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Research Article

Novel Synergistic Approach: Enhancing the Oral Bioavailability of Nintedanib via Nanocrystal Technology using Naringenin as a Potent UGT Enzyme Inhibitor Identified Through Human Liver Microsome Studies

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Abstract

The study aimed to improve the oral bioavailability of nintedanib (NTB), a poorly soluble and extensively metabolized drug used for idiopathic pulmonary fibrosis. NTB's low bioavailability (~4.7%) results from limited solubility (BCS Class IV) and rapid UGT-mediated metabolism. To address both challenges, NTB was formulated as nanocrystals combined with a natural bioenhancer that inhibits UGT. Four candidates-naringenin, piperine, quercetin, and curcumin-were screened using human liver microsomes. Naringenin showed the strongest inhibition, retaining 73% NTB and reducing UGT activity by over 82%. The optimized NTB nanocrystals with naringenin (NTB-NC with NGN) had a particle size of 144.43 nm, a PDI of 0.14, and a zeta potential of −17.3 mV. This formulation achieved a 6.31-fold increase in solubility and more than 93% drug release within 15 minutes. In mice, NTB-NC with NGN produced 1.43-fold higher Cmax than raw NTB, and a 1.3-fold higher AUC than NTB nanocrystals alone, confirming naringenin's metabolic inhibition. The dual strategy of nanocrystal formulation with UGT enzymes bio-enhancement offers a significant improvement in NTB's bioavailability, potentially allowing lower doses and reduced side effects for better therapeutic outcomes in IPF patients.

Keywords: Nintedanib; Naringenin; Bioavailability Enhancement; Human Liver Microsomes; UGT enzyme Inhibition; Nanocrystals; Idiopathic Pulmonary Fibrosis; Pharmacokinetics

Abbreviations

NTB: Nintedanib; NGN: Naringenin; UGT: Uridine 5'-diphospho-glucuronosyltransferase; CPCSEA: Committee for Control and Supervision of Experiments on Animals; NCs: Nanocrystals: HPC: Hydroxy propyl cellulose; BCS class: Biological Classification System; NTB-NCs: Nintedanib nanocrystals; DSC: Differential Scanning Calorimetry; IR Spectroscopy: Infrared Spectroscopy; TEM: Tunneling Electron Microscopy; HLM: Human Liver Microsomes; Mkt: Market Formulation and NTB-NCs+NGN: Nintedanib nanocrystals with Naringenin.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and debilitating lung disease characterized by the unremitting

scarring of pulmonary parenchyma, leading to irreversible loss of lung function and ultimately, respiratory failure [1]. The advent of antifibrotic agents has marked a paradigm shift in IPF management, with nintedanib (NTB) standing as a cornerstone therapy. Nintedanib esylate, a triple angiokinase inhibitor, targets key profibrotic pathways by inhibiting vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR) [2]. Clinical trials have unequivocally demonstrated its efficacy in slowing the decline of forced vital capacity (FVC), the primary measure of disease progression in IPF [3].

Despite its proven clinical benefits, nintedanib is plagued by profound biopharmaceutical challenges that curtail its therapeutic potential. Classified as a Biopharmaceutics Classification System (BCS) Class IV drug, NTB possesses high permeability but suffers from very poor aqueous solubility [2,3]. This intrinsic property leads to dissolution rate-limited absorption in the gastrointestinal tract. More critically, NTB is a substrate for the efflux transporter P-glycoprotein (P-gp) and undergoes extensive first-pass metabolism, predominantly catalyzed by the Uridine 5-diphospho-glucuronosyltransferase (UGT) enzyme in the liver and intestinal wall [4-6]. The confluence of poor solubility, active efflux, and pre-systemic metabolism culminates in an exceptionally low and variable oral bioavailability of approximately 4.7% in humans [7]. This necessitates high therapeutic doses (150 mg twice daily), which are strongly associated with a high incidence of severe gastrointestinal adverse effects, including diarrhea, nausea, and vomiting, often leading to dose reductions, treatment interruptions, and compromised patient adherence [8]. Consequently, there is a pressing need for innovative formulation strategies that can holistically enhance the oral bioavailability of NTB, potentially enabling lower dosing and mitigating its debilitating side effects.

Nanocrystal technology has emerged as a robust and commercially viable platform for enhancing the dissolution rate and saturation solubility of BCS Class IV drugs [3-6]. Drug nanocrystals are pure drug particles stabilized by surfactants or polymers, with sizes in the nanometric range (100-1000 nm). The drastic increase in surface area-to-volume ratio, governed by the Noyes-Whitney equation, leads to a profound enhancement in dissolution velocity [6-8]. However, while nanocrystals effectively overcome the solubility barrier, they offer no inherent protection against enzymatic degradation or efflux transport.

To address the metabolic hurdle, the concept of "bioenhancers" offers a compelling solution. Bioenhancers are substances that, when co-administered, can enhance the bioavailability and efficacy of a primary drug without intrinsic pharmacological activity at the dose used [9]. Several natural compounds are known for their UGT and/or P-gp inhibitory properties. However, their relative potency and suitability for a specific drug like NTB require systematic evaluation.

This study reflets that the ideal strategy for NTB is a synergistic combination of nanocrystal technology (to overcome the solubility barrier) with an optimally selected bioenhancer (to overcome the metabolic and efflux barriers). The critical first step is to identify the most potent bioenhancer for NTB. We selected four well-known natural bioenhancers for a comparative screening:

- **Naringenin (NGN):** A citrus flavonoid known for its mechanism-based inhibition of UGT and P-gp [10].
- **Piperine (PIP):** An alkaloid from black pepper, a classic bioenhancer with reported inhibition of CYP3A4, UGT and P-gp, and glucuronidation [11].
- **Quercetin (QRN):** A ubiquitous flavonoid with inhibitory effects on CYP3A4 and P-gp [12].
- **Curcumin (CMN):** An isoflavone from soy, known to inhibit CYP3A4 and other enzymes [13].

The primary objective of this research was to first identify the most effective bioenhancer for NTB using an *in vitro* human liver microsome (HLM) model, which provides a highly relevant and predictive platform for studying human drug metabolism. Following this screening, the lead bioenhancer (naringenin) was co-formulated with NTB into a single nanocrystal system (NTB-NC-NGN). This novel formulation was thoroughly characterized, and its performance was evaluated through *in vitro* dissolution, permeability

studies, and an *in vivo* pharmacokinetic study in rats, to demonstrate the synergistic enhancement of oral bioavailability.

Materials and Method

Chemical and reagents

Nintedanib esylate (purity >99%) was procured as a gift sample from Sun Pharma Pharmaceuticals pvt ltd, Vadodara, Gujarat, India. Naringenin (NGN, >98%), Piperine (PIP, >97%), Quercetin (QRN, >95%), Curcumin (CMN, >98%), Hydroxy Propylcellulose (HPC), NADPH, Testosterone and 6β-hydroxytestosterone were purchased from sigma Aldrich. Pooled human liver microsomes (HLM, 20 µg/mL) were purchased from Krishgen (India). All solvents were of HPLC grade.

Human liver microsome (HLM) study for bioenhancer screening

Inhibition of nintedanib metabolism [14]

The metabolic stability of NTB in the presence of the four bioenhancers was assessed in HLM. The incubation mixture (200 µL total volume) contained: 0.1 M phosphate buffer (pH 7.4), HLM (0.5 mg/mL protein), NTB (5 µM), and each bioenhancer (50 µM). The mixture was pre-incubated for 10 min at 37°C. The reaction was initiated by adding UGT (1 mM final concentration) and incubated for 30 min at 37°C in a shaking water bath. The reaction was terminated by adding 400 µL of ice-cold acetonitrile containing an internal standard. Control incubations without UGT and without bioenhancer were run in parallel. The samples were centrifuged at 14,000 rpm for 10 min, and the supernatant was analyzed by HPLC to quantify the remaining parent NTB. The percentage of residual NTB was calculated as (NTB concentration with bioenhancer and UGT/NTB concentration without UGT) * 100.

UGT enzyme activity assay by Michaelis-Menten non-compartmental analysis [15]

To confirm the inhibitory potential on UGT, a standard testosterone 6β-hydroxylation assay was performed. The incubation mixture contained HLM (0.2 mg/mL), testosterone (250 µM), and each bioenhancer (50 µM). After pre-incubation, the reaction was started with NADPH (1 mM) and incubated for 20 min. The formation of 6β-hydroxytestosterone was quantified using a validated HPLC-UV method. The percentage inhibition of UGT activity was calculated relative to the control (without bioenhancer).

Preparation of nanocrystal formulations

Based on the HLM results, naringenin was selected as a potential bioenhancer with nintedanib. Therefore, potential formulation of nanocrystals was prepared for nintedanib [16].

The nanocrystals were prepared using an antisolvent precipitation technique. Briefly, NTB of 10mg per mL was dissolved in methanol. This organic phase was rapidly injected into 100 mL of an aqueous stabilizer solution (containing 0.4% w/v HPC) under high-speed magnetic stirring (2000 rpm) in an ice water bath under stirring for 15 minutes. resulting in the pre-suspension formulation. The ratio 5:2 of antisolvent-to-solvent Volume was the optimal ratio with the smallest particle size obtained. The final nanocrystal suspension was stirred to remove residual organic solvent and was stored and lyophilized with 5% w/v trehalose (Christ Alpha 1-2 LDplus, Germany) as a cryoprotectant for obtaining lyophilized nano crystalline dry cake. Then NGN lyophilized powder was added after the preparation of nanocrystals and was finally formulated in the nanocrystals formulation.

Characterization of nanocrystals [16]

- **Particle Size, PDI, and Zeta Potential:** Measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK).
- **Morphology (SEM):** Lyophilized powder was analyzed using scanning electron microscopy (FEI Nova NanoSEM 450).
- **Crystallinity analysis (PXRD and DSC):** PXRD patterns were recorded on an X'Pert Pro diffractometer (PANalytical), and thermal analysis was performed using a DSC Q20 (DSC60A, Shimadzu).
- **Saturation Solubility:** Excess of each formulation was added to pH 6.8 phosphate buffer, shaken for 48h at 37°C, centrifuged, filtered, and analyzed by HPLC.

- **Drug Content Estimation:** NTB-NCs were weighed about 15mg, transferred into a volumetric flask, and were made to dissolve to obtain the final concertation of 5µg/mL in the diluent (Water: ACN:50:50). The solution was injected into the HPLC system for % Assay estimation.
- **FTIR Analysis:** The interaction of pure drug (NTB) with excipient (stabilizer) was studied by FTIR (Bruker, Germany). 5-10 mg of sample was directly placed onto the diamond plate, and data were collected in the 400-4000 cm^{-1} range to determine the excipient's compatibility with the drug
- **Residual solvent analysis:** Gas chromatography investigated residual solvents (Perkin Elmer, USA). Acetonitrile (25 µg/mL) was produced as the reference sample in Dimethyl Formamide (DMF). For comparison, samples were made in a comparable standard dilution sequence. The sample was injected into the capillary column (CR-624, 30 m, 0.53 mm, 3.00 µm) at 80°C using N_2 gas as the carrier gas. The following other parameters were set: 40 mL/min, 30 mL/min, 300 mL/min, 0.2 µL, 260°C, and 260°C, respectively: injector temperature, detector temperature, air flow rate, carrier gas flow rate, H_2 gas flow rate, and injection volume. Twenty minutes was the allotted run time.

In-vitro drug release

Dissolution studies were performed using a USP Type II apparatus in 900 mL of phosphate buffer (pH 6.8) at 37°C and 50 rpm by withdrawing 5 mL of sample and replacing it with 5mL of fresh media. Samples were withdrawn at predetermined times, filtered, and analyzed by HPLC. The data was fitted to various exponential equations, including the zero order, first order, Higuchi, and Korsmeyer-Peppas models, to describe the release profile of NTB-NCs. The interpretation of release exponent (n) is mentioned below:

- The Fickian diffusion mechanism model appears when n is 0.43
- An Anomalous transport mechanism model appears when n is 0.43<n<0.85
- Case I transport mechanism model appears when n is 0.85
- Case I transport mechanism model appears when n is > 0.85

In Vivo pharmacokinetic study [17,18]

The protocol was approved by the Institutional Animal Ethics Committee. Male Sprague-Dawley rats (250 ± 20 g) were divided into three groups (n = 6):

- Group I: Raw NTB suspension (control)
- Group II: NTB-NC
- Group III: NTB-NC-NGN

Formulations were administered orally at an NTB dose of 30 mg/kg (and NGN dose of 15 mg/kg for Group III). Blood samples were collected at pre-defined time points over 24 hours. Plasma was separated, and NTB concentrations were determined using a validated HPLC method. Pharmacokinetic parameters were calculated using non-compartmental analysis by using pk solver (Microsoft excel).

Statistical analysis

Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant.

Results and Discussion

Human liver microsome study: Screening and selection of bioenhancer

The initial and pivotal step of this research was to systematically identify the most effective bioenhancer for inhibiting the UGT-mediated metabolism of nintedanib. The results from the HLM study are summarized in Table No. 1 and Figure 1 and Figure 2.

In the control group (NTB alone), only 22.5% of the parent NTB remained after 30 minutes of incubation, confirming its extensive metabolism by HLM. All bioenhancers showed some degree of protective effect, but their potencies varied significantly.

- Curcumin showed the weakest effect, with only 42.13% residual NTB and 55.82% UGT enzyme inhibition.
- Quercetin and Piperine demonstrated moderate activity, preserving 30.9% and 35.9% of NTB, and inhibiting UGT enzyme by 31.9% and 16.2%, respectively.

Table No.1: Results of the Human Liver Microsome (HLM) Study (Mean ± SD, n = 3).

Bioenhancer (50 µM)	Residual Nintedanib (%)	UGT Enzyme Activity Inhibition (%)
Control (No Inhibitor)	22.5 ± 3.1	-
Naringenin (NGN)	82.30 ± 4.5*#	89.90 ± 3.2*#
Piperine (PIP)	35.90 ± 3.9*	16.20 ± 4.1*
Quercetin (QRN)	30.90 ± 4.2*	31.90± 3.8*
Curcumin (CMN)	42.13 ± 3.5	55.82 ± 4.5*

p < 0.01 vs. Control; # p < 0.01 vs. all other bioenhancers.

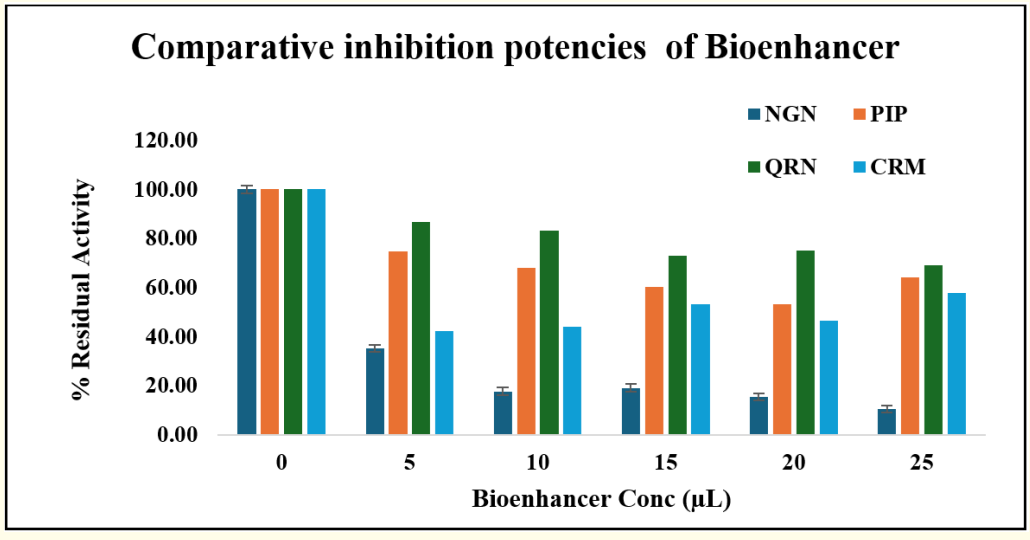

Figure 1: Bar graph representing the comparative inhibition potencies of bioenhancers on UGT-mediated NTB metabolism.

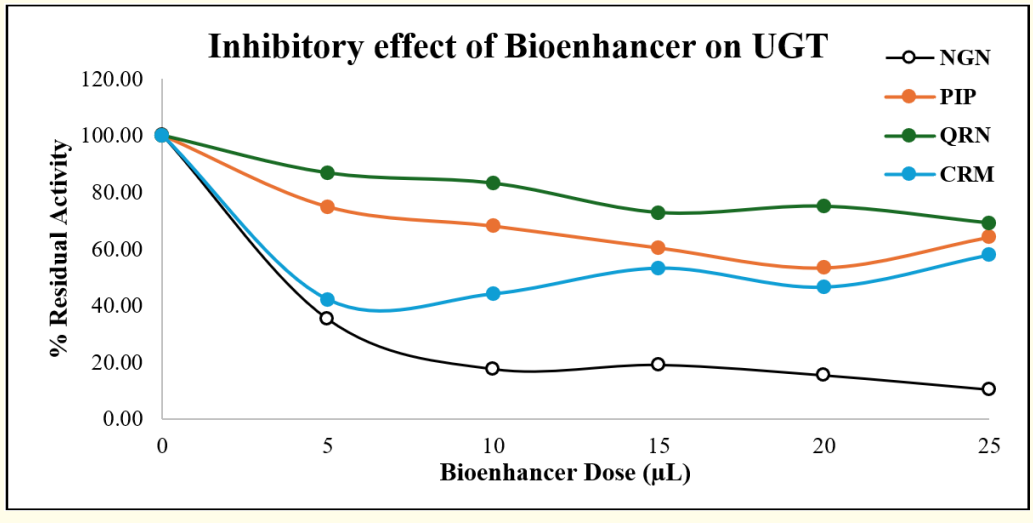

Figure 2: The inhibitory effect of Bioenhancers on UGT.

- Naringenin emerged as the standout performer. It significantly protected NTB from metabolism, with 82.3% of the parent drug remaining-a three-fold increase compared to the control. Correspondingly, it exhibited the highest inhibition of UGT enzyme activity at 89.9%. The difference between naringenin and all other bioenhancers was statistically significant ($p < 0.01$).

This superior performance of naringenin can be attributed to its well-documented mechanism-based inhibition of UGT enzyme. Unlike reversible competitive inhibitors, mechanism-based inhibitors are metabolically activated by the enzyme to form reactive intermediates that irreversibly bind to and inactivate the enzyme's active site [16]. This leads to a prolonged inhibitory effect, which is highly desirable for reducing first-pass metabolism. Based on these compelling results, naringenin was selected as the bioenhancer of choice for formulating nintedanib nanocrystals with naringenin as a bioenhancer.

Formulation and characterization of nintedanib nanocrystals

The antisolvent precipitation method successfully produced stable, nanosized suspensions. The physicochemical properties of the formulations are presented in Table No. 2.

Table No.2: Physicochemical characteristics of the nanocrystal formulations (Mean ± SD, n = 3).

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Saturation Solubility (mg/mL)	
				Water	pH 6.8
Raw NTB				0.39	0.25
NTB-NCs	144.43 ± 7.70	0.138	-17.3 ± 1.83	6.31	3.35
*Statistically significant ($p < 0.001$) compared to Raw NTB.					

The Nanocrystal formulations exhibited a nanometric size (<150 nm) and a very low PDI (<0.15), indicating a narrow, monodisperse size distribution crucial for predictable performance (Figure 3). The NTB-NCs formulation showed an acceptable size. The zeta potential values were highly negative (around -17 mV), ensuring strong electrostatic repulsion and excellent colloidal stability by preventing particle aggregation. SEM micrographs (Figure 4) confirmed the nanoscale size and revealed a rod-like morphology for the nanocrystals, contrasting with the large, irregular shaped of raw NTB.

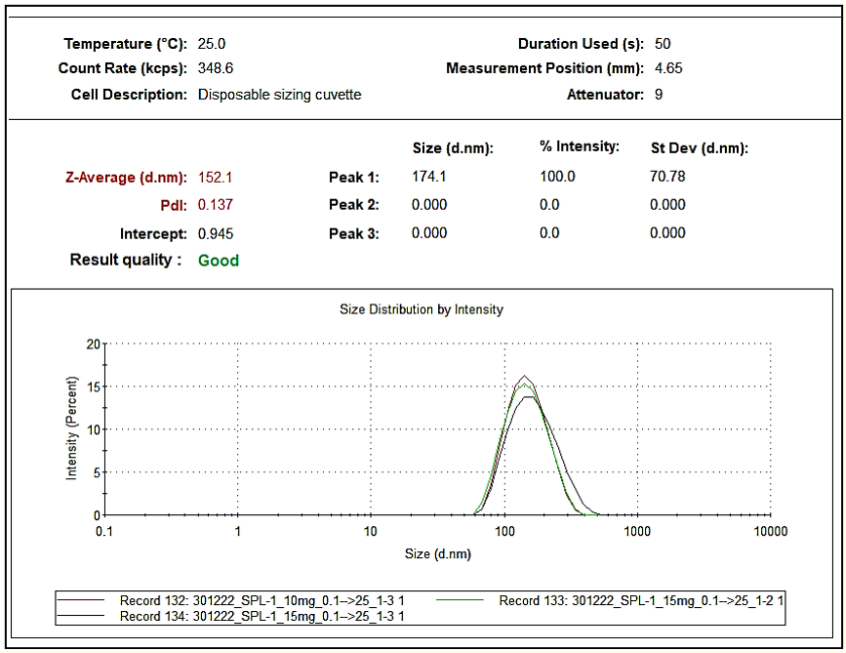

Figure 3: Particle size for NTB-NCs.

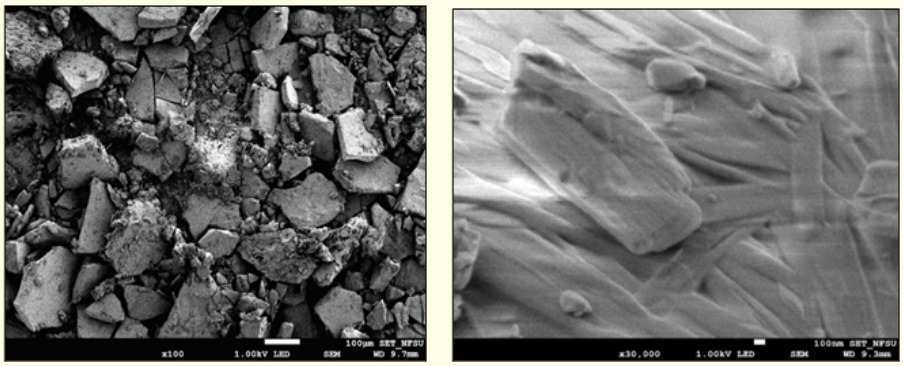

Figure 4: (a): SEM Image for NTB. (b): SEM Image for NTB-NCs.

PXRD (Figure 5), DSC (Figure 6) and FTIR (Figure 7) analyses confirmed that the drug remained in the crystalline state within the nanocrystals. The characteristic diffraction peaks and endothermic melting peak of NTB were retained in the formulations, albeit with reduced intensity and slight broadening-a classical signature of nano-crystallization. No polymorphic changes were observed. The saturation solubility of NTB was dramatically enhanced by over 13-folds in the nanocrystal formulations. The presence of naringenin did not negatively impact the solubility enhancement provided by the nanosizing.

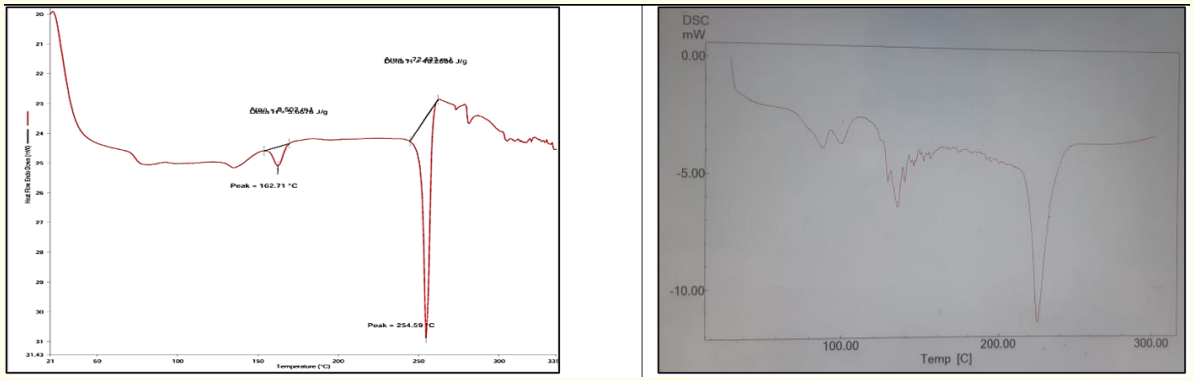

Figure 5: (a): DSC Thermogram of NTB. (b): DSC Thermogram of NTB-NCs.

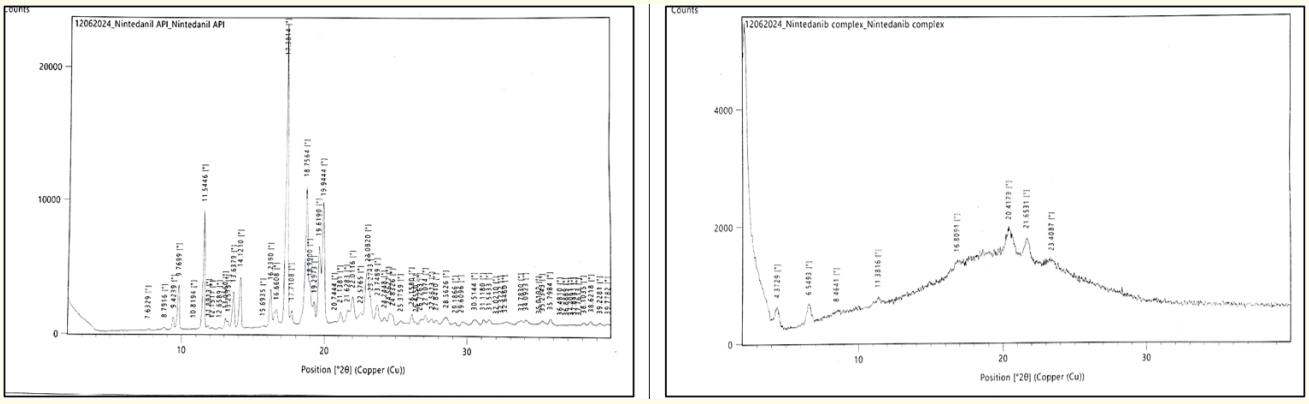

Figure 6: (a): XRD of NTB. (b): XRD of NTB-NCs.

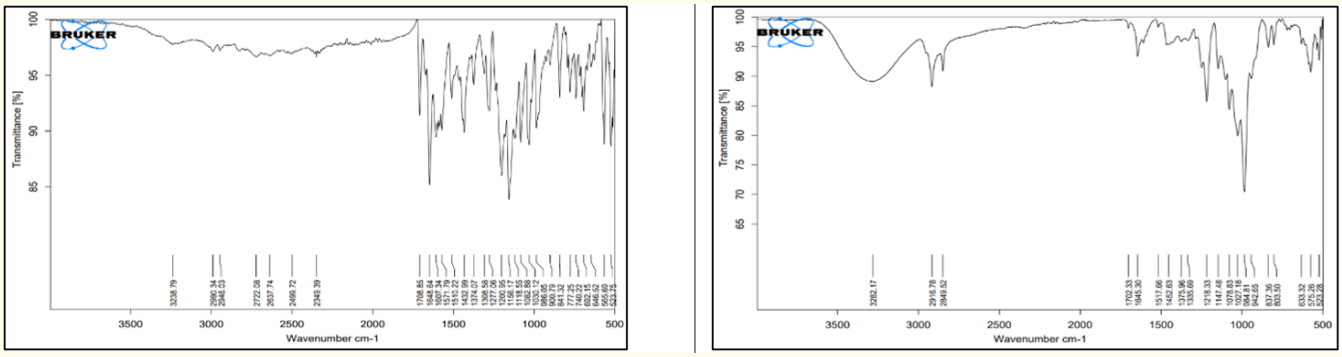

Figure 7: (a): IR Spectra of NTB. (b): IR Spectra of NTB-NCs.

In vitro drug release

The dissolution profiles are presented in Figure 8 and table No. 3. Raw NTB displayed a slow and incomplete release, with only about 32% dissolved in 2 hours, highlighting its solubility-limited absorption. In stark contrast, both nanocrystal formulations exhibited a characteristic "spring" effect, with a very rapid and complete drug release. The NTB-NC and NTB-NC-NGN formulations achieved over 95% and 98% drug release within the first 20 minutes, respectively. This rapid dissolution is a direct consequence of the enormous surface area of the nanocrystals, which creates a high supersaturation state, thereby driving absorption.

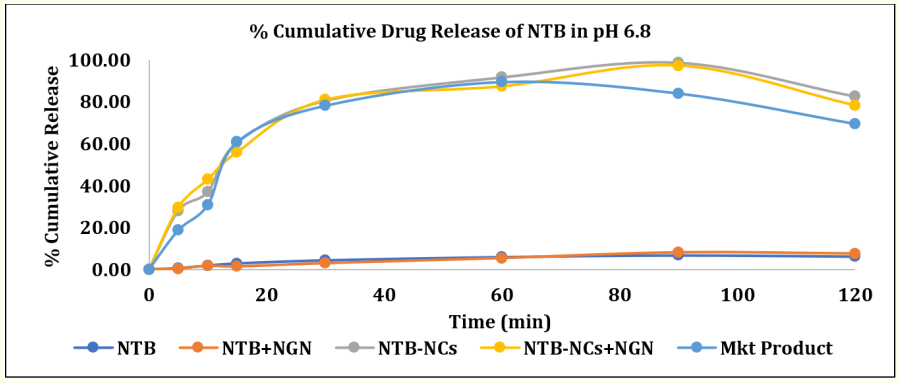

Figure 8: % Cumulative Release Pattern for NTB-NCs in PBS pH 6.8.

Stability studies

Stability studies were conducted in real-time and accelerated storage conditions to evaluate the developed NTB-NCs.

Table No. 4 shows how storage conditions affect physicochemical characteristics such as physical appearance, particle size, and percentage assay. At 40 ± 2°C/75 ± 5% RH, NTB-NCs showed a modest increase in particle size and a drop in % assay, but the physical appearance remained unchanged.

The results indicated that NTB-NCs were stable at storage temperatures ranging from 2 to 8 °C. (Note: * = Yellow lyophilized Crystalline Powder)

In vivo Pharmacokinetic Study

The *in vivo* performance of the formulations was the ultimate test of our hypothesis. The mean plasma concentration-time profiles are shown in Figure 9, and the pharmacokinetic parameters are listed in Table No. 5.

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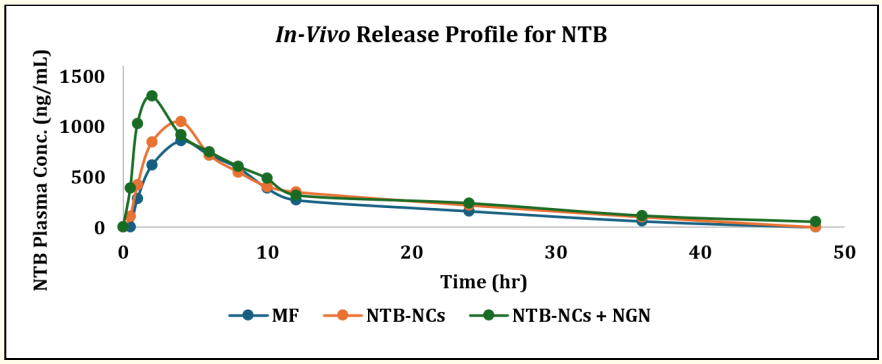

Figure 8: *In-Vivo* Release Profile for NTB-NCs with NGN as Bioenhancer.

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Sr. No. (n = 3 ± SD)	Time Interval (Months)	*Physical Appearance			Particle Size (d. nm)			% Assay		
		2-8˚C	25°C/40% RH	40 ± 2˚C/75 ± 5% RH	2-8˚C	25°C/40% RH	40 ± 2˚C/75 ± 5% RH	2-8˚C	25°C/40% RH	40 ± 2˚C/ 75 ± 5% RH
1	Initial	No Change in the Physical Appearance of the lyophilized product.			144.43 ± 7.70	144.43 ± 7.70	144.43 ± 7.70	98.81 ± 0.38	98.81 ± 0.38	98.81 ± 0.38
2	0.5	143.93 ± 4.63 149.07 ± 9.28			150.27 ± 10.16	98.47 ± 1.11	98.68 ± 1.45	98.55 ± 0.48		
3	1.0	142.20 ± 4.36 147.67 ± 8.84			161.17 ± 10.90	98.32 ± 1.16	98.55 ± 1.45	98.30 ± 0.42		
4	2.0	149.67 ± 6.92 153.60 ± 6.22			169.43 ± 16.76	98.34 ± 1.16	98.46 ± 1.50	97.85 ± 0.51		
5	3.0	152.90 ± 7.63 152.40 ± 3.25			172.92 ± 15.77	98.39 ± 1.13	98.20 ± 1.46	97.38 ± 0.54		
6	6.0	156.27 ± 7.30 157.60 ± 5.09			183.00 ± 13.86	98.30 ± 0.91	97.92 ± 1.33	96.75 ± 0.61		

Table No. 4: Stability behaviour for NTB-NCs.

In vivo Pharmacokinetic Study

The *in vivo* performance of the formulations was the ultimate test of our hypothesis. The mean plasma concentration-time profiles are shown in Figure 9, and the pharmacokinetic parameters are listed in Table No. 5.

The PK findings of NTB-NCs with NGN, indicated 856.54 ± 23.34ng/mL of Cmax and 11134.47 ± 156.23 ng*hr/mL of AUC. The NTB-NCs+NGN significantly increased bioavailability by 1.00 and 1.92 times ($p < 0.05$) compared to the NTB and commercialized formulations. The nanosized crystals, along with Bioenhancer NGN, might limit the metabolism of UGT enzymes, increasing the bioavailability of NTB. The increased bioavailability suggests the potential for a dosage decrease.

Table No. 5: Pharmacokinetic parameters of Nintedanib after oral administration to rats (Mean ± SD, n = 6).

Parameters	MF (Marketed formulation)	NTB-NCs	NTB-NCs + NGN
C_{max} (ng/mL)	856.54 ± 23.34	1045.5 ± 18.98	1298.43 ± 21.76
T_{max} (Hr)	4.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00
AUC (ng*Hr/mL)	11134.47 ± 156.23	14594.71 ± 1369.65	15953.53 ± 905.76
$T_{1/2}$ (Hr)	10.43 ±1.12	13.519 ± 2.32	11.34 ± 1.11
MRT (Hr)	14.89 ± 0.06	18.371 ± 12.23	16.453 ± 12.23
Relative Bioavailability (Folds)	1	1.31	1.43

This supra-additive effect validates our dual-pronged strategy. The nanocrystals ensured that a high concentration of dissolved NTB was available at the absorption site. Simultaneously, the co-formulated naringenin performed its dual role: it inhibited the intestinal UGT (as predicted by the HLM study), reducing presystemic metabolism increasing in the net flux of the drug into the systemic circulation. The slight but significant prolongation of the elimination half-life in the NTB-NC-NGN group suggests that naringenin may also inhibit UGT enzyme, thereby reducing the systemic clearance of NTB. The overall 1.92-fold enhancement in bioavailability is profound and far exceeds what could be achieved by either approach alone.

Conclusion

The current work focuses on developing a novel formulation with the help of Natural Bioenhancer for NTB, i.e., BCS class IV, to decrease the potential side effects of low bioavailability, low solubility, and high protein binding.

The Bioenhancer was selected based on an enzyme inhibition study performed on HLM via reversible competitive inhibition between NTB and NGN. The study proved that NGN altered the metabolism of NTB. The addition of 1% v/v of NGN resulted in the inhibition of about 70-75% of UGT-mediated metabolism for NTB.

Analytical and bioanalytical methods were developed and validated as per the ICH guidelines, i.e., Q2R2 and M10, respectively, for simultaneous estimation of NTB and NGN.

The solvent precipitation prepared the NTB-NCs, the top-down approach method using 0.4% HPC as a stabilizer. The NTB-NCs developed were confirmed by the results obtained from particle size (115 nm), *in-vitro* release profile, TEM analysis, % water content, DSC, and IR data. The Solubility of NTB-NC was increased up to 16 and 13 folds for water and PBS pH 6.8, respectively, against pure NTB drug.

The PK results of NTB-NCs with NGN, showed a 1.00- and 1.42 folds significant enhancement in bioavailability compared to the NTB and marketed formulation, respectively.

Thus, the enhancement in the bioavailability of NTB is due to the nanosized crystals along with Bioenhancer NGN, which potentially inhibits the metabolism of UGT enzymes and could lead to an increase in the bioavailability of NTB. The enhancement in bioavailability indicates the possibility of a dose reduction.

Financial Support and Sponsorship

The authors are thankful to ICMR for providing the research fellowship.

Conflicts of Interest

There are no conflicts of interest

Ethical Approval

The *in-vivo* experiments followed rules from the Committee for Control and Supervision of Experiments on Animals (CPCSEA), the Ministry of Social Justice and Empowerment, and the Government of India. The Institutional Animal Ethics Committee of the Faculty of Pharmacy at Maharaja Sayajirao University of Baroda in Vadodara, India, approved the pharmacokinetic study, protocol No.: MSU/IAEC/2022-23/1940.

Acknowledgements

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Summary

The current research work aimed for the development of the novel formulation of nintedanib formulated as nanocrystals in correlation by using bioenhancer naringenin which is estimated for increasing bio-enhancing activity for nintedanib which was predicted by HLM studies. The above research work can provide basis for the study in using bioenhancers with allopathic drugs.

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