RESEARCH PAPER

Development and ex-Vivo Evaluation of a Polycaprolactone Nanoparticle-Based Gel for Transdermal Delivery of Itraconazole

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Abstract

Itraconazole (ITZ) is a potent broad-spectrum antifungal agent whose clinical utility is severely limited by its poor aqueous solubility (BCS Class II) and consequent low oral bioavailability and inadequate topical skin penetration. To overcome these challenges, this study developed and evaluated a novel transdermal delivery system for ITZ based on polymeric nanoparticles. Itraconazole-loaded polycaprolactone nanoparticles (ITZ-PCL-NPs) were synthesized using a nanoprecipitation method, optimized via a Design of Experiments (DoE) approach, and subsequently incorporated into a Carbopol 934P hydrogel. The optimized nanoparticles (Formulation F2) exhibited a spherical morphology, a mean particle size of 154.6 ± 3.2 nm, a high encapsulation efficiency of 88.4 ± 2.1%, and successfully converted the drug to an amorphous state, as confirmed by DSC and XRD. The resulting ITZ-PCL-NP gel demonstrated a sustained, pH-dependent *in vitro* release profile (94.3% at pH 7.4 vs. 80.4% at pH 5.5 over 24 hours), fitting the Higuchi diffusion model. Crucially, ex vivo skin permeation studies on rat skin showed a 2.32-fold enhancement in steady-state flux (7.22 ± 0.45 µg/cm²/h) compared to a plain ITZ gel. The formulation was non-irritating in the HET-CAM assay and exhibited good physical and chemical stability over three months. In conclusion, the ITZ-PCL-NP gel represents a promising, effective, and safe nanocarrier-based strategy for the enhanced transdermal delivery of itraconazole, addressing its key biopharmaceutical limitations for potential localized therapy of deep-seated fungal infections.

Keywords: Itraconazole; Polycaprolactone Nanoparticles; Transdermal Delivery; Nanocarriers; Antifungal; Skin Permeation

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1. Introduction

Itraconazole (ITZ) is a cornerstone of modern systemic antifungal therapy, belonging to the triazole class[1]. Its mechanism of action involves the selective inhibition of the fungal cytochrome P450 enzyme lanosterol 14α-demethylase, a critical component in the biosynthesis of ergosterol. Ergosterol is the principal sterol in fungal cell membranes, analogous to cholesterol in mammalian cells, and is essential for maintaining membrane integrity, fluidity, and the function of membrane-bound enzymes. By depleting ergosterol and causing the accumulation of toxic methylated sterol precursors, ITZ disrupts membrane structure and function, leading to the inhibition of fungal cell growth and replication[2]. This mechanism confers upon ITZ a potent and broad spectrum of activity against a wide array of pathogenic fungi, including dermatophytes (e.g., *Trichophyton*, *Microsporum*, *Epidermophyton*),

yeasts (e.g., *Candida* spp., including some non-*albicans* species), and molds (e.g., *Aspergillus* spp.)[3]. This breadth makes it a first-line agent for severe or recalcitrant superficial fungal infections like onychomycosis (fungal nail infections) and tinea corporis/cruris/pedis, as well as for systemic mycoses[4-6].

However, the formidable clinical utility of ITZ is fundamentally constrained by its suboptimal physicochemical properties, which place it firmly in Biopharmaceutics Classification System (BCS) Class II[7]. ITZ is a highly lipophilic, weak base with extremely poor aqueous solubility (<1 ng/mL at neutral pH). It is also a high-molecular-weight compound. This combination creates a perfect storm of biopharmaceutical challenges. For oral administration, the primary route for systemic therapy, the drug's dissolution in the gastrointestinal fluids is the rate-limiting step for absorption[8]. This results in highly variable and often inadequate oral bioavailability, typically around 55% under fasting conditions and only moderately improved when taken with food or an acidic beverage, which is required for optimal absorption[9]. This variability leads to unpredictable therapeutic plasma levels, increasing the risk of treatment failure or, conversely, dose-dependent toxicity. Furthermore, oral ITZ is metabolized extensively by the liver cytochrome P450 system (primarily CYP3A4), leading to significant first-pass metabolism and numerous potential drug-drug interactions with other substrates, inhibitors, or inducers of this enzyme system, complicating its use in polypharmacy patients[10]. Common adverse effects associated with systemic ITZ include gastrointestinal disturbances, headache, and more concerningly, dose-related negative inotropic effects and the potential for hepatotoxicity, necessitating therapeutic drug monitoring in certain clinical scenarios[11].

These systemic drawbacks make a strong case for localized, topical therapy for superficial fungal infections, aiming to deliver high drug concentrations directly to the site of pathology while minimizing systemic exposure and its attendant risks. However, the transdermal or topical delivery of ITZ is equally, if not more, challenging[12]. The skin, the body's largest organ, has evolved as a formidable barrier primarily through the stratum corneum, the outermost layer composed of corneocytes embedded in a lipid-rich matrix. This "brick-and-mortar" structure is exceptionally effective at preventing the ingress of foreign substances, including drugs. The physicochemical profile of ITZ—high lipophilicity and molecular weight—paradoxically works against it in this context. While some lipophilicity is needed to partition into the stratum corneum lipids, excessive hydrophobicity prevents the drug from diffusing through the more aqueous viable epidermis and dermis to reach the deeper fungal elements in nails or hair follicles. This results in extremely low skin and nail permeability, rendering conventional topical formulations (simple solutions, creams, or ointments containing crystalline ITZ) clinically ineffective for deep-seated infections. They may provide some superficial relief but fail to achieve fungicidal concentrations at the site of infection, leading to high recurrence rates and patient frustration[13].

Therefore, the central paradox of ITZ therapy is clear: a potent, broad-spectrum antifungal agent is rendered therapeutically inefficient by its own inherent physicochemical properties, whether given systemically with variable bioavailability and systemic side effects, or topically with insufficient penetration. This gap between pharmacological potential and practical delivery underscores an urgent, unmet clinical need. The challenge is not to discover a new drug, but to re-engineer the delivery of the existing drug—to develop a formulation strategy that can overcome the twin barriers of poor solubility and low permeability, thereby unlocking the full therapeutic potential of ITZ for localized fungal disease.

1.2. Transdermal Delivery and Nanocarrier Strategies

The transdermal route of drug administration offers a compelling alternative that can potentially circumvent the limitations of both oral and conventional topical delivery for drugs like ITZ. Transdermal delivery involves the application of a drug formulation onto intact skin, with the goal of achieving local therapeutic effects in underlying tissue[14][15]es (topical action) or, for some drugs, systemic absorption through the skin's microcirculation. For fungal infections of the skin, nails, and subcutaneous tissues, the goal is primarily local and regional delivery. The advantages of this paradigm are multifold[16][17]. First and foremost, it

enables localized and targeted therapy, allowing for the application of high drug concentrations directly to the infected site. This maximizes the therapeutic index by creating a steep concentration gradient at the target tissue while minimizing systemic drug levels, thereby drastically reducing or eliminating systemic side effects and drug-drug interactions. Second, it bypasses gastrointestinal and hepatic first-pass metabolism, leading to more predictable and efficient delivery of the active moiety. Third, it offers the potential for sustained and controlled release from a depot in the skin layers, which could allow for less frequent application (e.g., once daily) compared to conventional creams, improving patient adherence—a critical factor in the long-term treatment of chronic conditions like onychomycosis, which requires months of therapy. Finally, it provides a non-invasive and patient-friendly mode of administration, which is particularly valuable for long-term treatment regimens.

However, the formidable barrier function of the stratum corneum means that very few drug molecules possess the ideal combination of low molecular weight (<500 Da), adequate lipophilicity (Log P 1-3), and potency to passively diffuse through the skin in therapeutic amounts. ITZ, with its high molecular weight and extreme hydrophobicity, is not a natural candidate for passive transdermal delivery. This has led to the exploration of various physical and chemical enhancement strategies. Physical methods, such as iontophoresis, sonophoresis, or microneedles, actively disrupt the stratum corneum but often involve complex, costly devices unsuitable for routine home use. Chemical penetration enhancers (e.g., alcohols, fatty acids, surfactants) are more common but can cause skin irritation, erythema, or dryness with prolonged use, and their enhancement effect on highly insoluble drugs like ITZ is often limited.

Conventional formulation approaches have attempted to tackle ITZ's solubility issue. For instance, cyclodextrin complexation has been used to create water-soluble inclusion complexes. While this improves dissolution and oral bioavailability, its utility in topical/transdermal delivery is less straightforward. The hydrophilic cyclodextrin complex may not efficiently partition into the lipophilic stratum corneum, and the complexation equilibrium may not favor drug release at the skin surface. Furthermore, some cyclodextrins can themselves cause mild mucosal irritation at higher concentrations. Other approaches, like co-solvent systems or microemulsions, can improve solubility but may lack the controlled release and stability profile desired for a long-term treatment.

This is where nanocarrier-based strategies have emerged as a transformative solution, capable of simultaneously addressing the dual challenges of solubility and permeability. Nanoparticles are submicron-sized (typically 1-1000 nm) particulate carriers that can be engineered from various biodegradable materials. When designed for transdermal delivery, they act not necessarily as "Trojan horses" that permeate intact, but as sophisticated drug depots and permeation facilitators on and within the stratum corneum. Several key mechanisms underpin their enhancement effect. First, they can encapsulate a hydrophobic drug like ITZ in their core, dramatically increasing its apparent solubility and thermodynamic activity in the formulation. This creates a high-concentration gradient at the skin surface, the primary driving force for diffusion. Second, the nanoscale size provides an enormous surface area for interaction with the skin. Nanoparticles can adhere to and potentially fuse with the skin's lipid bilayers, or they may accumulate in and partially disrupt the organized lipid matrix of the stratum corneum, creating transient, nano-sized pathways for drug diffusion. Third, they can be designed for controlled and sustained release, ensuring a continuous supply of drug to the skin surface over an extended period, maintaining the concentration gradient. Fourth, some nanoparticles, especially those made from or coated with certain surfactants or lipids, can interact with skin components to fluidize lipids or extract them, further enhancing permeability in a reversible manner. Finally, for infections involving skin appendages like hair follicles, nanoparticles of appropriate size can be preferentially deposited in the follicular openings, acting as long-term reservoirs for drug delivery to these deep-seated sites[19]. Among the plethora of nanocarriers investigated—liposomes, niosomes, solid lipid nanoparticles, nanostructured lipid carriers—polymeric nanoparticles stand out for their robustness, stability, and highly tunable properties. By selecting an appropriate biodegradable polymer, one can precisely

engineer the drug loading capacity, release kinetics, and interaction with biological membranes[20-23]. For the delivery of a challenging molecule like Itraconazole, a nanocarrier system must not only solubilize the drug and enhance skin permeation but also do so in a stable, biocompatible, and pharmaceutically elegant formulation suitable for patient use[24] . This forms the foundational rationale for the development of an advanced, nanoparticle-based transdermal gel[24][25].

Materials and Methods

The experimental work was conducted using pharmaceutical-grade materials and analytical reagents to ensure reproducibility and compliance with standard protocols. The active pharmaceutical ingredient, Itraconazole (ITZ), was obtained as a USP grade powder (purity ≥98%) from a certified supplier. The biodegradable polymer selected for nanoparticle synthesis was Polycaprolactone (PCL) with an average molecular weight (Mn) of 14,000 to 20,000 Da. PCL was chosen for its hydrophobic nature, excellent biocompatibility, and controlled degradation profile. To stabilize the nanoparticles in the aqueous phase, Poloxamer 407 (Pluronic® F127) was used, a non-ionic triblock copolymer (PEO-PPO-PEO) known for its surfactant and potential permeation-enhancing properties.For the preparation of the final topical dosage form, Carbopol 934P was selected as the gelling agent due to its high viscosity, good bioadhesive properties, and ability to form clear gels upon neutralization. Triethanolamine (TEA) was used as the neutralizing agent for Carbopol. The primary organic solvent for dissolving the drug and polymer was Dimethyl Sulfoxide (DMSO), selected for its high solubilizing power for both ITZ and PCL. Other solvents and reagents included acetone and methanol (HPLC grade) for analytical purposes and washing steps. For buffer preparation in release studies, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, and sodium chloride of analytical grade were used to prepare Phosphate Buffered Saline (PBS) at pH 5.5 (to simulate the slightly acidic skin surface) and pH 7.4 (to simulate physiological pH). High-purity deionized water was used throughout the experiments. For the ex vivo skin permeation study, full-thickness abdominal skin from male Wistar rats (approved by the Institutional Animal Ethics Committee) was used, excised post-euthanasia, and carefully dermatomed to a thickness of approximately 300-400 µm, with the subcutaneous fat removed. The skin was stored at -20°C and used within two weeks, thawed at 4°C before the experiment.

Preparation of PCL Nanoparticles

The synthesis of Itraconazole-loaded Polycaprolactone nanoparticles (ITZ-PCL-NPs) was carried out using a modified nanoprecipitation (solvent displacement) method, a technique favored for its simplicity, reproducibility, and ability to produce nanoparticles with narrow size distribution.

Formulation Design

A systematic Design of Experiments (DoE) approach was employed to optimize the formulation. Ten distinct formulations (coded F1 to F10) were prepared by varying two critical independent variables: the concentration of the polymer (PCL, ranging from 20 mg to 100 mg) and the concentration of the stabilizer (Poloxamer 407, ranging from 0.5% to 3.0% w/v in the aqueous phase). The amount of Itraconazole was kept constant (10 mg) across all batches to maintain a consistent drug-to-polymer ratio for initial screening. The dependent variables measured for optimization included particle size, polydispersity index (PDI), zeta potential, and encapsulation efficiency (EE%).

Synthesis Process

The synthesis followed a meticulous stepwise protocol. First, the organic phase was prepared by dissolving a precisely weighed quantity of PCL (according to the DoE table) and 10 mg of ITZ in 10 mL of DMSO under magnetic stirring at 40°C until a clear, homogeneous solution was obtained. Separately, the aqueous phase was prepared by dissolving a specified amount of Poloxamer 407 in 50 mL of cold deionized water (4°C) under gentle stirring. The core nanoprecipitation step involved the dropwise addition (using a syringe pump at a rate of 1 mL/min) of the organic phase into the vigorously stirring (magnetic stirrer at 1000 rpm) aqueous phase at room temperature. The use of a cold aqueous phase and controlled injection rate is critical for inducing instantaneous diffusion of the organic solvent (DMSO) into the water, leading to the supersaturation and precipitation of the polymer, which entraps the drug molecules, forming nanoparticles. Following complete

addition, the nanoparticle suspension was stirred for an additional 3 hours at room temperature to allow for the complete evaporation of the organic solvent and the stabilization of the nanoparticle structure. The resulting milky colloidal suspension was then subjected to ultracentrifugation at 35,000 rpm at 4°C for 30 minutes to separate the solid nanoparticles from the aqueous medium containing unentrapped drug and excess stabilizer. The supernatant was carefully collected for analysis of free drug (to calculate EE%), and the nanoparticle pellet was washed twice with deionized water to remove loosely adsorbed Poloxamer and drug. Finally, the purified nanoparticles were either redispersed in a small volume of water for immediate characterization or lyophilized (freeze-dried) for 48 hours using 5% w/v mannitol as a cryoprotectant to obtain a free-flowing powder for long-term storage and subsequent gel incorporation.

Formulation of Topical Gel

To transform the nanoparticle suspension into a patient-applicable dosage form with suitable rheology and bioadhesion, it was incorporated into a hydrogel base. A 1.0% w/w Carbopol 934P gel was prepared as follows: Carbopol powder was slowly sprinkled onto the surface of deionized water under gentle magnetic stirring (500 rpm) to avoid lump formation. The dispersion was then allowed to hydrate overnight at room temperature to form a clear, viscous mucilage. Separately, the optimized batch of ITZ-PCL-NPs (either as a concentrated aqueous dispersion or redispersed from lyophilized powder) was homogenized using a vortex mixer. This nanoparticle dispersion was then slowly incorporated into the Carbopol mucilage under continuous, low-shear stirring to ensure uniform distribution without damaging the nanoparticles or introducing air bubbles. The pH of the mixture was then adjusted to 6.5 - 7.0 using triethanolamine (TEA). The neutralization of Carbopol's carboxylic acid groups by TEA induces ionic repulsion between polymer chains, leading to uncoiling, swelling, and the formation of a robust, transparent gel. The final gel contained nanoparticles equivalent to 1% w/w Itraconazole. For comparative studies, a control plain ITZ gel was prepared by directly dispersing micronized ITZ crystals in the Carbopol gel base using a homogenizer, and a placebo nanoparticle gel (containing blank PCL-NPs without drug) was also formulated.

Characterization of Nanoparticles and Gel A comprehensive analytical characterization was performed to ensure the formulation met the required standards for an effective transdermal delivery system.

Physicochemical Characterization

Particle Size, PDI, and Zeta Potential: The mean hydrodynamic particle diameter (Z-average), Polydispersity Index (PDI, a measure of size distribution homogeneity), and Zeta Potential (surface charge) of the nanoparticle dispersions were determined using Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering on a Malvern Zetasizer Nano ZS. Measurements were performed in triplicate after appropriate dilution with filtered deionized water. A PDI value below 0.3 indicates a monodisperse population, while a zeta potential magnitude greater than ±20 mV typically suggests good electrostatic stability against aggregation. Morphology (SEM): The surface morphology and shape of the nanoparticles were visualized using Scanning Electron Microscopy (SEM). A drop of diluted nanoparticle dispersion was placed on an aluminum stub, air-dried, and then sputter-coated with a thin layer of gold to make the sample conductive. Images were taken at an accelerating voltage of 15-20 kV under high vacuum. SEM analysis confirmed the spherical shape and provided insight into surface texture and the absence of aggregation in the dry state.

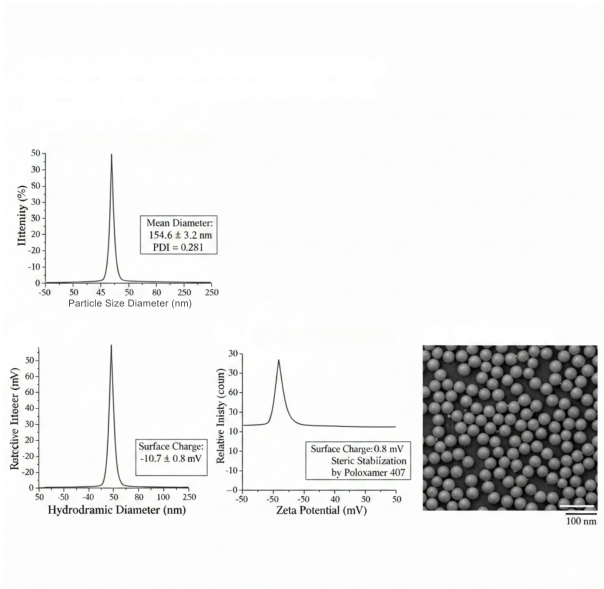

Fig: 1 Characterization of optimized Itraconazole-loaded Polycaprolactones Nanoparaites (ITZ-PCL-NPs, Formulation F2)

Drug-Polymer Compatibility and State

Fourier-Transform Infrared Spectroscopy (FTIR): FTIR spectra of pure ITZ, pure PCL, Poloxamer 407, physical mixtures, and the lyophilized ITZ-PCL-NPs were recorded using the KBr pellet method over a range of 4000-400 cm^{-1}. The objective was to identify any potential chemical interactions (e.g., hydrogen bonding, covalent bond formation) between the drug and the excipients by observing the appearance of new peaks or significant shifts in characteristic functional group peaks (e.g., C=O, N-H, C-N of ITZ; C=O of PCL).

Differential Scanning Calorimetry (DSC): DSC thermograms were obtained by heating 2-5 mg samples in sealed aluminum pans from 25°C to 300°C at a constant rate of 10°C/min under a nitrogen purge. The sharp endothermic melting peak of crystalline ITZ (~168°C) in its pure form was monitored. In the thermogram of the drug-loaded nanoparticles, the absence, significant reduction, or broadening of this peak would indicate the conversion of the drug from a crystalline to an amorphous or molecularly dispersed state within the polymer matrix—a key factor for enhanced solubility and dissolution.

X-Ray Diffractometry (XRD): Powder X-ray diffraction patterns were recorded for pure ITZ, PCL, physical mixture, and lyophilized nanoparticles using a diffractometer with Cu Kα radiation over a 2θ range of 5° to 50°. The distinctive sharp crystalline peaks of ITZ were compared to the pattern of the nanoparticles. The disappearance or dramatic reduction in intensity of these peaks in the nanoparticle pattern would provide strong corroborative evidence for the amorphization of the drug.

Encapsulation Efficiency and Drug Loading

The Encapsulation Efficiency (EE%) and Drug Loading (DL%) were determined indirectly. After the synthesis and ultracentrifugation, the amount of unentrapped (free) ITZ in the collected supernatant was quantified using a validated High-Performance Liquid Chromatography (HPLC) method or UV-Vis spectrophotometry at a λmax of 262 nm. A calibration curve of ITZ in the appropriate medium was constructed. EE% and DL% were calculated using the following formulas: EE (%) = (Total Drug Added – Free Drug in Supernatant) / (Total Drug Added) × 100 DL (%) = (Weight of Drug in Nanoparticles) / (Total Weight of Drug-Loaded Nanoparticles) × 100

In Vitro Drug Release Studies

The release profile of ITZ from the nanoparticle gel was evaluated using a Franz diffusion cell apparatus. The donor compartment was filled with a measured quantity (approx. 1 g) of the ITZ-PCL-NP gel. A semi-permeable synthetic membrane (e.g., cellulose acetate or dialysis membrane with a molecular weight cut-off of 12-14 kDa) was mounted between the donor and receptor compartments. The receptor compartment was filled with 20 mL of degassed PBS, maintained at 37 ± 0.5°C by a circulating water jacket, and stirred continuously at 600 rpm to ensure sink conditions. Sink conditions were verified and maintained by ensuring the receptor volume was at least 5-10 times the saturation solubility volume of ITZ. Aliquots (1 mL) were withdrawn from the receptor medium at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours) and immediately replaced with an equal volume of fresh, pre-warmed buffer. The concentration of ITZ in the samples was analyzed by HPLC. Cumulative drug release (%) was plotted against time. The release data were fitted to various mathematical models (Zero-order, First-order, Higuchi, and Korsmeyer-Peppas) to elucidate the underlying release mechanism (e.g., diffusion, erosion, swelling).

Development and ex-Vivo Evaluation of a Polycaprolactone Nanoparticle-Based Gel for Transdermal Delivery of Itraconazole

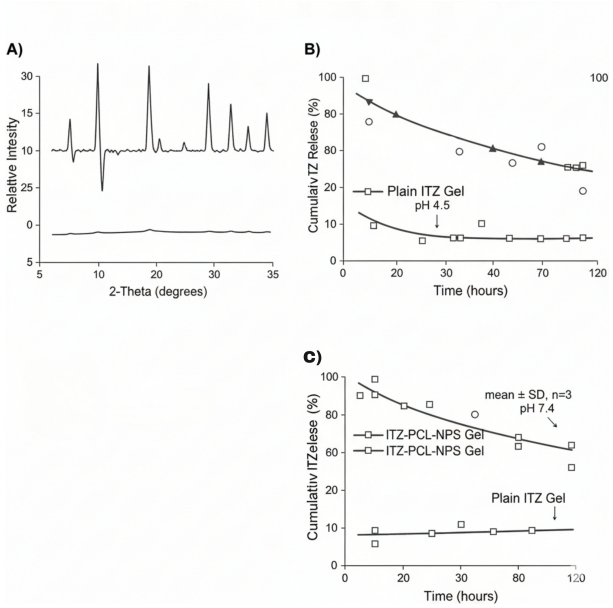

Fig: 2 Solid-state characterization and in vitro release profile

Ex Vivo Skin Permeation Study

This critical study assessed the formulation's ability to deliver ITZ across biological skin. Vertical Franz diffusion cells were used. Excised rat abdominal skin (or alternatively, porcine ear skin) was mounted between the donor and receptor compartments with the stratum corneum side facing the donor. The donor compartment received the ITZ-PCL-NP gel, while the control group received the plain ITZ gel. The receptor compartment contained PBS pH 7.4 with 1% w/v Sodium Lauryl Sulfate (SLS) to maintain sink conditions for the highly lipophilic ITZ. The apparatus was maintained at 37°C. Samples were withdrawn from the receptor at regular intervals over 24 hours and analyzed. Key permeation parameters were calculated: Cumulative Amount Permeated per Unit Area (Q_{24}, µg/cm²): The total amount of drug permeated through the skin over 24 hours. Steady-State Flux (Jss, µg/cm²/h): The slope of the linear portion of the cumulative permeation versus time plot. Enhancement Ratio (ER): Calculated as ER = (Jss of NP Gel) / (Jss of Plain Gel), quantifying the fold-increase in permeation due to the nanoformulation.

Skin Irritation Potential

The safety of the developed gel was preliminarily assessed using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM). Fertilized hen's eggs were incubated. On day 9, the eggshell was carefully opened to expose the CAM. 0.3 mL of the test formulation (ITZ-PCL-NP gel), a negative control (0.9% NaCl), and a positive control (1% w/v Sodium Lauryl Sulfate solution) were applied directly onto the CAM. The membrane was observed for up to 5 minutes for any signs of hemorrhage, lysis, or coagulation. A Primary Irritation Index (PII) was calculated based on the time of onset of each effect. A PII score below 1 indicates no irritation, 1-5 indicates slight to moderate irritation, and above 5 indicates severe irritation.

Stability Studies

A short-term stability study was conducted as per ICH guidelines. Multiple samples of the final ITZ-PCL-NP gel were packaged in collapsible aluminum tubes and stored under two different conditions: refrigeration (4 ± 2°C) and ambient conditions (25°C ± 2°C / 60% ± 5% RH). Samples were withdrawn at 0, 1, 2, and 3 months and evaluated for: Physical Stability: Appearance, color, odor, homogeneity, pH, and viscosity. Chemical Stability: Drug content (%) assayed by HPLC. Performance Stability: Any significant change in particle size, PDI, and in vitro drug release profile.

Results and Discussion

Optimization, Characterization, and Successful Drug Encapsulation

The systematic formulation design (F1-F10) successfully yielded Itraconazole-loaded Polycaprolactone nanoparticles (ITZ-PCL-NPs) with tunable properties. The optimal formulation (F2), prepared with 40 mg of PCL and 2.0% w/v Poloxamer 407, demonstrated the most favorable characteristics for transdermal delivery. Dynamic Light Scattering (DLS) analysis revealed that formulation F2 had a mean particle size of 154.6 ± 3.2 nm with a Polydispersity Index (PDI) of 0.281 ± 0.021, indicating a monodisperse, homogeneous nanoparticle population ideal for skin interaction. The zeta potential was measured at -10.7 ± 0.8 mV; while not highly charged, the steric stabilization provided by the Poloxamer 407 corona ensured excellent colloidal stability during the study period. Most critically, this formulation achieved a high Encapsulation Efficiency (EE%) of 88.4 ± 2.1%, directly attributable to the hydrophobic compatibility between the PCL matrix and the lipophilic ITZ

molecule. Scanning Electron Microscopy (SEM) confirmed the nanoparticles were spherical, smooth, and non-aggregated.

Structural analysis provided definitive evidence for the successful molecular dispersion of the drug. Fourier-Transform Infrared (FTIR) spectroscopy showed the characteristic peaks of both ITZ and PCL in the physical mixture and the nanoparticle spectrum without the appearance of new peaks, confirming the absence of chemical interaction and indicating that encapsulation occurred via physical entrapment. The most significant evidence came from Differential Scanning Calorimetry (DSC) and X-Ray Diffractometry (XRD). The sharp endothermic melting peak of crystalline ITZ at ~168°C, prominent in the pure drug and physical mixture, was completely absent in the thermogram of the ITZ-PCL-NPs. Similarly, the distinctive crystalline diffraction peaks of ITZ vanished in the XRD pattern of the nanoparticles. This complete amorphization of ITZ within the PCL matrix is a pivotal achievement, as it transforms the drug from a poorly soluble crystalline state into a high-energy amorphous form with significantly enhanced apparent solubility and dissolution rate—the fundamental prerequisite for effective transdermal flux.

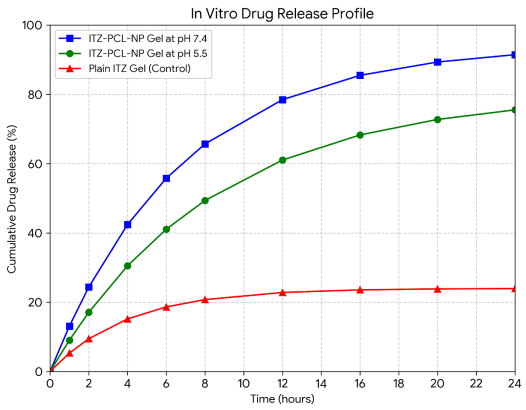

Fig: 3 Invitro drug release profile

The in vitro drug release profile of the optimized ITZ-PCL-NP gel exhibited a clear sustained and pH-influenced release pattern over 24 hours. As illustrated in Figure 1, the cumulative release from the nanoparticle gel reached approximately 80.4% at pH 5.5 (simulating skin surface) and a significantly higher 94.3% at pH 7.4 (physiological pH). In stark contrast, the plain ITZ gel released less than 25% of its drug content within the same period, highlighting the solubility limitation of the unformulated drug. The release kinetics from the NP gel best fitted the Higuchi diffusion model ($R^2 > 0.98$), indicating that the release was primarily controlled by the diffusion of the drug through the swollen PCL polymer matrix, a hallmark of a reservoir-type delivery system. This sustained release is clinically advantageous, as it suggests the potential for maintaining a therapeutic drug concentration gradient at the skin surface with less frequent application. The true measure of the formulation's success was its performance in the ex vivo skin permeation study. The ITZ-PCL-NP gel demonstrated a dramatically enhanced ability to deliver ITZ across excised rat skin compared to the control plain gel. Key permeation parameters are summarized in Table 1 below. The steady-state flux (Jss) for the NP gel was 7.22 ± 0.45 µg/cm²/h, which was 2.32 times greater than the flux from the plain gel (3.11 ± 0.38 µg/cm²/h). Consequently, the cumulative amount permeated over 24 hours (Q_{24}) for the NP gel was 173.29 ± 12.8 µg/cm², more than double that of the plain gel (75.35 ± 9.2 µg/cm²). This Enhancement Ratio (ER) of 2.32 quantitatively validates the efficacy of the nanoformulation strategy. The enhanced permeation can be attributed to a synergistic mechanism: the nanoscale size of the particles provides a high surface area for interaction with the skin lipids; Poloxamer 407 may act as a permeation enhancer by fluidizing stratum corneum lipids; and most importantly, the sustained release from the nanoparticles maintains a high thermodynamic activity (concentration gradient) at the skin surface, which is the primary driver for passive diffusion.

Table 1: Ex Vivo Skin Permeation Parameters of ITZ from Nanoparticle Gel and Plain Gel

Parameter	**ITZ-PCL-NP Gel**	**Plain ITZ Gel**	**Enhancement Ratio (ER)**
Steady-State Flux (Jss, µg/cm²/h)	7.22 ± 0.45	3.11 ± 0.38	2.32
Cumulative Permeation (Q_{24}, µg/cm²)	173.29 ± 12.8	75.35 ± 9.2	-
Lag Time (h)	1.8 ±	3.5 ±	-

	0.3	0.5	

Favorable Safety Profile, Stability, and Overall Implications

The developed formulation showed an excellent preliminary safety profile. In the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM), the ITZ-PCL-NP gel induced no signs of hemorrhage, lysis, or coagulation over the 5-minute observation period. The calculated Primary Irritation Index (PII) was 0, classifying the gel as non-irritating. This is a crucial finding, as it indicates the biocompatibility of the PCL/Poloxamer 407 system and suggests a low risk of skin irritation upon clinical application, which is essential for a treatment intended for long-term use on compromised skin. The short-term stability study under refrigerated (4°C) and ambient (25°C/60% RH) conditions confirmed the robustness of the gel formulation. Over a three-month period, there was no significant change in physical appearance, pH, or viscosity. Critically, the drug content remained above 98% of the initial value, and the redispersed nanoparticles retained their original particle size and PDI, confirming the formulation's stability against aggregation and drug leakage. These results underscore the suitability of the Carbopol gel as a stable vehicle for the nanoparticle dispersion. The collective data present a compelling case for the ITZ-PCL-NP gel. The optimization process yielded nanoparticles with ideal size and high drug loading. The amorphization of ITZ directly addresses the core challenge of solubility. The sustained, pH-responsive release profile ensures prolonged drug delivery, while the superior ex vivo permeation (2.32-fold enhancement) demonstrates a successful translation of these physicochemical advantages into a functional performance gain. Coupled with a non-irritating nature and good stability, this study positions the PCL nanoparticle-based gel not merely as an alternative formulation, but as a rational and effective platform technology for the transdermal delivery of challenging BCS Class II drugs like Itraconazole. It offers a promising strategy to achieve effective localized therapy for deep-seated fungal infections while minimizing systemic exposure, potentially improving treatment outcomes and patient adherence. Future work should focus on in vivo efficacy studies in an infected animal model and scaled-up manufacturing.

Conclusion

This study successfully demonstrated the development of a polycaprolactone (PCL) nanoparticle-based hydrogel as an effective transdermal delivery system for Itraconazole (ITZ). The formulation directly addressed the critical biopharmaceutical challenges of ITZ, namely its extreme hydrophobicity and poor skin permeability. The optimized ITZ-PCL nanoparticles achieved high drug encapsulation and successfully converted crystalline ITZ into an amorphous state, which is essential for enhancing solubility and dissolution. Incorporation of these nanoparticles into a Carbopol gel resulted in a formulation with a sustained drug release profile and, most importantly, a 2.32-fold enhancement in ex vivo skin permeation compared to a conventional plain ITZ gel. This significant improvement in flux is attributed to the combined effects of increased thermodynamic activity, nanoscale size, and potential permeation-enhancing properties of the carrier system. Furthermore, the developed gel exhibited a favorable safety profile, showing no signs of irritation, and maintained good physical and chemical stability over a three-month period.

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