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Exploratory study on physicochemical properties of nitazoxanide nanocrystalline suspension obtained by evaporative solvent-antisolvent technique

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Abstract

Nitazoxanide (NTZ) is a broad-spectrum antiparasitic drug with low bioavailability due to its poor aqueous solubility, classified as a Biopharmaceutical Classification System (BCS) class II drug. This study aimed to improve the understanding of NTZ nanocrystalline suspensions produced via the solvent-antisolvent technique. NTZ was extracted from commercially available coated tablets and characterized using DSC, TG, UV-Vis, and FTIR, revealing the presence of impurities likely from excipients. Nanocrystalline suspensions were prepared using three different stabilizers (Lecithin, Poloxamer 188, and Tween® 80) and evaluated for particle size distribution (PSD) and polydispersity index (PDI) over a one-week stability study. Poloxamer 188 was the most effective in reducing particle size (934.7 nm), though Lecithin achieved a more favorable PDI (0.289), indicating better particle uniformity. These findings suggest the solvent-antisolvent technique is a promising approach for producing NTZ nanocrystalline suspensions, but further reduction in particle size and exploration of alternative stabilizers are needed to optimize stability and performance.

Keywords: Nitazoxanide; Nanocrystalline suspension; Solven-antisolvent; Bottom-up; Nanoparticles; Pharmaceutical Technology

1. Introduction

Nitazoxanide (NTZ), 2-acetyloxy-*N*-(5-nitro-2-thiazolyl)benzamide, is a broad-spectrum antiparasitic, antibacterial and antiviral agent. NTZ is practically insoluble in water, and it is classified as a BCS class II drug, what makes it difficult to be orally absorbed [1,2]. For that reason, higher doses of NTZ are necessary in formulation which leads to some adverse effects including abdominal pain, diarrhea, headache and nausea [2]. The NTZ molecular structure is presented in Figure 1.

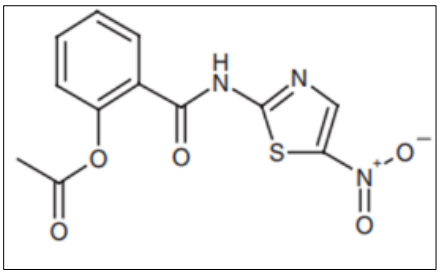

Figure 1 Molecular structure of NTZ. Adapted from Hemphill A et al., 2006 [2]

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To obtain a safe, stable, and effective drug product, the active pharmaceutical ingredient must be thoroughly studied. Preformulation studies are essential for establishing compatibility with excipients and providing information about the drug's physical and chemical properties. Techniques such as DSC, TG, UV-Vis, and FTIR spectroscopy are widely used in preformulation studies, including for the identification of drug molecules [3].

Lipophilic drugs with limited water solubility, such as NTZ, are commonly present in marketed formulations. These characteristics pose significant challenges in the formulation and clinical use of oral pharmaceutical dosage forms. Due to their poor solubility, such drugs often dissolve slowly in biological fluids, resulting in inconsistent absorption and, consequently, low bioavailability. This can necessitate the use of higher doses, which may lead to concerns regarding both safety and efficacy [4]. Several techniques have been applied to enhance drug solubility, such as micronization and nanonization to reduce particle size, formation of polymorphs, cocrystals, complex formation, and the preparation of drug dispersions in carriers [5,6].

These techniques can be grouped into two categories. The first is top-down methods, which involve the mechanical reduction of particle size from bulk material [7–9]. The second category is bottom-up technologies, which involve controlled precipitation, where a drug solution is added to an antisolvent phase. This approach, known as the solvent-antisolvent technique, requires careful selection of the antisolvent, as it is a critical step in achieving nanoscale particles [10].

At the nanoscale, van der Waals forces and Brownian motion play essential roles in the interactions and stability of nanoparticles. Van der Waals forces, which are weak attractive forces between particles, come into effect when nanoparticles are brought close together due to the random movement caused by Brownian motion. As these particles move and collide, their proximity allows van der Waals forces to induce attraction, making nanoparticles prone to aggregation. This tendency to aggregate often leads to flocculation, where clusters of particles form, compromising the stability of the nanoparticle dispersion [11,12]. To enhance the stability of supersaturated nanoparticle solutions and prevent natural agglomeration, the use of stabilizers, surfactants, or a combination of both is recommended [13]. In this study, three different surfactants — Tween® 80, Poloxamer 188, and Lecithin — were evaluated to determine the most effective stabilizer for NTZ-containing nanocrystalline suspension.

2. Material and Methods

2.1. Materials

Nitazoxanide film-coated tablets (Annita® 500 mg) were purchased from a local pharmacy. Methanol, dimethyl sulfoxide (DMSO), and acetonitrile grade for HPLC were purchased from Mallinckrodt Baker. Mannitol, Tween® 80, Poloxamer 188 and Lecithin with analytical grade were gently donated by the University of São Paulo.

2.2. Methods

2.2.1. Extraction of Nitazoxanide (NTZ)

Nitazoxanide (NTZ) was extracted from commercially available film-coated tablets (Annita® 500 mg). The tablets were finely pulverized using a mortar and pestle until a yellowish powder. A volume of 250 mL of methanol was added to the powder, and the mixture was transferred to a suitable glass beaker shielded from light. The beaker was then placed in an ultrasonic bath for 10 minutes to facilitate extraction. Afterward, the solution was filtered through a 0.45 µm nylon membrane to obtain the filtrate. The solvent was evaporated using a speed-vacuum system, yielding a solid material with a substantial amount of nitazoxanide. The theoretical yield of isolated NTZ (iNTZ) was estimated to be 1500 mg.

2.2.2. iNTZ characterization

UV-Vis Spectroscopy

UV-Vis analysis of the isolated nitazoxanide (iNTZ) was performed using a Shimadzu UV-2550 spectrophotometer. A 12 µg/mL solution of the iNTZ was prepared by dissolving a small quantity of the extract in an appropriate volume of methanol. The solution was placed in an ultrasonic bath to ensure complete dissolution. The analysis was conducted over a wavelength range of 200 nm to 400 nm, with methanol serving as the blank.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained using a Shimadzu IR-Prestige-21 spectrometer. The spectra of the iNTZ were recorded across the wavelength range of 500 cm^{-1} to 4000 cm^{-1}.

Differential scanning calorimetry (DSC)

DSC measurements were conducted using an Exstar DSC 7020 (Hitachi Instruments) under a nitrogen atmosphere with a flow rate of 100 mL/min. Approximately 2 mg of the iNTZ were placed in an aluminum pan and hermetically sealed. The temperature was programmed to increase at a rate of 10 °C/min, ranging from 25 °C to 600 °C.

Thermogravimetry (TG)

Accurately 5 mg of iNTZ were placed into a platinum pan. The TG measurements were obtained by a TG/DTA STA7200 (Hitachi Instruments). A nitrogen atmosphere was used with an output of 100mL/min and the temperature were set between 25 °C and 600 °C with a heating rate of 10°C/min.

2.2.3. Stability of NTZ

The stability of NTZ was evaluated under hydrolytic and photolytic conditions at room temperature. Small amounts of the NTZ methanolic solution were either mixed with deionized water or exposed to light, and samples were analyzed at the start of the experiment and after 24 hours of exposure. The quantification of NTZ was performed using a Shimadzu HPLC system, consisting of an LC-9A pump, Rheodyne model 7125 injector, SPD-6AV UV-Vis detector, SCL-6B control unit, and Chromatopack C-R4A software. A C18 column (250 mm / 4.6 µm) was used for separation, with a mobile phase of acetonitrile:water (1:1 v/v).

After exposure, samples were dissolved in acetonitrile and injected into the HPLC system. The final concentration of the prepared samples was 30 µg/mL of NTZ. The NTZ peak, with a retention time of 7.5 minutes, was integrated, and the amount of NTZ present in each sample was quantified.

2.2.4. Preparation of NTZ Nanocrystalline Suspension via Solvent-Antisolvent Technique

A bottom-up technique, known as the evaporative solvent-antisolvent method, was employed to produce a nanocrystalline suspension of NTZ. This approach involves dissolving the isolated drug in a suitable solvent, followed by the addition of an antisolvent, which induces the precipitation of the drug as nanoparticles. The objective of this technique is to achieve controlled nanoparticle formation, enhancing the drug's solubility and bioavailability through size reduction [14]. Three solutions of NTZ, each with a final concentration of 10 mg/mL, were prepared in DMSO. Separately, three antisolvent solutions were prepared, with deionized water as the antisolvent and mannitol as a cryoprotectant for subsequent lyophilization. For each antisolvent solution, a different surfactant (Lecithin, Poloxamer 188, or Tween® 80) was used. Initially, the antisolvent solutions were stirred at 7,000 rpm until they became completely clear. Then, the magnetic stirrer speed was increased to 10,000 rpm, and an aliquot of the NTZ solvent phase was rapidly injected into the antisolvent phase. The mixture was stirred for an additional 5 minutes to ensure thorough mixing [10]. The antisolvent phase was prepared using 90 mL of deionized water, 500 mg of mannitol, and 50 mg of each surfactant. The antisolvent phase, containing the nanocrystalline suspension of NTZ, was then lyophilized under light protection. Following lyophilization, the nanoparticles were re-suspended and analyzed using a Zetasizer Nano ZS to determine the Particle Size Distribution (PSD) and Polydispersity Index (PDI).

3. Results and Discussion

3.1. Extraction of NTZ

The theoretical amount of NTZ was calculated to be 1500 mg. After re-crystallization, 705 mg of iNTZ were obtained, corresponding to a yield of only 47% (w/w). This low yield can be attributed to the gentle extraction process, which was carefully performed to minimize the extraction of other components from the marketed film-coated tablets, particularly those with lipophilic properties.

3.2. Characterization of iNTZ

According to literature, the absorption peak of NTZ is typically observed at 345 nm [15]. However, the UV-Vis spectrum of the NTZ sample obtained in this study, measured between 200 nm and 400 nm, shows a peak at 353.6 nm (Figure 2). This shift can be attributed to the lack of a purification process, suggesting the presence of impurities from the excipients

in the marketed tablets. Another possible explanation is partial degradation of NTZ, as it is known to be sensitive to light, which can lead to degradation and cause spectral shifts. Impurities can also influence the spectrum, contributing to the peak shift [16].

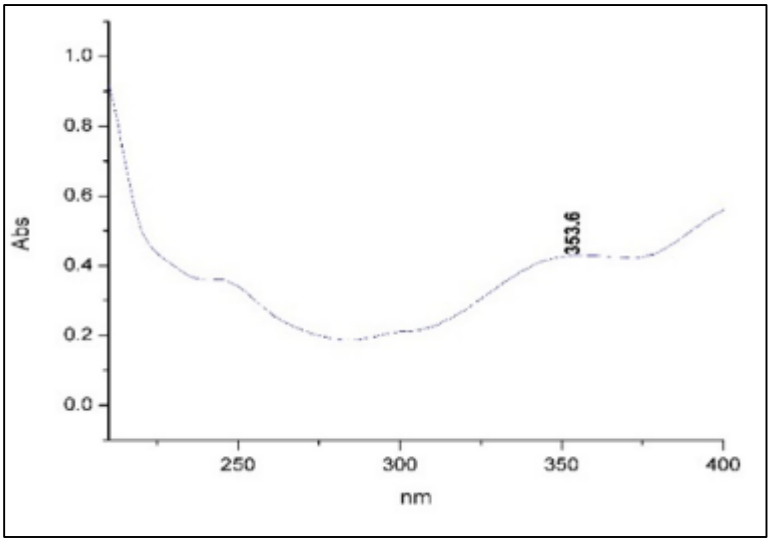

Figure 2 UV-Vis spectrum of NTZ extract

The FTIR spectrum was obtained in the mid-range between 500 cm^{-1} and 4000 cm^{-1}, revealing several peaks that can be used to identify the presence of NTZ in iNTZ. The key peaks corresponding to the characteristic functional groups of NTZ are clearly visible in the iNTZ IR spectrum (Figure 3), and these peaks serve as markers for the identification of the NTZ molecule.

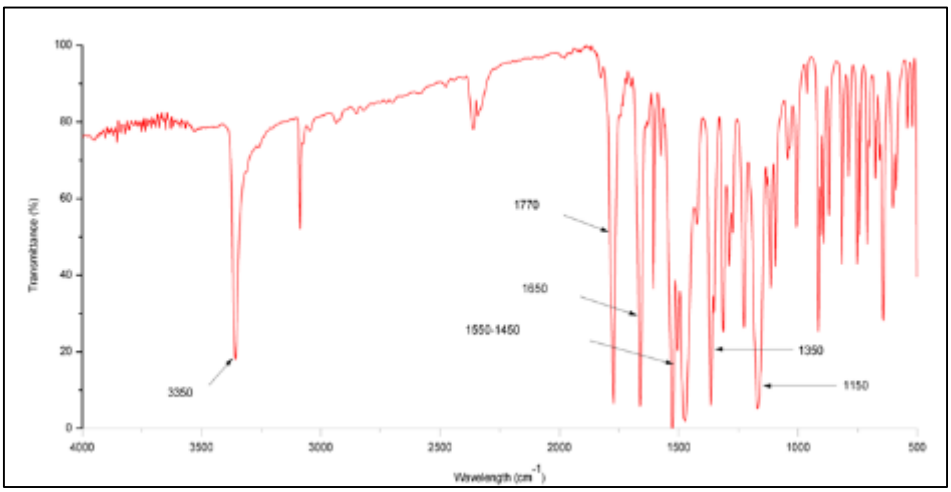

Figure 3 FTIR Spectrum of Nitazoxanide recorded using a KBr pellet

The FTIR spectrum of the iNTZ sample reveals key functional groups, confirming the presence of the amide (N-H at 3350 cm^{-1}, C=O at 1650 cm^{-1}) and nitro groups (NO_2 at 1550-1450 cm^{-1}, C-N at 1350 cm^{-1}), which align with the expected NTZ structure [17]. However, the peaks at 1770 cm^{-1} and 1150 cm^{-1}, indicative of ester bonds, suggest possible impurities or degradation products, likely due to NTZ's light sensitivity or residual excipients from the formulation. While the primary structure of NTZ is confirmed, the presence of these unexpected peaks emphasizes the need for further purification or analysis to identify the source of these impurities.

The thermal analysis of the iNTZ sample aligns with literature, which indicates NTZ melts with decomposition around 175-202°C [18]. The observed broad endothermic event between 175°C and 195°C suggests the presence of impurities, as expected due to the lack of purification. The decomposition onset at 200°C and an intense exothermic peak at 240°C further confirm that iNTZ behaves similarly to pure NTZ, with the wider melting range attributed to impurities.

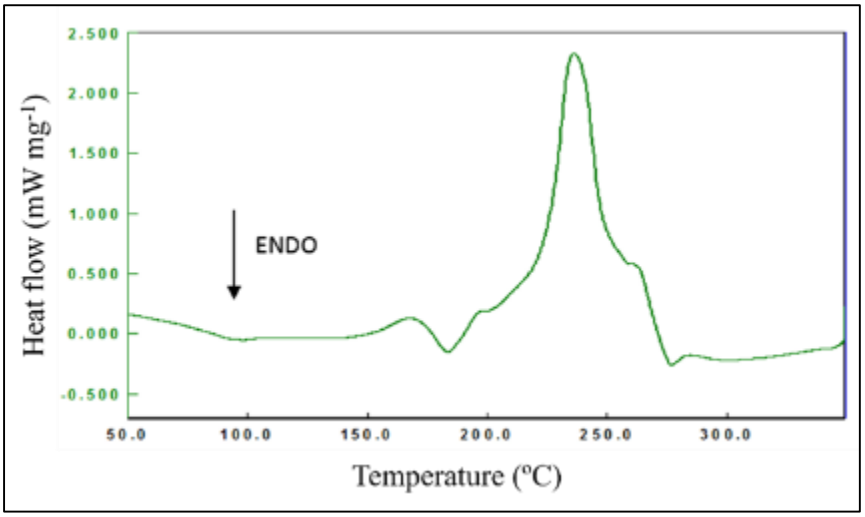

Figure 4 DSC curve of iNTZ.

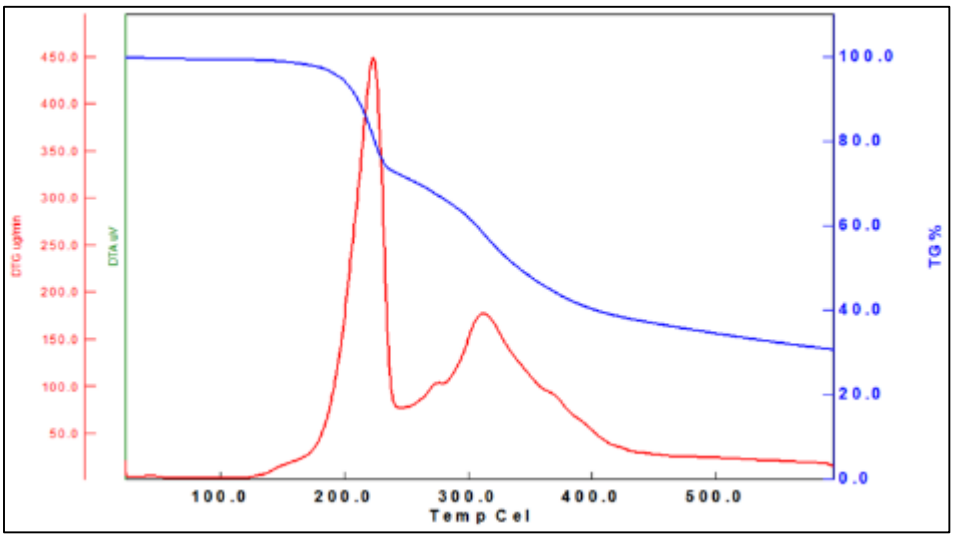

Figure 5 TG curve of iNTZ

3.3. NTZ stability

The stability data for the NTZ sample are shown in Table 1, expressed as the percentage of NTZ remaining. After 24 hours of light exposure, the degradation was minimal, with only a 2.7% loss, indicating that NTZ is relatively stable under photolytic conditions. In contrast, hydrolytic conditions proved to be more critical, resulting in a 9% (w/w) loss due to significant decomposition. These findings suggest that aqueous environments are more detrimental to NTZ stability, highlighting the importance of selecting appropriate storage conditions. To ensure stability, both the NTZ sample and its nanocrystalline suspension should not be stored in aqueous media and may benefit from stabilization strategies such as lyophilization.

Table 1 Stability of the NTZ exposed samples.

Stress condition	Amount of NTZ after 24 hours (%)	Loss after 24 hours (%)
Water	91.0%	9.0%
Light	97.3%	2.7%

3.4. Nanocrystalline suspension of NTZ via solvent-antisolvent technique

Following lyophilization, the nanoparticles were re-suspended to evaluate the Particle Size Distribution (PSD), and a one-week stability study was conducted. The particle sizes were similar across the three formulations containing Lecithin, Poloxamer 188, or Tween® 80. However, the formulations with Lecithin and Tween® 80 exhibited both nanoscale and microscale particles, with some particles exceeding 1000 nm in size. In contrast, the formulation stabilized with Poloxamer 188 showed an average particle size of 934.7 nm. The PSD for all three nanocrystalline suspensions is presented in Figure 6.

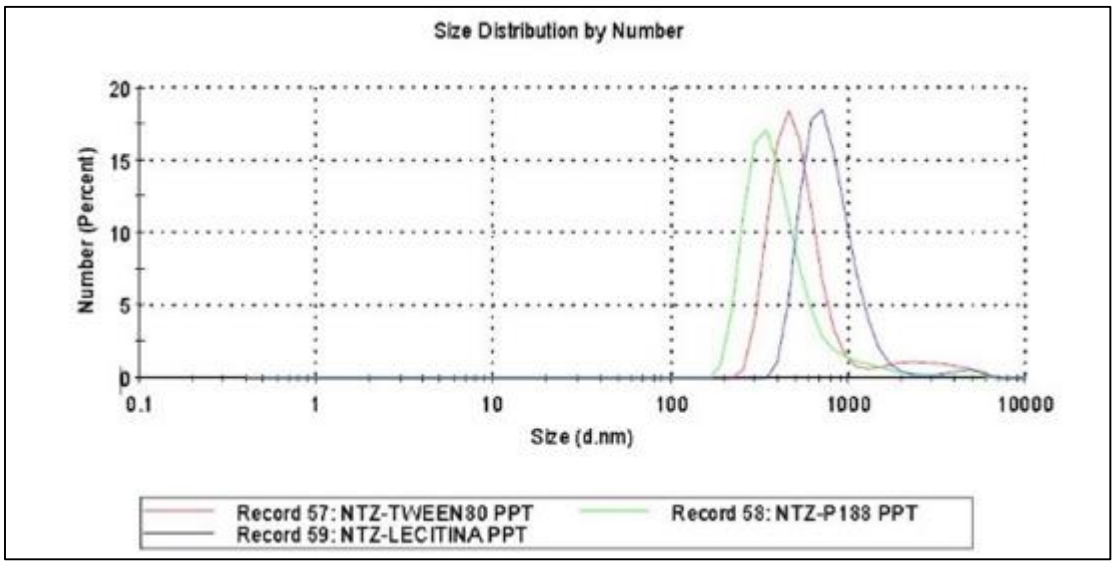

Figure 6 PSD of nanocrystalline suspension containing Lecithin, Poloxamer 188 or Tween® 80

3.5. Stability of NTZ Nanocrystalline Suspensions

During the one-week stability study, a slight decrease in the average Particle Size Distribution (PSD) was observed across all nanocrystalline suspensions. This reduction could be attributed to the partial dissolution of loosely stabilized particles and their subsequent re-formation into smaller, more stable particles. Another possible explanation is the reorganization of surfactant layers, which may have enhanced stabilization over time, contributing to the observed decrease in PSD. The formulation containing lecithin showed the most notable improvement in the Polydispersity Index (PDI), decreasing from 0.379 to 0.289, which suggests a more uniform particle size distribution. In contrast, the formulations stabilized with Poloxamer 188 and Tween® 80 showed no significant change in PDI. After one week, the Lecithin-based formulation was the only one to achieve a PDI below 0.3, indicating a relatively monodisperse system with better particle homogeneity. The amphiphilic properties of lecithin likely contributed to this enhanced stability, as it can form a stable interface around the nanoparticles, reducing aggregation and promoting particle uniformity. These findings, shown in Table 3, suggest that Lecithin is the most effective stabilizer among those tested. Further optimization of the nanocrystalline suspension may include experimenting with stabilizer combinations, increasing stirring intensity, or adjusting process temperature to achieve smaller PSDs while maintaining or enhancing stability over time.

Table 2 Comparison of PSD and PDI after one-week of storage

	First Week		**Second Week**	
Stabilizer	**Average PSD (nm)**	**PDI**	**Average PSD (nm)**	**PDI**
Lecithin	1208.7	0,379	985,3	0,289
Tween® 80	1274,7	0,472	1066	0,472
Poloxamer 188	934,7	0,541	922,8	0,612

4. Conclusion

This study demonstrated the successful application of various characterization techniques, including DSC, TG, UV-Vis, and FTIR, to evaluate the physicochemical properties of nitazoxanide (NTZ) extracted from marketed coated tablets. The presence of impurities, likely from excipients, was evident in the thermal and spectroscopic analyses, though the primary NTZ structure remained intact. The solvent-antisolvent technique proved effective in producing NTZ nanocrystalline suspensions, with Lecithin showing the best stabilization properties, as indicated by a reduction in both PSD and PDI over the one-week stability study. Formulations containing Poloxamer 188 and Tween® 80 also exhibited stable suspensions, though they were less effective at reducing particle size and polydispersity compared to Lecithin. Further optimization of process parameters such as stirring speed, temperature, and stabilizer combinations is necessary to enhance particle size distribution and overall suspension stability. This research provides valuable insights for improving NTZ formulations and underscores the potential of the solvent-antisolvent technique for developing nanocrystalline suspensions.

Compliance with ethical standards

Disclosure of conflict of interest

There exists no form of conflicting interest among authors.

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