted benzene respectively.

The physical mixture of NIT with HPMC-E6 (Fig. 3b), which constitutes the NS, exhibited peaks at wave numbers 1699.29, 1213.23, 1529.55, 3089.96, 754.17, and 704.02 cm^{-1}, which are in close proximity to characteristic peaks present in FTIR spectra of plain NIT (Fig. 3a). This indicates compatibility between NIT and HPMC-E6 (42).

The FTIR spectra of physical mixture of NIT with CCS (Fig. 3c) showed peaks at wave numbers 1697.36, around 1215, 1531.48, 3089.96, around 750 and 704.02 cm^{-1}, which are in accordance with the characteristic peaks of plain NIT (Fig. 3a).

The FTIR spectra of a physical mixture of NCs with MCC and CCS (Fig. 3d) presented peaks around 1701, 1215, 1529.55, 3100, 754.17, and 704.02 cm^{-1}, that are very close to the characteristic peaks given by NIT. This result suggested that the integrity of NIT has been preserved on the lyophilization of NS and no interaction between NCs and tablet excipients was also inferred.

Thus, the characteristic peaks of the NIT were observed in both NCs (a physical mixture of NIT with HPMC-E6) and N-ODT (a physical mixture of NCs with MCC and CCS), revealing the compatibility of the NIT with all the formulation excipients.

p-XRD Analysis

The powder X-ray diffraction (p-XRD) pattern of both plain NIT and NCs is depicted in Fig. 4. The p-XRD pattern of plain NIT (Fig. 4a) exhibited typical high-energy diffraction peaks at 2θ values between 8° and 30° with three most significant peaks at 9.944°, 11.469°, and 13.087°. These peaks imply the crystalline structure of the NIT (4). The NCs p-XRD pattern (Fig. 4b) also displayed distinguishing high-energy diffraction peaks at 2θ values

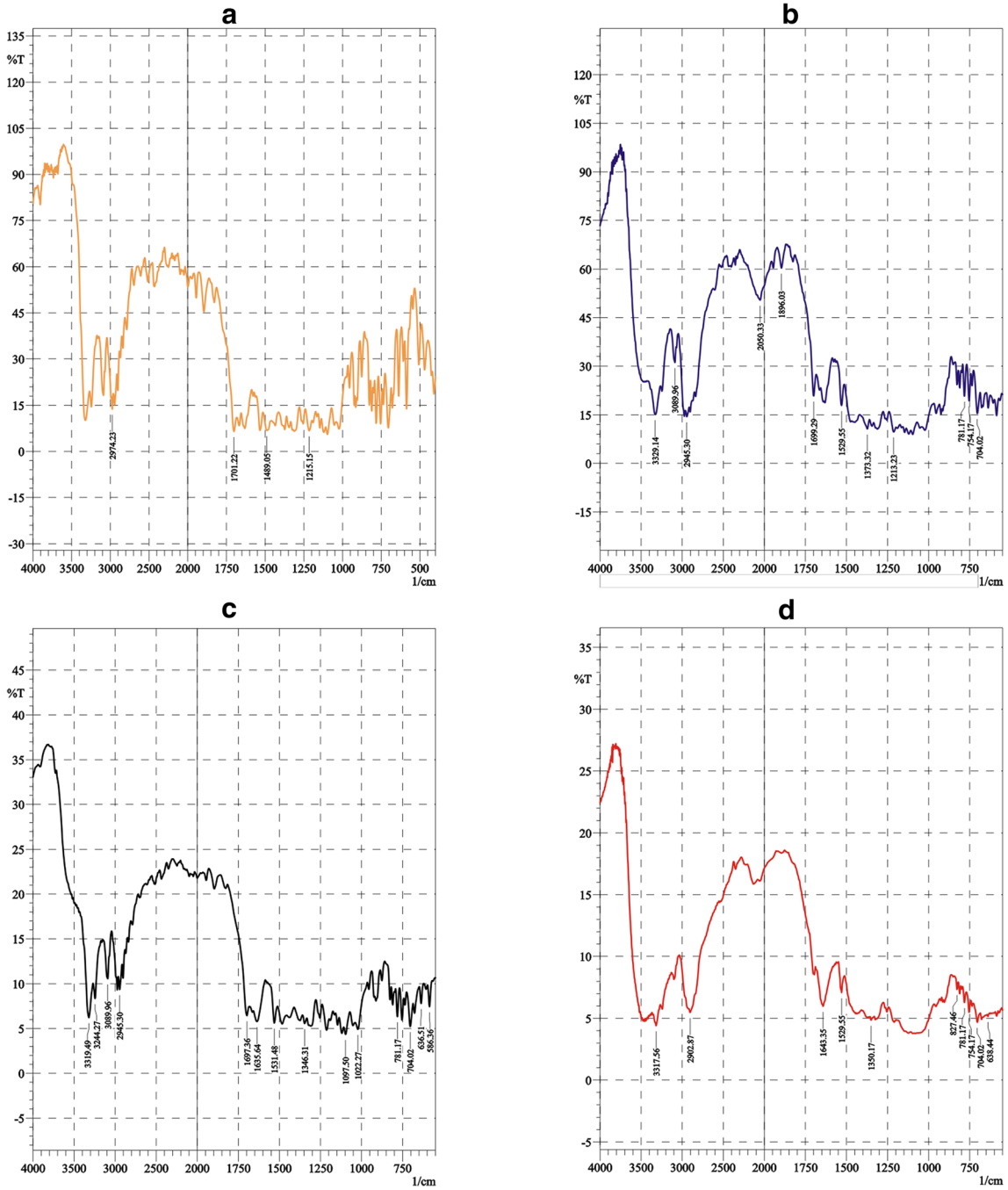

Fig. 3. FTIR spectra of **a** plain NIT, **b** physical mixture of NIT and HPMC-E6, **c** physical mixture of NIT and CCS, and **d** physical mixture of NCs with MCC and CCS

between 8° and 32° with the three most significant peaks at 2θ values of 10.158°, 11.528°, and 13.215°, which are in accordance with the p-XRD pattern of plain NIT. The obtained results clearly revealed the perseverance of the crystalline structure of NIT on lyophilization. However, there is a difference in intensity and width of the peaks in the p-XRD pattern of lyophilized powder. The broadened peaks imply lower crystalline size of NCs and the decline in the intensity of peaks can be ascribed to the change in the crystallinity (34,38,43,44, and).

Characterization of NCs

Particle Size Determination

The mean particle size and PDI of the optimized batch of NCs was 570.33 ± 19.59 nm and 0.057 ± 0.007 respectively (Fig. 5b). The PDI of 0.057 implies narrow particle size distribution and thus indicates the stability of the formulation. Figure 5 also reveals reduction in particle size of plain NIT from 6599.47 nm (Fig. 5a) to 570.33 nm as NIT NCs.

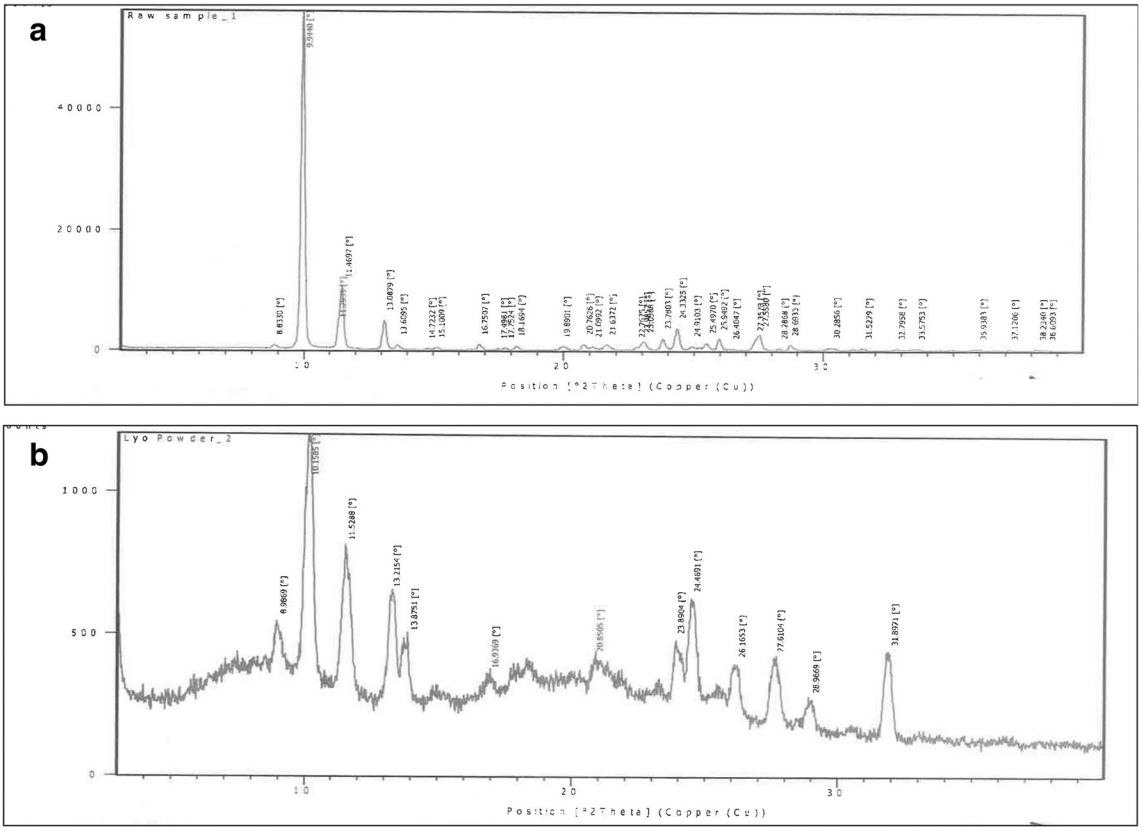

Fig. 4. The X-ray diffraction patterns of **a** plain NIT and **b** NCs

Drug Content

The percent NIT content in the NCs (% entrapment efficiency) was found to be 78.4 ± 2.3% and percent drug loading was found to be 33.33 ± 1.1%. These parameters mainly depend on the polymeric stabilizer content present in the formulation. As the % content of stabilizer increases to a certain extent drug particle size decreases with increment in the drug loading. But as stabilizer concentration increases further, drug loading keeps on increasing with an increase in mean particle diameter too. At high concentration of stabilizer viscosity of the solution enhances, that in turn hinders the transmission of ultrasonic vibrations and thus the diffusion between the solvent and antisolvent during precipitation, and this leads to an undesired increment in drug particle size. Therefore, an optimum concentration of stabilizer with lowest drug particle size and optimum drug loading capacity was chosen (4,45).

Saturation Solubility

The water solubility of NIT from NCs was found to be 50.46 ± 0.47 μg/mL, which is much higher than plain NIT (1.93 ± 0.25 μg/mL). Thus, the solubility of NIT (as NCs) was found to be significantly ($p < 0.01$) increased (26.14 times) in comparison to plain NIT. The remarkable increase in solubility of NIT as NCs could be due to the reduction in the size of NIT crystals to nano-scale and thus enhancement in effective surface area of drug crystals coming in contact with dissolution medium.

Stability Study

No significant difference was observed in the physical appearance (Yellow fluffy dry powder), particle size and PDI of NCs after 3 months (631.8 ± 22.34 nm with PDI of 0.145 ± 0.051) and 6 months (673.2 ± 18.56 nm with PDI of 0.237 ± 0.072) of storage at 40 ± 2°C/75 ± 5% RH. The obtained results indicate good stability of NCs.

Optimization of N-ODT

Effect of Formulation Variables on Dispersion Time

Contour plots (Fig. 6a) and 3D surface response plot (Fig. 6b) indicate the effect of concentration of diluent (B) and compression force (C) on the dispersion time of the tablet. The increase in the B caused a decrease in the dispersion time (negative effect) while the increase in C caused an increase in the dispersion time (positive effect). Among the prepared batches, F3 was selected as an optimized batch, showing minimum dispersion time (39 s). The assessment of results through ANOVA rendered F-value to be 131.79 and p value to be 0.0011, which denotes the significance of the linear model. The best-fitted model for dispersion time (R^2 = 0.9943) was found to be a linear model. The polynomial equation was generated on the basis of the best-fitted model, wherein the (+) sign indicates the increase in the response and the (-) sign indicates the decrease in the response.

The final equation in terms of coded factors for dispersion time is:

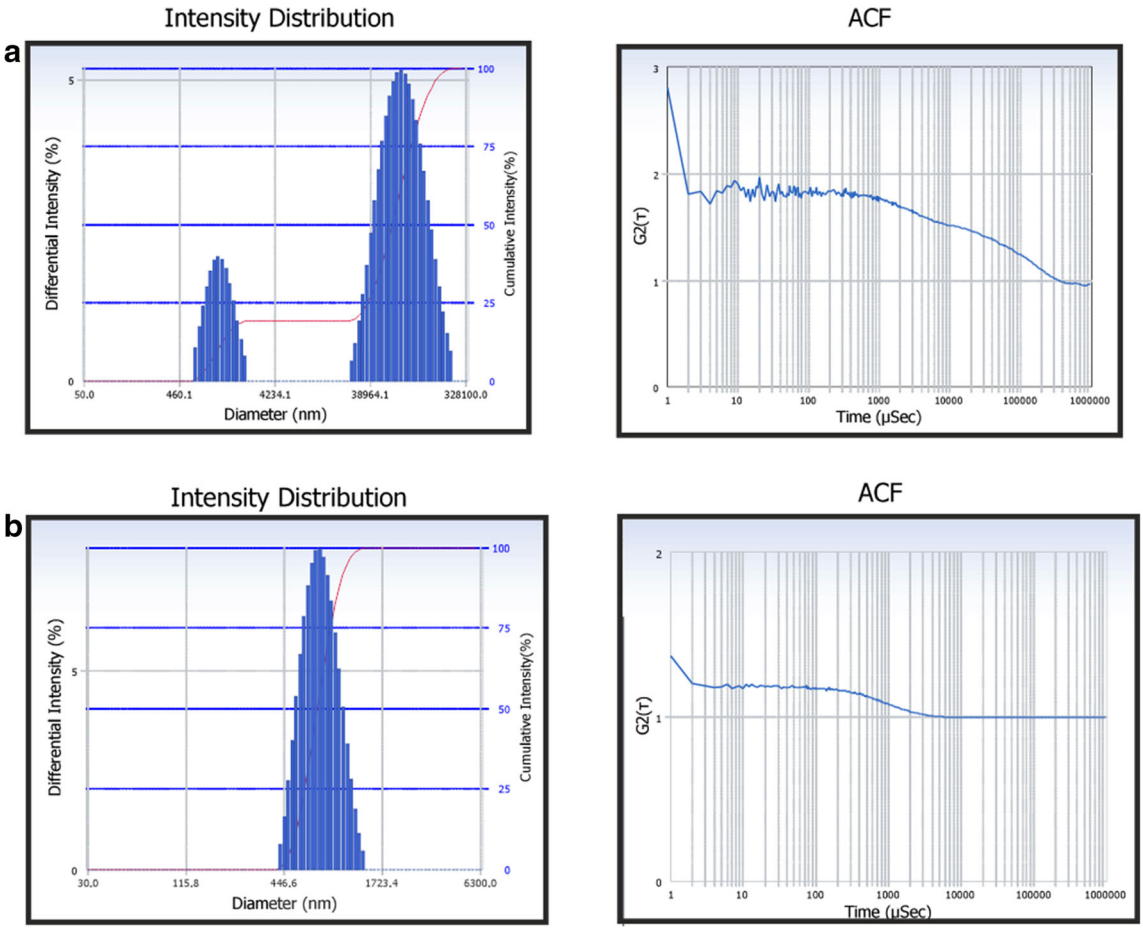

Fig. 5. **a** Particle size of plain NIT and **b** particle size of NCs after reconstitution

$$\text{Dispersion time} = +67.38 + 4.37*A + 1.87*B + 18.13*C + 8.63*BC$$

Pre-compression Evaluation of NCs Blend

The derived properties such as bulk density, tapped density and angle of repose of NCs blend were found to be 0.434 ± 0.004 g/mL, 0.514 ± 0.004 g/mL and 28.83 ± 0.75° respectively. Besides, Carr's index and Hausner's ratio were found to be 15.56% and 1.18 respectively. The angle of repose is one of the essential properties of the material that provide information regarding cohesion (internal friction) of the particles. The higher angle of repose indicates cohesion while the lower value indicates non-cohesion between the powder particles and thus ensures good flow-ability. The angle of repose of NCs blend was found in the range of 25–30°, revealing excellent flow-ability. Moreover, both Carr's index and Hausner's ratio values were found to be within the standard limit, indicating good flow property and compressibility of the blend (46,47).

Post-compression Evaluation of N-ODT

Diameter and Thickness

The diameter and thickness of optimized N-ODT were found in the range of 6.9–7.3 mm and 2.7–3.2 mm respectively. The values of both were within the limits.

Hardness and Friability

Hardness is a critical quality-control parameter affecting friability and disintegration time of ODTs. The optimized N-ODT displayed hardness within the intended limit (3.46 ± 0.115 kg/cm^2). The N-ODT showed % friability of 0.69 ± 0.072%, which is in accordance with the prescribed limit (less than 1%) as per the I.P. This suggests good mechanical resistance (28).

Weight Variation and Content Uniformity

In the weight variation study, the average weight of N-ODTs was found to be 352.71 mg, which is within the ±5% deviation criteria as specified by I.P. The content uniformity of N-ODT was found to be 105.6 ± 5.12%, which is within the acceptable limit of 85 to 115%. Thus, the obtained results are within the intended limit and consistent with the previous reports (32).

Dispersion Time

Dispersion time plays an imperative role in ODT development. The dispersion time of ODT is desired to be less than 1 min. Optimized N-ODT (batch F3) displayed an *in vitro* dispersion time of 39 ± 1.13 s, which complies with the prescribed limit. A rapid dispersion of N-ODT can be

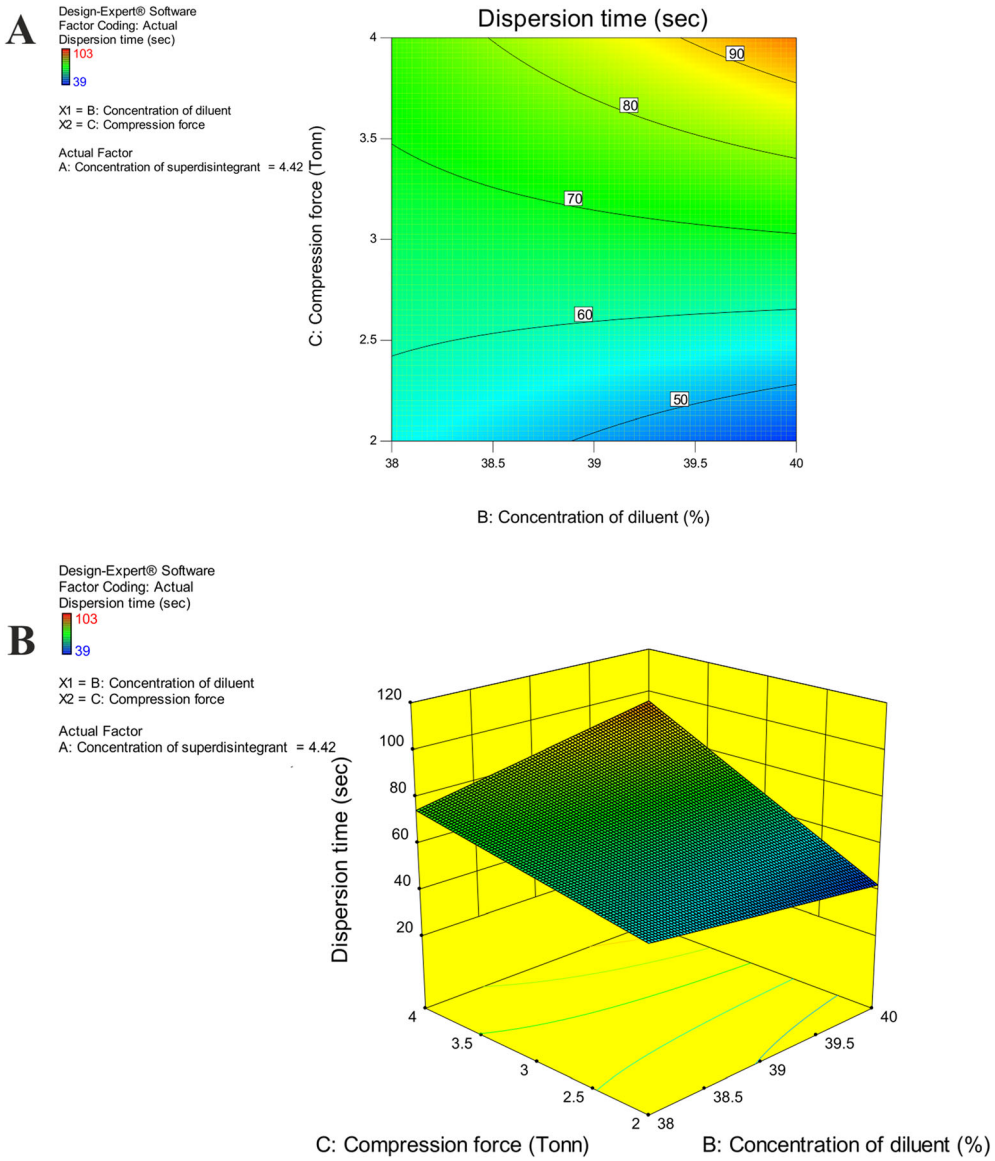

Fig. 6. **a** Contour plot and **b** response surface plot for independent variables of N-ODT with dispersion time as a response

attributed to the optimized concentration of superdisintegrant and diluent in the formulation (48).

Disintegration Time

The disintegration time (DT) marks a crucial contribution in drug absorption in the buccal cavity, thereby fostering bioavailability. The optimized N-ODT showed DT of 33.91 ± 0.94 s, revealing rapid drug release which further facilitates better absorption and bioavailability of NIT. The quick disintegration of the tablet could be a result of the combined effect of superdisintegrant (CCS), directly compressible diluent (MCC) and filler (lactose anhydrous) present in optimized concentration. MCC can undergo plastic deformation at low compression forces and results in compact generation. At the same time as a disintegrant, it draws liquid into compacts by capillary action and causes separation of bonded particles (4). Lactose increases the porosity of tablet compacts in combination with MCC and thus aids in rapid tablet disintegration (49).

Wetting Time

The wetting time of N-ODT was 44.66 ± 1.527 s, which indicates the porous nature of the tablet hydrophilic matrix. The short wetting time can be ascribed to the presence of superdisintegrant which absorbs large amounts of water and swells, thereby causing swift rupture of tablet. Moreover, tablets prepared using lyophilized powder (NCs) also facilitate wetting by absorbing a large amount of water when exposed to an aqueous environment. The obtained results are concurrent with previous literature data (37).

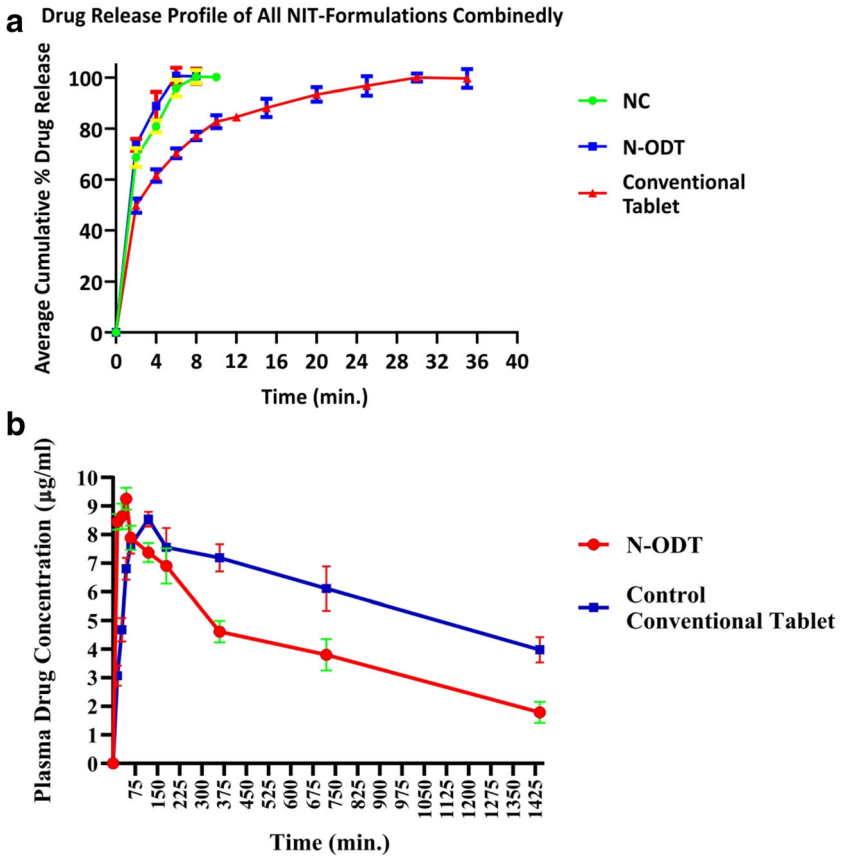

Fig. 7. **a** *In vitro* drug release profile of NIT formulations and **b** Plasma drug concentration-time profile of NIT formulations

In Vitro Dissolution Study of NIT Formulations

The *in vitro* release profile of NIT from N-ODT was studied by using USP type II dissolution apparatus and was compared with the NIT release from NCs and conventional NIT tablet (Fig. 7a). The *in vitro* release of NIT from NCs and N-ODT was found to be 100.28 ± 2.64% (in 8 min) and 100.61 ± 3.3% (in 6 min) respectively. In contrast, the conventional NIT tablet took significantly ($p < 0.05$) more time (30 min) to release almost the same amount of NIT. Thus, the release of NIT from both N-ODT and NCs was found to be rapid and remarkably higher in comparison to conventional NIT tablets. Significantly higher *in vitro* release of NIT from NCs could be due to enhanced effective surface area of NCs available for dissolution. Moreover, the remarkable release of NIT from N-ODT could be due to combined technological aspects of nano sizing of drug crystals along with faster water uptake through the porous tablet structure formed due to the incorporation of superdisintegrant and other excipients in the formulation, resulting in rapid disintegration and dissolution of the tablet.

In Vivo Pharmacokinetic Study

The pharmacokinetic behavior of N-ODT was studied by using male white rabbits and compared with conventional NIT tablet. The key PK parameters like C_{max}, T_{max}, $t_{½}$, MRT, and [$AUC_{0\text{-}24}$] were analyzed and the results are listed in Table IV. The time versus plasma drug concentration data obtained from the study is presented in Fig. 7b. We observed a significant ($p < 0.05$) difference in the T_{max} and C_{max} values of N-ODT in comparison to conventional NIT tablets. Rapid

Table IV. Pharmacokinetic Parameters of NIT Formulations

Pharmacokinetic parameter	N-ODT	Conventional tablet
C_{max} (µg/mL)	9.25 ± 0.39	8.54 ± 0.26
T_{max} (minute)	45 ± 0.00	120 ± 0.00
$t_{½}$ (minute)	655.175 ± 23.35	1250.217 ± 42.59
MRT (minute)	957.095 ± 38.65	1838.817 ± 45.33
[$AUC_{0\text{-}24}$] (µg min/mL)	5793.22 ± 112.47	8535.14 ± 154.81

Values are mean ± SD, $n = 3$

Table V. Stability Study of Optimized N-ODT

Test		Results			
		storage period			
		Day 0	1 month	3 months	6 months
Physical appearance		Light yellow	Light yellow	Light yellow	Light yellow
Hardness test (kg/cm^2)		3.49 ± 0.142	3.52 ± 0.184	3.53 ± 0.147	3.55 ± 0.131
Disintegration time (s)		33.94 ± 1.875	33.15 ± 2.39	34.69 ± 1.572	35.22 ± 1.713
NIT content uniformity (%)		105.6 ± 5.12	105.19 ± 4.82	104.12 ± 5.47	104.55 ± 4.63
Dissolution study	Time in min	Dissolution profile (% CDR)			
		Storage period			
		Day 0	1 month	3 months	6 months
	2	73.91 ± 1.39	70.99 ± 2.72	72.51 ± 0.795	72.961 ± 1.442
	4	86.22 ± 2.82	87.67 ± 1.81	88.38 ± 1.55	89.427 ± 0.75
	6	99.93 ± 1.646	101.42 ± 1.19	100.72 ± 1.24	99.837 ± 2.67

Values are mean ± SD, $n = 3$

systemic delivery and generation of faster onset of action for NIT has been inferred, as the C_{max} of 8.54 ± 0.26 μg/mL achieved by control conventional tablet with T_{max} of 120 min has been reduced to 15 min in case of developed N-ODT formulation with nearly same plasma NIT concentration (8.45 ± 0.28 μg/mL). Furthermore, the N-ODT demonstrated a notably higher C_{max} (9.25 ± 0.39 μg/mL) and reduced T_{max} (45 min) than that of the conventional tablet (8.54 ± 0.26 μg/mL in 120 min), indicating improved bioavailability. Another important advantage of the developed formulation is its fast clearance as observed through lower concentrations at the later time-points compared to the conventional tablet. The enhanced oral bioavailability of NIT from N-ODT could be attributed to the increased solubility and thus improved dissolution rate of the drug. Besides, the pre-gastric drug absorption (minimization of the first pass hepatic metabolism) may be another important reason for the increased bioavailability.

In Vitro Stability Study of N-ODT

No significant difference in the physical appearance, hardness, disintegration time, content uniformity, and dissolution profile was observed in N-ODT after 1, 3, and 6 months of storage at 40 ± 2°C/75 ± 5%RH (Table V). Thus, the obtained results clearly revealed good *in vitro* stability of N-ODT.

CONCLUSION

In the present research, NIT NS was prepared successfully by the antisolvent sonoprecipitation technique using HPMC-E6 as a stabilizer. The NS was optimized using 3^2 optimal response surface design. Further, the optimized NS was lyophilized to NCs successfully and NCs exhibited perseverance of integrity and crystallinity of NIT even on lyophilization. The reconstituted NCs showed retention of drug particle size in nanometer range. Besides, the saturation solubility study of NCs displayed significantly improved water solubility of NIT than plain NIT. The NCs-loaded ODTs were prepared by direct compression method using CCS as a superdisintegrant and optimized by 2^3 FFD. The optimized N-ODT demonstrated very less dispersion and disintegration time (< 1 min). Moreover, the *in vitro* dissolution study of both NCs and N-ODT displayed faster and complete release of NIT within 9 min, which is much superior to conventional NIT tablets. The *in vivo* pharmacokinetic study in the rabbits exhibited significantly improved oral bioavailability of NIT from N-ODT in comparison to the conventional NIT tablets. Furthermore, both NCs and N-ODT were found to be stable on storage at 40 ± 2°C/75 ± 5%RH. Thus, N-ODT could be an impending approach for efficient delivery of poorly soluble drugs including NIT with improved bioavailability to treat critical diseases successfully.

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Data Availability Applicable

DECLARATIONS

Ethics Approval and Consent to Participate Applicable (IAEC Approval No. - MES/COP/IAEC/04/2017-18)

Consent for Publication Not applicable

Competing Interest The authors declare no competing interests.

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