https://doi.org/10.33472/AFJBS.6.6.2024. 5958-5977

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Formulation, Characterization, and Evaluation of Itraconazole-Encapsulated Polymeric Nanoparticles

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Article Info

Volume 6, Issue 6, June 2024

Received: 23 April 2024

Accepted: 27 May 2024

Published: 20 June 2024

doi: 10.33472/AFJBS.6.1.2024.5958-5977

ABSTRACT:

This comprehensive study designed polymeric nanoparticles of itraconazole and evaluated the properties and performance of Itraconazole-loaded polymeric nanoparticles, focusing on their formulation, stability, and release kinetics. The integrity of itraconazole within the nanoparticles was validated by Fourier Transform Infrared Spectroscopy (FT-IR) analysis, which revealed no appreciable chemical changes after encapsulating. Drug release rates and bioavailability are directly impacted by drug/polymer ratios, as demonstrated by the differences in particle sizes that were found through investigation. Zeta potential measurements showed that the presence of negative surface charges prevented aggregation, resulting in adequate stability. The entrapment efficiency of several formulations was also evaluated in the study; some showed excellent efficiency, indicating ideal formulation circumstances. Studies on the release of drugs in vitro showed controlled release profiles that were noticeably better than those of the pure drug, indicating increased therapeutic efficacy. The formulation's capacity to increase itraconazole's bioavailability—a critical factor for medications with poor solubility—was further validated by solubility testing. The formulation's potential to enhance absorption across various intestinal segments was highlighted by ex vivo intestinal permeability experiments. Studies on stability revealed that the mixture remained more stable at lower temperatures, which has important storage and handling implications.

Keywords: Polymeric Nanoparticles, Itraconazole Drug Delivery, Eudragit L 100, Drug Release Kinetics, Solubility Enhancement

INTRODUCTION

Polymeric nanoparticles have emerged as a groundbreaking technology in the field of drug delivery, offering significant advantages over traditional methods. These nanoscale carriers, typically ranging from 10 to 1000 nanometers in size, are composed of biodegradable and biocompatible polymers. Their unique properties make them ideal for improving the delivery and efficacy of various therapeutic agents, including poorly soluble drugs, peptides, proteins, and nucleic acids. The use of polymeric nanoparticles in drug delivery addresses several limitations associated with conventional drug administration. Traditional delivery methods often suffer from poor bioavailability, rapid clearance from the body, and non-specific distribution, which can lead to suboptimal therapeutic outcomes and increased side effects. In contrast, polymeric nanoparticles can enhance the solubility of hydrophobic drugs, protect the encapsulated drug from degradation, and provide controlled and sustained release profiles. These features help to maintain therapeutic drug levels in the bloodstream for extended periods, reducing the frequency of dosing and improving patient compliance (Castro et al., 2022,

Zielińska et al., 2020, Crucho and Barros, 2017, Senthilnathan et al., 2015). One of the most significant advantages of polymeric nanoparticles is their ability to target specific tissues or cells. This targeting capability can be achieved through passive or active targeting mechanisms. Passive targeting exploits the enhanced permeability and retention (EPR) effect, which allows nanoparticles to accumulate preferentially in tumor tissues due to their leaky vasculature. Active targeting involves functionalizing the surface of nanoparticles with ligands, such as antibodies, peptides, or small molecules, that bind selectively to receptors on the target cells. This targeted delivery approach minimizes systemic side effects and maximizes the therapeutic efficacy of the drug (Jawahar and Meyyanathan, 2012, Grenha, 2012, Rao and Geckeler, 2011, Lu et al., 2011, Tuncel and Demir, 2010).

The design and formulation of polymeric nanoparticles involve careful selection of polymers and fabrication techniques. Commonly used polymers include poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and polyethylene glycol (PEG), which are chosen for their biodegradability and biocompatibility. Various fabrication methods, such as nanoprecipitation, emulsification-solvent evaporation, and ionic gelation, allow for the fine-tuning of particle size, drug loading capacity, and release kinetics. The versatility in design enables the development of nanoparticles tailored to specific therapeutic needs. Polymeric nanoparticles have shown great promise in the delivery of anticancer drugs. Chemotherapeutic agents often have narrow therapeutic windows and severe side effects due to non-specific distribution (Morachis et al., 2012, Locatelli and Comes Franchini, 2012). By encapsulating these drugs in polymeric nanoparticles, it is possible to enhance their solubility, protect them from premature degradation, and achieve controlled release. This not only increases the drug's efficacy against cancer cells but also reduces toxicity to healthy tissues. Additionally, the ability to functionalize nanoparticles with targeting ligands further improves the precision of drug delivery to tumor cells, enhancing therapeutic outcomes (Jawahar and Meyyanathan, 2012, Grenha, 2012, Rao and Geckeler, 2011, Lu et al., 2011, Tuncel and Demir, 2010).

In the realm of infectious diseases, polymeric nanoparticles have been employed to deliver antibiotics, antiviral agents, and vaccines. Encapsulation of antibiotics in nanoparticles can overcome issues of poor solubility and stability, ensuring sustained release and improved therapeutic levels at the site of infection. For antiviral therapy, nanoparticles can protect sensitive agents from degradation and enhance their uptake by infected cells. In vaccine delivery, nanoparticles can serve as adjuvants, enhancing the immune response and providing controlled release of antigens to mimic natural infection processes. The use of polymeric nanoparticles is also expanding into the delivery of genetic materials, such as DNA, RNA, and siRNA, for gene therapy applications. These nanoparticles can protect nucleic acids from enzymatic degradation, facilitate cellular uptake, and enable controlled release, making gene therapy more effective and safer. Despite the promising advantages, the development and clinical translation of polymeric nanoparticles face several challenges. These include scalability of production, regulatory hurdles, and ensuring long-term stability and biocompatibility. Additionally, understanding the interactions between nanoparticles and biological systems is crucial for optimizing their design and function. In closing, polymeric nanoparticles represent a versatile and powerful platform for drug delivery, capable of overcoming many limitations of traditional methods. Their ability to enhance drug solubility, provide controlled release, and target specific tissues opens new avenues for the treatment of various diseases (Morachis et al., 2012, Locatelli and Comes Franchini, 2012). Ongoing research and development are likely to further advance this technology, making it an integral part of future therapeutic strategies.

Itraconazole, an antifungal agent, presents several challenges that make it an ideal candidate for formulation into polymeric nanoparticles. As a BCS Class II drug, Itraconazole is characterized by poor water solubility, which significantly limits its bioavailability and

therapeutic efficacy when administered through conventional routes. Enhancing its solubility and ensuring sustained release can significantly improve its clinical outcomes. Polymeric nanoparticles offer a solution to the solubility problem by increasing the surface area of the drug and facilitating better interaction with aqueous environments. Encapsulation of Itraconazole in polymeric nanoparticles can protect the drug from premature degradation and enhance its stability, thus ensuring a more consistent therapeutic effect (De Beule and Van Gestel, 2001, Piérard et al., 2000). Moreover, the controlled release properties of polymeric nanoparticles can maintain therapeutic drug levels over extended periods, reducing the frequency of administration and improving patient compliance. The ability of polymeric nanoparticles to target specific tissues or cells can be particularly beneficial for antifungal therapies, which often require prolonged treatment courses and high drug concentrations at the site of infection. By functionalizing nanoparticles with ligands that target fungal cells, it is possible to increase the concentration of Itraconazole at the infection site while minimizing systemic exposure and associated side effects. Additionally, the use of biodegradable and biocompatible polymers like PLGA, PCL, and PEG ensures that the nanoparticle formulation is safe for long-term use and can be metabolized by the body without causing adverse reactions (Piérard et al., 2000). These attributes make polymeric nanoparticles a promising vehicle for delivering Itraconazole more effectively and safely, enhancing its therapeutic potential in treating fungal infections. Therefore Considering all the facts, this present study was designed to prepare polymeric nanoparticles of itraconazole via nanoprecipitation techniques and characterize the same in various ways.

MATERIAL AND METHODS

Materials

Itraconazole was generously provided as a gift sample by WellCare Pharma, located in Baddi, India. Eudragit L 100 was also gifted by TTK Pharmaceuticals in Chennai. Additionally, Pluronic F68 and Polyvinyl alcohol were purchased from Sigma Aldrich, Mumbai, India. All other chemicals and solvents employed in this study were of analytical grade, ensuring their suitability and reliability for experimental procedures.

Preparation of the polymeric nanoparticles

The preparation of Itraconazole-loaded polymeric nanoparticles involves the following steps. Various formulations were prepared using different ratios of drug to polymer, as outlined in the composition table (Torres-Flores et al., 2019). Itraconazole, at a constant weight of 10 mg, was used in conjunction with varying weights of the polymer Eudragit L 100. The weight of Eudragit L 100 ranged from 100 mg to 700 mg, corresponding to drug-to-polymer ratios from 1:10 to 1:70. For formulations PNF1 through PNPNF7, a 1% concentration of Pluronic F68 was included. The process began by dissolving the specified amounts of Itraconazole and Eudragit L 100 in 20 ml of methanol, forming the organic phase. This organic phase was then slowly injected into an aqueous phase containing 40 ml of water with 1% Pluronic F68 under continuous stirring. This led to the formation of nanoparticles as the polymer precipitated out. For formulations PNF8 through PNF14, 1% Polyvinyl alcohol was used instead of Pluronic F68. In these cases, the aqueous phase consisted of 40 ml of water with 1% Polyvinyl alcohol. The same procedure was followed where the organic phase containing Itraconazole and Eudragit L 100 in methanol was slowly injected into the aqueous phase under constant stirring. After the nanoparticle formation, the mixture was stirred continuously for 3-4 hours to ensure complete evaporation of the methanol, leaving behind the Itraconazole-loaded polymeric nanoparticles. These nanoparticles were then collected and characterized for further analysis. This method ensures reproducibility and consistency in the fabrication of Itraconazole-loaded polymeric nanoparticles across different formulations (Torres-Flores et al., 2019, Salatin et al., 2017).

Table 1. Composition table indicating the constituents of Itraconazole loaded polymeric nanoparticles

Code for Formulation	**Drug: Polymer Ratio**	**Weight of Drug (mg)**	**Polymer**	**Weight of Polymer (mg)**	**Concentration of Pluronic F68 (%)**	**Concentration of Polyvinyl Alcohol (%)**
PNF1	01:10	10	Eudragit L 100	100	1	
PNF2	01:20	10	Eudragit L 100	200	1	
PNF3	01:30	10	Eudragit L 100	300	1	
PNF4	01:40	10	Eudragit L 100	400	1	
PNF5	01:50	10	Eudragit L 100	500	1	
PNF6	1:60	10	Eudragit L 100	600	1	
PNPNF7	1:70	10	Eudragit L 100	700	1	
PNF8	01:10	10	Eudragit L 100	100		1
PNF9	01:20	10	Eudragit L 100	200		1
PNF10	01:30	10	Eudragit L 100	300		1
PNF11	01:40	10	Eudragit L 100	400		1
PNF12	01:50	10	Eudragit L 100	500		1
PNF13	1:60	10	Eudragit L 100	600		1
PNF14	1:70	10	Eudragit L 100	700		1

Characterization

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy was employed to characterize the chemical structure and potential interactions between Itraconazole and the polymers used in the nanoparticle formulations. FT-IR spectra were recorded using an FT-IR spectrometer (Bruker Tensor 27). Samples of pure Itraconazole, Eudragit L 100, and the Itraconazole-loaded nanoparticles (PNF7) were prepared by mixing with potassium bromide (KBr) and compressing into thin pellets. The spectra were scanned over a wavelength range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Key functional groups were identified by analyzing characteristic absorption peaks. The spectrum of pure Itraconazole was analyzed to determine the presence of specific functional groups, such as C=O stretching vibrations, N-H bending, and aromatic C=C stretching. Similarly, spectra of Eudragit L 100 and the nanoparticle formulations were examined for characteristic peaks. Comparative analysis of the FT-IR spectra was conducted to identify any shifts or changes in peak positions, indicating potential

interactions between Itraconazole and the polymer matrix. Such interactions could include hydrogen bonding or van der Waals forces, which may affect the drug's stability and release profile. The FT-IR analysis thus provided crucial insights into the compatibility and molecular interactions within the nanoparticle system, ensuring the integrity and efficacy of the formulated drug delivery system (Torres-Flores et al., 2019, Salatin et al., 2017).

Determination of Particle Size, Polydispersity Index, and Zeta Potential

The particle size, polydispersity index (PDI), and zeta potential of the Itraconazole-loaded polymeric nanoparticles were determined using dynamic light scattering (DLS) and electrophoretic light scattering techniques, respectively, with a Zetasizer Nano ZS (Malvern Instruments, UK) (Torres-Flores et al., 2019, Salatin et al., 2017). The particle size distribution and PDI of the nanoparticles were measured to assess the uniformity and stability of the formulations. A small volume of the nanoparticle suspension was diluted with deionized water to an appropriate concentration before measurement. The sample was placed in a disposable sizing cuvette, and the mean particle size (expressed in nanometers) and PDI were recorded. The PDI value provided an indication of the width of the particle size distribution, with values closer to 0.1 indicating a more uniform and monodisperse population of nanoparticles. The zeta potential of the nanoparticles was measured to evaluate their surface charge, which is a critical parameter influencing the stability of colloidal dispersions. A diluted suspension of the nanoparticles was injected into a zeta potential measurement cell. The zeta potential (expressed in millivolts) was determined by applying an electric field and measuring the velocity of the particles. Higher absolute values of zeta potential (typically above ±30 mV) indicated greater electrostatic repulsion between particles, suggesting better stability against aggregation. The nanoparticle suspension was diluted with deionized water to achieve a suitable concentration for measurement. The diluted sample was placed in a disposable sizing cuvette and inserted into the Zetasizer Nano ZS. Measurements were taken at 25°C, and the average particle size and PDI were recorded. The diluted nanoparticle suspension was injected into the zeta potential measurement cell. The cell was placed in the instrument, and measurements were taken at 25°C. The zeta potential values were recorded and analyzed. The data obtained from these measurements provided essential information on the physical characteristics of the nanoparticles, which are critical for their performance in drug delivery applications. Stable, uniformly sized nanoparticles with appropriate surface charge are crucial for ensuring consistent drug release and bioavailability.

Determination of % Entrapment Efficiency

The entrapment efficiency (EE) of Itraconazole-loaded polymeric nanoparticles was determined to evaluate the effectiveness of the drug encapsulation process (Torres-Flores et al., 2019, Salatin et al., 2017). This parameter is crucial as it indicates the proportion of the drug successfully incorporated into the nanoparticles relative to the total amount of drug used in the formulation. A high entrapment efficiency signifies that the formulation process is effective and that a significant amount of the drug is available for therapeutic action. To begin, a known quantity of the nanoparticle suspension was subjected to high-speed centrifugation at approximately 15,000 rpm for 30 minutes. This step was necessary to separate the free (unencapsulated) drug from the encapsulated drug within the nanoparticles. After centrifugation, the supernatant, which contained the free drug, was carefully collected for analysis, ensuring that the nanoparticle pellet remained undisturbed at the bottom of the centrifuge tube. The concentration of Itraconazole in the supernatant was determined using a UV-visible spectrophotometer. The supernatant was appropriately diluted to fall within the linear range of the spectrophotometer, and the absorbance was measured at the specific wavelength corresponding to Itraconazole. A calibration curve, prepared from standard solutions of Itraconazole, was used to calculate the amount of free drug present in the supernatant. Next, the total drug content within the nanoparticles was determined. This

involved dissolving a known amount of the nanoparticle pellet (obtained after centrifugation) in methanol, followed by analysis using a UV-visible spectrophotometer. The absorbance readings were compared against the calibration curve to quantify the total amount of Itraconazole encapsulated within the nanoparticles. The entrapment efficiency was calculated using the formula:

Entrapment Efficiency (%) = (Total drug – Free drug)/Total drug *100

In this formula, the "Total Drug" represents the initial amount of Itraconazole used in the formulation, while the "Free Drug" is the amount of Itraconazole found in the supernatant. By subtracting the free drug from the total drug, the amount of drug encapsulated within the nanoparticles was determined, and this value was expressed as a percentage of the total drug used. This method provides a reliable measure of the amount of drug successfully encapsulated within the nanoparticles. High entrapment efficiency is desirable as it indicates minimal drug wastage and ensures that a higher dose of the drug is available for therapeutic action. This, in turn, enhances the efficacy of the drug delivery system, making it more effective for clinical applications

***In Vitro* Drug Release Study**

The in vitro drug release profile of Itraconazole-loaded polymeric nanoparticles was assessed using a dialysis bag diffusion technique (Torres-Flores et al., 2019, Salatin et al., 2017, Chidambaram and Burgess, 1999). This method is essential for evaluating the release kinetics and overall performance of the drug delivery system, providing insights into how the drug is released over time under simulated physiological conditions. To begin, dialysis bags with a molecular weight cutoff of approximately 12,000–14,000 Da were pre-soaked in distilled water for 12 hours. This pre-soaking step ensured that the bags were fully hydrated and free of any preservatives that might interfere with the drug release study. A known amount of the nanoparticle suspension was then carefully placed into the prepared dialysis bags, which were securely sealed at both ends to prevent any leakage during the experiment. The dialysis bags containing the nanoparticle suspension were immersed in a beaker containing 100 ml of phosphate buffer (pH 6.5) with 0.1% sodium lauryl sulfate (SLS). This dissolution medium was chosen to simulate the intestinal fluid environment and to maintain sink conditions, ensuring that the concentration of Itraconazole in the medium did not reach saturation. The beaker was placed in a thermostatically controlled water bath maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. This setup aimed to mimic the physiological temperature and agitation of the gastrointestinal tract. Samples were collected at predetermined time intervals, such as 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours. For each sampling point, 1 ml of the dissolution medium was withdrawn and immediately replaced with an equal volume of fresh buffer to maintain a constant volume and concentration gradient. The withdrawn samples were filtered to remove any particulate matter, ensuring that only the dissolved drug was measured. The concentration of Itraconazole in each sample was determined using a UV-visible spectrophotometer at the appropriate wavelength. A calibration curve of Itraconazole in phosphate buffer pH 6.5 was used to quantify the drug concentration in the samples accurately. By measuring the absorbance of the samples and referencing the calibration curve, the amount of Itraconazole released at each time point was calculated. The cumulative percentage of drug released was then calculated for each time point and plotted against time to obtain the release profile of Itraconazole from the nanoparticles. The release data was further analyzed to determine the release kinetics by fitting it to various mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. This analysis provided insights into the mechanism of drug release and the overall efficiency of the drug delivery system. This comprehensive in vitro drug release study provided a detailed understanding of the drug release behavior from the nanoparticles. Such information is crucial for predicting the in vivo

performance of the formulation and optimizing the drug delivery system to achieve the desired therapeutic outcomes.

Lyophilization

Lyophilization, or freeze-drying, is a crucial technique for the stabilization and preservation of nanosuspensions, converting them into a dry powder form that can be easily reconstituted (Torres-Flores et al., 2019, Salatin et al., 2017). This process enhances the stability, shelf-life, and handling of nanoparticle formulations, making them more suitable for long-term storage and clinical use. The process begins with the preparation of the nanosuspension using methods such as nanoprecipitation or high-pressure homogenization. Once the nanosuspension is characterized for particle size, polydispersity index, and zeta potential, cryoprotectants like mannitol, trehalose, or sucrose are added. These cryoprotectants, typically in concentrations ranging from 1% to 10% (w/v), protect the nanoparticles from aggregation and degradation during the freeze-drying process. The nanosuspension is then aliquoted into appropriate containers and subjected to rapid freezing, often using a cryogenic freezer or liquid nitrogen. Rapid freezing helps to form small ice crystals, maintaining the structural integrity of the nanoparticles. The frozen samples are then placed in a lyophilizer for the primary drying phase, where the temperature is lowered below the eutectic point, and the pressure is reduced to create a vacuum. Under these conditions, ice sublimates directly into vapor without passing through the liquid phase, effectively removing most of the water from the nanosuspension. Following the primary drying phase, the secondary drying phase begins. During this phase, the temperature is gradually increased to remove any residual water molecules bound to the nanoparticles. This step is critical for achieving a low final moisture content, which is essential for the long-term stability of the lyophilized product. Once the lyophilization process is complete, the containers are sealed under vacuum or inert gas to prevent moisture uptake and then stored under recommended conditions.

Solubility Measurement

The solubility measurement studies were conducted to compare the solubility of Itraconazole-loaded polymeric nanoparticles and pure Itraconazole in distilled water and phosphate buffer (pH 6.5) with 0.1% sodium lauryl sulfate (SLS). These studies provide insight into the solubility enhancement achieved through nanoparticle formulation. A known weight equivalent to 10 mg of Itraconazole (pure drug) and the prepared nanoparticles were separately introduced into 25 ml stoppered conical flasks containing the respective solvents. The samples were agitated using a mechanical shaker for 24 hours at room temperature to ensure complete interaction between the drug and the solvent. This extended agitation period was necessary to reach equilibrium solubility conditions. After 24 hours, an aliquot from each flask was carefully withdrawn and filtered to remove any undissolved particles. The filtered solutions were then diluted appropriately to fall within the linear range of the UV-visible spectrophotometer. The concentration of Itraconazole in each solution was determined by measuring the absorbance at the specific wavelength corresponding to Itraconazole. The absorbance values were compared against a calibration curve prepared from standard solutions of Itraconazole to quantify the solubility. This method allowed for the precise determination of the solubility of both the pure drug and the nanoparticle formulation in the two different solvents. The solubility enhancement provided by the nanoparticle formulation was assessed by comparing the solubility data. Increased solubility of the Itraconazole-loaded nanoparticles in both distilled water and phosphate buffer pH 6.5 with 0.1% SLS indicated successful improvement in drug solubility, which is essential for enhancing bioavailability and therapeutic efficacy. The results of these solubility measurement studies underscore the effectiveness of polymeric nanoparticles in

enhancing the solubility of poorly soluble drugs like Itraconazole, thereby supporting their potential use in advanced drug delivery systems (Torres-Flores et al., 2019, Salatin et al., 2017).

Surface Morphology: Scanning Electron Microscopy (SEM)

The surface morphology of Itraconazole-loaded polymeric nanoparticles was examined using Scanning Electron Microscopy (SEM) (Torres-Flores et al., 2019, Salatin et al., 2017). This analysis provides detailed insights into the shape, surface texture, and structural integrity of the nanoparticles, which are crucial parameters for understanding their behavior in biological systems and ensuring consistent drug delivery. To prepare the samples for SEM analysis, a small volume of the nanoparticle suspension was dropped onto an aluminum stub. The samples were then air-dried or subjected to mild vacuum drying to remove any remaining solvent without causing aggregation or morphological changes. Once dried, the samples were coated with a thin layer of gold or platinum using a sputter coater. This conductive coating is essential for preventing charging under the electron beam and for enhancing the quality of the SEM images. The coated samples were placed in the SEM chamber and observed under various magnifications.

Intestinal Permeability Studies: Ex Vivo study

Ex vivo permeation studies of Itraconazole-loaded polymeric nanoparticles were conducted using rat intestines to evaluate the drug's permeability and absorption characteristics (Nunes et al., 2016). The Institutional Animal Ethical Committee approved the study, and 12 male Wistar albino rats, each fasted for 18–20 hours, were used in the experiments. The rats were anesthetized before isolating their intestinal segments. The intestinal segments were carefully removed and washed with phosphate saline buffer (pH 7.4) containing 0.1% sodium lauryl sulfate (SLS) to clear any mucus and lumen contents. One end of each intestinal segment was tied with a suture thread, and Itraconazole-loaded polymeric nanoparticles or pure Itraconazole (equivalent to 10 mg) were injected into the lumen of the isolated segments using a syringe. The other end of the segment was then tied securely. The prepared intestinal segments were placed in a beaker containing 100 ml of phosphate saline buffer (pH 7.4) with 0.1% SLS. The buffer was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide to maintain an appropriate oxygenation level. The beaker was kept at a constant temperature of 37°C ± 2°C with continuous stirring at 100 rpm to simulate physiological conditions. Samples were collected at predetermined intervals of 15, 30, 60, 90, and 120 minutes. At each time point, an aliquot of the medium was withdrawn and immediately replaced with fresh buffer to maintain a constant volume. The collected samples were filtered to remove any particulate matter before analysis. The concentration of Itraconazole in the samples was determined using a UV-visible spectrophotometer at a wavelength of 362 nm. The cumulative amount of drug permeated through the intestinal segments was calculated and compared between the Itraconazole-loaded nanoparticles and the pure drug. This study provided crucial insights into the permeability and absorption characteristics of Itraconazole when delivered via polymeric nanoparticles. Enhanced permeability and absorption of the drug-loaded nanoparticles, as compared to the pure drug, would indicate a significant improvement in the bioavailability of Itraconazole, supporting the potential of nanoparticle-based drug delivery systems in improving therapeutic outcomes.

Stability Studies

Stability studies were conducted for the selected Itraconazole-loaded polymeric nanoparticles formulation (PNF7) following modified ICH guidelines (Narayan and Choudhary, 2017, González-González et al., 2022). These studies aimed to assess the stability of the formulation under different storage conditions over a period of one month. The PNF7 formulation was divided into three sets of samples. These sets were stored under different conditions to simulate various environmental scenarios that the formulation might encounter during storage and handling. The storage conditions were as follows:

Refrigeration: 4°C ± 2°C
Accelerated Conditions: 40°C ± 2°C / 70% ± 5% RH
To monitor the stability, the drug content and entrapment efficiency of the nanoparticles were estimated periodically. At specific intervals, samples were withdrawn from each storage condition for analysis. The drug content of the nanoparticles was determined by dissolving a known amount of the nanoparticle suspension in methanol. The resulting solution was analyzed using a UV-visible spectrophotometer at the appropriate wavelength for Itraconazole. Entrapment efficiency was assessed by separating the free drug from the nanoparticles through centrifugation. The supernatant containing the free drug was analyzed using a UV-visible spectrophotometer.

Statistical analysis

All the data are presented as mean ± SD for 3 replicates. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test as post hoc and student t test was also utilized for statical analysis here necessary. All the analysis were performed using Microsoft excel as well as GraphPad Prism software.

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy was utilized to investigate the chemical structure and potential interactions between Itraconazole, excipients, and the polymeric nanoparticles. The FT-IR spectra of the pure drug (Itraconazole), the individual excipients, and the physical mixture of the drug with excipients were recorded and analyzed. In the spectral analysis, the characteristic peaks of pure Itraconazole were identified. These peaks correspond to specific functional groups and bonds within the molecule. Key absorption bands included the C=O stretching vibration, N-H bending, and aromatic C=C stretching vibrations. These peaks served as reference points for comparing the spectra of the drug-excipient mixtures and the drug-loaded nanoparticles. The FT-IR spectra of the Itraconazole-loaded polymeric nanoparticles were then compared to the spectra of the pure drug and the physical mixture. After thorough analysis, no significant differences were observed in the characteristic peaks of Itraconazole. This indicated that the primary functional groups of the drug remained intact and there were no significant chemical interactions or modifications during the nanoparticle formulation process. The absence of significant shifts or changes in the characteristic peaks suggests that the drug was physically encapsulated within the polymer matrix rather than chemically bonded. This is a desirable outcome, as it implies that the therapeutic efficacy of Itraconazole is preserved in the nanoparticle formulation. Overall, the FT-IR analysis confirmed that the encapsulation process did not alter the chemical structure of Itraconazole, ensuring that the drug maintained its integrity and therapeutic properties within the polymeric nanoparticles. This information is crucial for validating the formulation process and ensuring the stability and effectiveness of the drug delivery system

Determination of Particle Size, Polydispersity Index, and Zeta Potential

The particle size, polydispersity index (PDI), and zeta potential of the formulations PNF7, PNF 8, PNF11, and PNF14 were determined to evaluate the physical characteristics and stability of the Itraconazole-loaded polymeric nanoparticles. The particle sizes of the formulations were measured using dynamic light scattering (DLS). The particle size of PNF7 was found to be 196.6 nm, PNF8 was 242.9 nm, PNF11 was 214.5 nm, and PNF14 was 302.6 nm. These variations in particle size can be attributed to differences in drug/polymer ratios, surfactant concentration, and the volume of the external phase used during the formulation process. The particle size directly impacts the drug release rate and bioavailability, with smaller particles typically offering faster release rates. The PDI is a measure of the dispersion homogeneity, with values ranging from 0 to 1. A PDI value close to 0 indicates a homogenous dispersion, while

values greater than 0.3 suggest high heterogeneity. The PDI values for formulations PNF7, PNF 8, PNF11, and PNF14 were 0.136, 0.169, 0.163, and 0.176, respectively. These results indicate that all four formulations had relatively homogenous dispersions, which is desirable for consistent drug delivery and stability. The zeta potential of the nanoparticles was measured to assess their surface charge and potential stability. The zeta potential values for formulations PNF7, PNF 8, PNF11, and PNF14 were -21.3 mV, -14.1 mV, -20.1 mV, and -14.8 mV, respectively. The negative zeta potential values are likely due to the presence of terminal carboxylic groups in the polymers used. High absolute values of zeta potential (either positive or negative) are essential to ensure a high energy barrier between particles, which prevents aggregation and promotes stability. Although the zeta potential values observed were moderately high, they indicate sufficient electrostatic repulsion to maintain stability in the nanoparticle formulations. In summary, the particle size, polydispersity index, and zeta potential measurements provide critical information about the physical characteristics of Itraconazole-loaded polymeric nanoparticles. Formulations PNF7, PNF 8, PNF11, and PNF14 demonstrated suitable particle sizes and relatively homogeneous dispersions, with adequate zeta potential values to ensure stability. These attributes are essential for the effective delivery and sustained release of Itraconazole, enhancing its therapeutic efficacy.

Determination of % Entrapment Efficiency

The entrapment efficiency of Itraconazole-loaded polymeric nanoparticles across different formulations (PNF1 to PNF14) was analyzed and compared. The entrapment efficiency, expressed as a percentage (Mean ± SD), provides insight into the proportion of the drug successfully encapsulated within the nanoparticles relative to the total drug used in the formulation. Formulations PNF1, PNF2, PNF3, PNF4, PNF8, and PNF9 showed relatively low entrapment efficiencies, ranging from 33.32% to 37.98%. This suggests that these formulations had lower encapsulation capabilities, possibly due to suboptimal drug-to-polymer ratios or insufficient interaction between the drug and the polymer matrix. Formulations PNF5, PNF10, PNF11, and PNF12 exhibited moderate entrapment efficiencies, with values between 37.89% and 49.77%. These formulations showed improved drug encapsulation compared to the low-efficiency group, indicating more favorable conditions for drug-polymer interaction and encapsulation. Formulations PNF6, PNF7, PNF13, and PNF14 demonstrated high entrapment efficiencies, ranging from 55.27% to 67.68%. These high values suggest optimal formulation parameters, including appropriate drug-to-polymer ratios, surfactant concentrations, and external phase volumes that facilitated maximum drug encapsulation within the nanoparticles. The entrapment efficiency varied significantly among the different formulations, indicating the critical influence of formulation parameters on the encapsulation process. With an entrapment efficiency of 67.68% ± 2.61, PNF7 emerged as one of the best formulations, suggesting that the specific conditions used in this formulation (e.g., polymer concentration, surfactant level) were highly effective in achieving maximum drug encapsulation. Higher polymer concentrations generally correlated with higher entrapment efficiencies (e.g., PNF6, PNF7, PNF13, PNF14). This trend highlights the importance of sufficient polymer availability to encapsulate the drug molecules effectively. The presence and concentration of surfactants likely played a role in stabilizing the nanoparticles and preventing drug leakage, thus enhancing entrapment efficiency. The standard deviation (SD) values indicate the consistency of the encapsulation process. Lower SD values in formulations like PNF5 and PNF14 suggest a more reproducible and reliable encapsulation process compared to those with higher SD values. The results underscore the importance of optimizing formulation parameters to achieve high entrapment efficiency. Formulations such as PNF7, with high entrapment efficiency, are preferable for drug delivery applications as they ensure a larger proportion of the drug is encapsulated, potentially enhancing therapeutic efficacy. Future studies should focus on further

optimizing these parameters and understanding the underlying mechanisms to develop robust and efficient nanoparticle-based drug delivery systems.

Table 2. Entrapment Efficiency of Itraconazole loaded polymeric nanoparticles

Sl. No.	Code for Formulation	Entrapment Efficiency as percentage (Mean ± SD)
1	PNF1	34.11±2.71
2	PNF2	33.32±2.37
3	PNF3	35.78±2.55
4	PNF4	37.98±2.74
5	PNF5	49.77±1.25
6	PNF6	61.89±2.21
7	PNF7	67.68±2.61
8	PNF8	34.99±2.87
9	PNF9	35.78±1.94
10	PNF10	37.89±1.47
11	PNF11	43.79±2.23
12	PNF12	47.93±2.82
13	PNF13	55.27±2.31
14	PNF14	63.56±1.41

***In Vitro* Drug Release Study**

The in vitro drug release study for various formulations of Itraconazole-loaded polymeric nanoparticles (PNF1 to PNF14) was conducted over a period of 12 hours. The cumulative percentage of drug release was measured at different time intervals, providing insights into the release kinetics and efficiency of each formulation. In the first hour, all formulations exhibited a relatively low percentage of drug release, ranging from approximately 4.29% to 6.66%. This initial burst release can be attributed to the release of the drug located on or near the surface of the nanoparticles. Over the 12-hour period, the cumulative drug release varied significantly among the formulations. For example, at the 12-hour mark, PNF1 (1:10) showed a release of 65%, whereas PNF7 (1:70) exhibited a lower release of 55%. Formulations with higher drug-to-polymer ratios (e.g., PNF1, PNF2, PNF3) tended to show higher cumulative release percentages compared to those with lower ratios (e.g., PNF12, PNF13, PNF14). The drug-to-polymer ratio significantly impacted the release profile. Formulations with lower polymer content (e.g., PNF1, PNF8) released the drug more quickly compared to those with higher polymer content (e.g., PNF14, PNF7). This is likely due to the lower barrier for drug diffusion in formulations with less polymer. After the initial burst release phase, most formulations entered a more steady release phase, where the rate of drug release gradually decreased. This steady phase is indicative of the controlled release from the polymer matrix. PNF7, despite having a high entrapment efficiency (67.68%), showed a slower drug release profile (55% at 12 hours) compared to other formulations like PNF1 (65% at 12 hours). This suggests that PNF7's formulation, with a higher polymer concentration, effectively controlled the release rate, potentially offering a more sustained release. The study underscores the importance of optimizing formulation parameters to achieve the desired drug release profile. Formulations like PNF7, with controlled release properties, are advantageous for sustained therapeutic effect, reducing the need for frequent dosing.

Table 3. In Vitro Drug Release Study (PNF1-PNF7)

Time (hours)	PNF1 (1:10)	PNF2 (1:20)	PNF3 (1:30)	PNF4 (1:40)	PNF5 (1:50)	PNF6 (1:60)	PNF7 (1:70)
0	0	0	0	0	0	0	0
1	6.19±0.73	5.89±0.82	5.05±0.34	5.71±0.59	4.48±0.48	4.83±0.29	4.65±0.57
2	13.66±0.95	11.08±0.65	11.74±0.68	10.76±0.77	9.8±0.45	9.06±0.31	8.98±0.39
3	18.49±0.58	17.36±0.36	16.37±0.92	15.94±0.45	14.15±0.87	13.4±0.52	12.54±0.27
4	24.21±0.62	23.04±0.47	22.88±0.53	21.12±0.31	20.28±0.68	18.42±0.48	17.74±0.36
5	29.54±0.77	28.79±0.29	27.53±0.75	26.72±0.44	25.53±0.62	23.53±0.87	22.86±0.54
6	34.81±0.69	33.08±0.83	32.45±0.28	31.95±0.91	30.48±0.41	28.7±0.55	27.53±0.63
7	40.32±0.84	38.51±0.47	37.93±0.39	36.42±0.57	35.27±0.77	33.83±0.61	31.54±0.88
8	45.68±0.59	44.61±0.29	42.57±0.66	41.27±0.48	40.95±0.53	38.33±0.47	36.39±0.52
9	50.86±0.71	49.56±0.37	47.42±0.33	46.97±0.59	45.03±0.36	43.51±0.54	41.91±0.61
10	55.78±0.49	54.1±0.63	52.05±0.55	51.43±0.43	50.92±0.68	48.15±0.79	46.41±0.73
11	61.49±0.61	59.73±0.48	57.69±0.36	56.68±0.34	55.6±0.57	53.93±0.62	51.14±0.47
12	65.29±0.33	64.21±0.77	62.14±0.41	60.68±0.52	59.91±0.38	57.02±0.49	55.32±0.64

Table 4. In Vitro Drug Release Study (PNF8-PNF14)

Time (hours)	PNF8 (1:10)	PNF9 (1:20)	PNF10 (1:30)	PNF11 (1:40)	PNF12 (1:50)	PNF13 (1:60)	PNF14 (1:70)
0	0	0	0	0	0	0	0
1	6.66±0.77	5.11±0.60	5.6±0.43	5.51±0.85	4.81±0.37	4.36±0.60	4.29±0.73
2	13.14±0.39	11.26±0.29	11.12±0.34	10.77±0.93	9.05±0.54	9.58±0.36	8.81±0.44
3	18.55±0.68	16.26±0.49	17.88±0.45	15.33±0.53	14.39±0.88	13.44±0.35	12.42±0.64
4	24.63±0.47	22.07±0.65	23.63±0.32	21±0.41	20.84±0.36	18.25±0.77	18±0.53
5	29.88±0.53	28.62±0.72	27.31±0.74	26.41±0.55	25.92±0.63	23.32±0.39	22.63±0.66
6	34.09±0.75	33.33±0.31	32.76±0.55	31.39±0.83	30.51±0.69	28.8±0.28	27.19±0.88
7	40.91±0.54	38.07±0.83	37.01±0.68	36.5±0.44	35.46±0.59	33.88±0.42	31.92±0.31

8	45.26±0.34	44.74±0.59	42.85±0.36	41.22±0.63	40.42±0.61	38.22±0.68	36.78±0.44
9	50.61±0.66	49.77±0.44	47.66±0.53	46.8±0.42	45.23±0.71	43.7±0.61	41.16±0.53
10	55.48±0.48	54.9±0.29	52.29±0.47	51.24±0.33	50.25±0.53	48.24±0.39	46.55±0.62
11	61.46±0.77	59.1±0.39	57.04±0.68	56.27±0.55	55.7±0.35	53.2±0.72	51.26±0.47
12	65.24±0.41	64.09±0.54	62.26±0.55	60.94±0.36	59.28±0.61	57.9±0.43	55.95±0.68

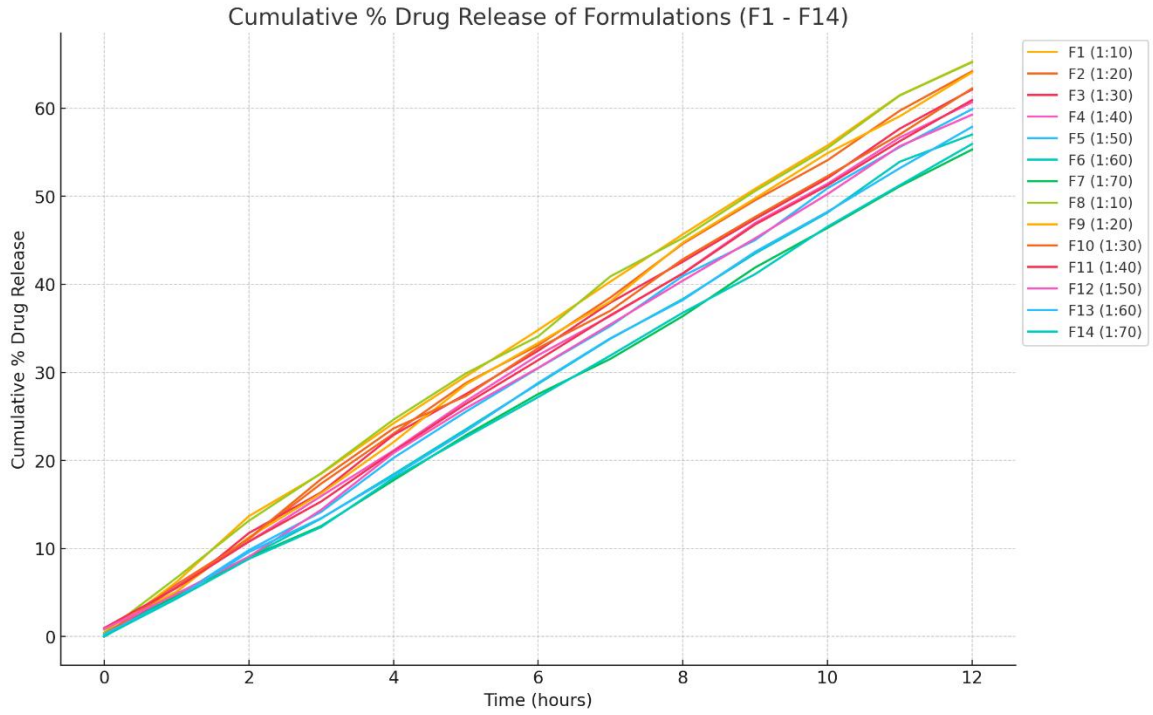

Figure 1. In Vitro Drug Release Study (PNF1-PNF14)

Drug Release Kinetics

The drug release kinetics of various Itraconazole-loaded polymeric nanoparticle formulations (PNF1 to PNF14) were evaluated using five mathematical models: Zero-order kinetics, First-order kinetics, Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model. The correlation coefficients (R^2) and kinetic constants provide insights into the drug release mechanisms and the efficiency of each formulation. The R^2 values for zero-order kinetics were high across all formulations, ranging from 0.9801 to 0.9911. This suggests a consistent drug release rate over time, independent of concentration, which is ideal for maintaining steady therapeutic levels. Formulations PNF2 (0.9911) and PNF1 (0.9891) exhibited particularly high R^2 values, indicating a strong fit to zero-order kinetics and suggesting these formulations can maintain a consistent drug release profile. The first-order kinetics analysis revealed R^2 values between 0.9762 and 0.9912, indicating that in some formulations, the drug release rate is proportional to the remaining drug concentration. Formulation PNF7 had the highest R^2 value (0.9912), suggesting a decrease in release rate as the drug concentration diminishes, which is typical for formulations with less controlled release characteristics The Higuchi model, which describes drug release as a diffusion process based on Fick's law, showed high R^2 values (0.9824 to 0.9904). This indicates that diffusion is a significant mechanism in the drug release

process. Formulations PNF5 (0.9904) and PNF3 (0.9894) showed the highest R^2 values, suggesting that these formulations provide a predictable release pattern primarily driven by diffusion. The R^2 values for the Korsmeyer-Peppas model ranged from 0.9931 to 0.9981, with n values indicating the mechanism of drug release. Most formulations had n values between 0.64 and 0.70, suggesting a combination of diffusion and erosion mechanisms. Formulation PNF2 (n = 0.6771) and PNF7 (n = 0.6481) demonstrated a strong fit, implying controlled release characteristics with both diffusion and polymer erosion playing roles. The Hixson-Crowell model, which considers changes in surface area and diameter of particles, had R^2 values ranging from 0.9886 to 0.9946. This suggests that drug release involves a reduction in particle size. Formulations PNF7 and PNF14 showed high correlation coefficients (0.9946), indicating a significant influence of particle erosion on drug release. The kinetic analysis indicates that different formulations exhibit varied drug release mechanisms. Formulations like PNF2, PNF7, and PNF14 showed excellent fit across multiple models, highlighting their potential for controlled and sustained drug release. High R^2 values in the zero-order and Higuchi models suggest that these formulations can achieve a consistent and predictable release, which is advantageous for maintaining therapeutic drug levels over an extended period. Formulations PNF7 and PNF14, with high fits to both the Korsmeyer-Peppas and Hixson-Crowell models, demonstrate that their drug release is influenced by both diffusion and erosion mechanisms. This complex release behavior is desirable for achieving a prolonged therapeutic effect with reduced dosing frequency. The kinetic analysis supports the potential of Itraconazole-loaded polymeric nanoparticles for providing controlled and sustained drug release. Formulations such as PNF7 and PNF14, which show a combination of diffusion and erosion mechanisms, are particularly promising for achieving extended drug release and improving therapeutic efficacy and patient compliance.

Table 5. Drug Release Kinetics

Formulation Code	Zero order Kinetics		First order Kinetics		Higuchi Model		Korsmeyer-Peppas model		Hixson Crowell	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H h^{-1}	R^2	n value	R^2	K_{HC} $h^{-1/3}$
PNF1	0.9891	5.593	0.9822	-0.0409	0.9834	24.559	0.9961	0.7021	0.9926	-0.1202
PNF2	0.9911	5.286	0.9842	-0.0369	0.9824	23.249	0.9981	0.6771	0.9946	-0.1092
PNF3	0.9831	5.085	0.9882	-0.0339	0.9894	22.509	0.9971	0.6811	0.9936	-0.1052
PNF4	0.9851	4.9	0.9872	-0.0319	0.9864	21.689	0.9961	0.6641	0.9936	-0.0992
PNF5	0.9801	4.724	0.9902	-0.0299	0.9904	21.019	0.9971	0.6621	0.9936	-0.0942
PNF6	0.9861	4.486	0.9872	-0.0279	0.9824	20.019	0.9931	0.6741	0.9916	-0.0872

PNF7	0.9851	4.193	0.9912	-0.0249	0.9844	18.789	0.9971	0.6481	0.9946	-0.0792
PNF8	0.9831	5.569	0.9762	-0.0419	0.9844	24.379	0.9971	0.6471	0.9886	-0.1222
PNF9	0.9851	5.45	0.9812	-0.0399	0.9854	23.919	0.9981	0.6591	0.9916	-0.1172
PNF10	0.9871	5.168	0.9842	-0.0359	0.9834	22.739	0.9971	0.6501	0.9926	-0.1082
PNF11	0.9861	4.999	0.9852	-0.0339	0.9844	22.079	0.9981	0.6571	0.9926	-0.1022
PNF12	0.9871	4.749	0.9852	-0.0299	0.9824	21.079	0.9971	0.6801	0.9916	-0.0942
PNF13	0.9871	4.599	0.9882	-0.0289	0.9834	20.469	0.9971	0.6741	0.9936	-0.0902
PNF14	0.9871	4.338	0.9912	-0.0259	0.9834	19.379	0.9971	0.6581	0.9946	-0.0832

Solubility studies

The solubility studies were conducted to compare the solubility of pure Itraconazole and the Itraconazole-loaded polymeric nanoparticle formulation PNF7 in distilled water and phosphate buffer (pH 6.5) with 0.1% sodium lauryl sulfate (SLS) over a period of 4 hours. The results, expressed in µg/ml, provide insight into the effectiveness of nanoparticle formulation in enhancing drug solubility. The solubility of pure Itraconazole in distilled water was found to be 33.78 ± 2.723 µg/ml. This low solubility is characteristic of Itraconazole, a BCS class II drug known for its poor water solubility. The solubility of Itraconazole in the PNF7 formulation was significantly higher, measured at 12189 ± 3186 µg/ml. This drastic increase indicates that the nanoparticle formulation dramatically enhances the solubility of Itraconazole in distilled water. The high standard deviation, however, suggests some variability in the measurements, which may need further optimization for consistency. In the phosphate buffer with 0.1% SLS, the solubility of pure Itraconazole improved to 47.68 ± 2.114 µg/ml. The presence of SLS, a surfactant, aids in increasing the solubility of hydrophobic drugs like Itraconazole by reducing surface tension and improving wetting. The solubility of Itraconazole in the PNF7 formulation in phosphate buffer was 264.87 ± 3.783 µg/ml. Although this is lower than the solubility in distilled water, it is still significantly higher than that of the pure drug. The formulation's effectiveness in enhancing solubility is evident, with a more consistent solubility value and lower standard deviation compared to its performance in distilled water. The solubility studies highlight the significant impact of nanoparticle formulation on the solubility of Itraconazole. The PNF7 formulation increased the solubility of Itraconazole by several orders of magnitude in both distilled water and phosphate buffer with SLS. This enhancement can be attributed to the increased surface area of the nanoparticles and the presence of the polymer matrix, which helps to solubilize the drug more effectively. The improvement in solubility is crucial for enhancing the bioavailability of Itraconazole, as higher solubility can lead to better absorption

in the gastrointestinal tract. The solubility in phosphate buffer with SLS is particularly relevant for simulating intestinal conditions, indicating that the PNF7 formulation could improve the drug's bioavailability in vivo. However, the variability in solubility measurements in distilled water suggests that further optimization of the nanoparticle formulation process is needed to achieve more consistent results. This could involve refining the preparation methods or adjusting the polymer-to-drug ratio to ensure uniformity. The nanoparticle formulation PNF7 significantly enhances the solubility of Itraconazole, addressing one of the major challenges associated with its bioavailability.

Table 6. Solubility studies

Time (Hrs)	**4 hrs**	
Solvent Used	Distilled water	Phosphate buffer pH 6.5 with 0.1% SLS
Solubility (µg/ml) - Pure Drug	33.78±2.723	47.68±2.114
Solubility (µg/ml) - Formulation PNF7	12189±3186	264.87±3.783

Surface Morphology: Scanning Electron Microscopy (SEM)

The SEM Photograph of the selected best formulation PNF7 Eudragit L 100 with 1% Pluronic F68 were shown in Figure 2. The results indicated that the formulated nanoparticles revealed almost spherical in shape with relative smooth surface**.**

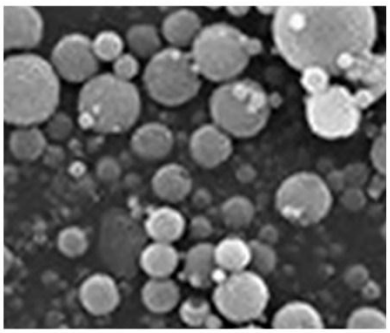

Figure 2. SEM photograph of the PNF7

Intestinal Permeability Studies: Ex Vivo study

Ex vivo intestinal permeability studies were conducted to compare the permeability of pure Itraconazole and the Itraconazole-loaded polymeric nanoparticle formulation PNF7 through different segments of the small intestine (duodenum, jejunum, and ileum). The cumulative amount of drug permeated (mg) was measured, providing insights into the effectiveness of the nanoparticle formulation in enhancing drug absorption. The cumulative amount of pure Itraconazole permeated through the duodenum was 0.3253 ± 0.002 mg. The cumulative amount of Itraconazole permeated from the PNF7 formulation was significantly higher at 0.9042 ± 0.003 mg. The cumulative amount of pure Itraconazole permeated through the jejunum was 0.3401 ± 0.003 mg. The cumulative amount of Itraconazole permeated from the PNF7 formulation was 0.9153 ± 0.011 mg. The cumulative amount of pure Itraconazole permeated through the ileum was 0.3210 ± 0.012 mg. The cumulative amount of Itraconazole permeated from the PNF7 formulation was 0.9131 ± 0.002 mg. Across all segments of the small intestine, the Itraconazole-loaded nanoparticle formulation PNF7 exhibited significantly higher cumulative drug permeation compared to the pure drug. This enhanced permeability is indicative of the improved absorption potential of the nanoparticle formulation. The substantial

increase in permeation with PNF7 can be attributed to several factors, including the increased surface area of the nanoparticles, improved solubility, and potentially enhanced interaction with the intestinal mucosa. While the pure drug showed marginal differences in permeability among the different intestinal segments, the PNF7 formulation demonstrated consistently high permeability across all segments. The jejunum, known for its extensive surface area and rich blood supply, exhibited the highest permeability for both the pure drug and the nanoparticle formulation. This suggests that the jejunum might be the most efficient site for drug absorption when using nanoparticle formulations. The significantly higher permeability of the PNF7 formulation underscores the potential of nanoparticle-based delivery systems to overcome the limitations of poorly permeable drugs like Itraconazole. Enhanced permeability can lead to better bioavailability, reducing the required dosage and improving therapeutic efficacy. This is particularly beneficial for drugs with poor water solubility and limited intestinal absorption. The ex vivo intestinal permeability studies highlight the substantial improvement in drug permeation achieved with the Itraconazole-loaded nanoparticle formulation PNF7 compared to the pure drug. This enhancement is consistent across different segments of the small intestine, indicating that nanoparticle formulations can effectively improve drug absorption throughout the gastrointestinal tract. These findings support the use of nanoparticle-based delivery systems for enhancing the bioavailability and therapeutic effectiveness of Itraconazole and similar drugs. Further in vivo studies are warranted to validate these results and explore the clinical benefits of this advanced drug delivery approach.

Table 7: Ex vivo intestinal permeability studies

Small Intestinal Segments	**Cumulative Amount of Drug Permeated (mg)**	
	Pure Drug	**Formulation PNPNF7**
Duodenum	0.3253±0.002	0.9042±0.003
Jejunum	0.3401±0.003	0.9153±0.011
Ileum	0.3210±0.012	0.9131±0.002

Stability Studies

The stability studies of the optimized Itraconazole-loaded polymeric nanoparticle formulation (PNF7) were conducted over one month under two different storage conditions: 4°C and 40°C/70% RH. At 4°C: The drug content decreased slightly from 92.51% ± 0.10 to 90.01% ± 0.05, indicating good stability. At 40°C/70% RH, the drug content showed a more significant decrease to 87.88% ± 1.11, suggesting that higher temperatures and humidity levels impact the stability of the drug. At 4°C, entrapment efficiency showed a slight decrease from 62.82% ± 1.34 to 60.79% ± 1.07. At 40°C/70% RH, entrapment efficiency decreased more notably to 58.81% ± 1.92, indicating that the encapsulation integrity is compromised at higher temperatures and humidity. Formulation PNF7 maintains better stability at 4°C compared to 40°C/70% RH. Lower temperatures preserve drug content and entrapment efficiency, whereas higher temperatures and humidity accelerate degradation and reduce encapsulation efficiency.

Table 8. Stability studies of optimized formulation (PNF7)

Evaluation Parameter	**Storage Temperature**	**0 Day**	**1 Month**
% Drug content	4 ^{0}C	92.51±0.10	90.01±0.05
	40 ^{0}C /70%RH	92.51±0.10	87.88±1.11
% Entrapment efficiency	4 ^{0}C	62.82±1.34	60.79±1.07
	40 ^{0}C /70%RH	62.82±1.34	58.81±1.92

Comparison between Cumulative % drug release of pure drug and PNF7

The PNF7 formulation offers a marked improvement in the cumulative drug release of Itraconazole, indicating its potential for enhanced bioavailability and sustained therapeutic action compared to pure drug (Figure 5). This controlled release system could lead to more effective and patient-friendly Itraconazole therapy.

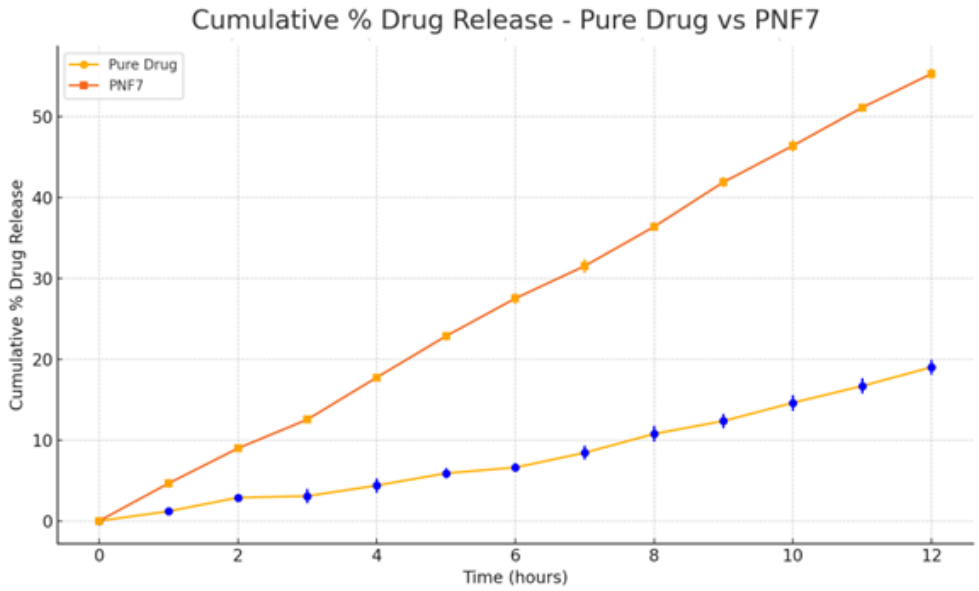

Figure 3. A comparative analysis indicating improvement in Cumulative % drug release of PNF7

CONCLUSION

The conclusions of this present study highlighted the possibility of using polymeric nanoparticles as an efficient drug delivery method for the drug itraconazole, which has low permeability and solubility. Therapeutic efficacy was ensured by the drug's chemical structure remaining unaltered during the encapsulation process, as demonstrated by the FT-IR study. Evaluations of the nanoparticles' zeta potential and particle size demonstrated their stability and ability to provide a regulated release. Formulations such as PNF7 were found to be ideal due to their favourable release kinetics and high entrapment efficiencies. This improved the drug's bioavailability and potential for therapeutic use. The formulation's efficacy in clinical applications is supported by the solubility improvements seen in both simulated intestinal fluids and aqueous solutions. *Ex vivo* investigations provided additional evidence for the formulation's remarkable capacity to enhance drug absorption across the intestinal barrier, indicating enhanced bioavailability *in vivo*. The significance of suitable storage conditions in preserving the integrity of the formulation was brought to light by stability studies. In conclusion, these nanoparticle systems offered a promising method for improving the efficacy and distribution of bioactive substances like itraconazole, which may change treatment paradigms by improvising the drug release and bioavailability.

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