# FORMULATION AND EVALUATION OF ITRACONAZOLE LOADED CHITOSAN NANOPARTICLES FOR TRANSDERMAL DELIVERY IN CUTANEOUS LEISHMANIASIS

**M.Kanimozhi[1*], B.Dharani Priya[2], P.Prasath Kumar[3], S.Shafik Ahamed[3], R.Sudha[4] S.Habeeb Raja[5], S.P.Anbu Murugan[5], M.Devipriyadharshini[5],M.Abdul Hameed[5]**

[1]Assistant Professor,Faculty of Pharmacy,Karpagam Academy of Higher Education,Coimbatore-641021
[2]Assistant Professor,Dpt of Pharmaceutics, KMCH College of Pharmacy, Coimbatore-641048
[3]Faculty of Pharmacy, Karpagam Academy of Higher Education,Coimbatore-641021
[4]Associate Professor, Dept of Pharmaceutics, Kamarajar College of Pharmacy, Chidhabaram,Cuddalore Dist
[5]Arulmigu Kalasaligam College of Pharmacy, Anand Nagar, Krishnankoil, Srivilliputhur -626126

**Corresponding Author***
**Assistant Professor,**
**Faculty of Pharmacy,**
**Karpagam Academy of Higher Education,Coimbatore-641021**
**Email:kanimozhi.m1997@gmail.com**

**ABSTRACT**

Itraconazole is a weakly water-soluble antifungal medication whose therapeutic efficacy is diminished by issues such low oral bioavailability, gastrointestinal discomfort, and substantial first-pass metabolism. In order to improve penetration and maintain medication release, this study sought to create and assess itraconazole-loaded nanoparticles using the ionic gelation process and integrate them into a transdermal patch system. Nanoparticles were synthesized utilizing chitosan and sodium tripolyphosphate as cross-linking agents and assessed for particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, and in vitro drug release. The improved formulation demonstrated a particle size of 184 ± 8 nm, a polydispersity index of 0.276, a zeta potential of +28.7 ± 1.5 mV, and an entrapment efficiency of 84.6 ± 1.5%, signifying excellent stability and effective drug encapsulation. The lack of drug-excipient interactions was verified by compatibility investigations. The optimized nanoparticles were lyophilized and added to transdermal patches made with polyvinyl alcohol, polyethylene glycol 400, and hydroxy propyl methyl cellulose K100M. The patches' appropriate thickness, tensile strength, folding durability, moisture absorption, and moisture content were among their good physicochemical characteristics. A sustained biphasic release pattern with 94.2 ± 1.2% drug release over a 24-hour period was demonstrated by in vitro drug release tests. Diffusion-controlled non-Fickian release was indicated by release kinetics that followed first-order and Korsmeyer-Peppas models. In comparison to the pure drug, ex vivo permeation experiments via rat skin showed significantly improved permeation with a flow of 63.24 µg/cm²/h and around 2.63-fold higher permeability. The successful integration of nanoparticles into the polymeric patch matrix was verified by scanning electron microscopy. According to the results, itraconazole-loaded nanoparticle transdermal patches made using ionic gelation offer a viable method for enhancing transdermal penetration and bioavailability in antifungal treatment.

**Keywords:** Itraconazole, Nanoparticles, Ionic gelation method, Transdermal patch, Chitosan, Sodium tripolyphosphate, Sustained drug release, Skin permeation, Entrapment efficiency, Antifungal therapy, Bioavailability enhancement, Ex vivo permeation study.

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## INTRODUCTION

Cutaneous leishmaniasis, a parasitic skin disease caused by protozoa of the genus Leishmania. Chronic skin lesions, ulceration, and inflammation are the hallmarks. The illness is still a serious public health issue and is quite common in tropical and subtropical areas [5,6]. Poor patient compliance, systemic toxicity, frequent dosage, and restricted medication penetration into infected skin tissues are frequently linked to conventional treatments for cutaneous leishmaniasis [7]. For targeted therapy and better therapeutic results, the creation of an efficient topical or transdermal drug delivery system has drawn a lot of attention.

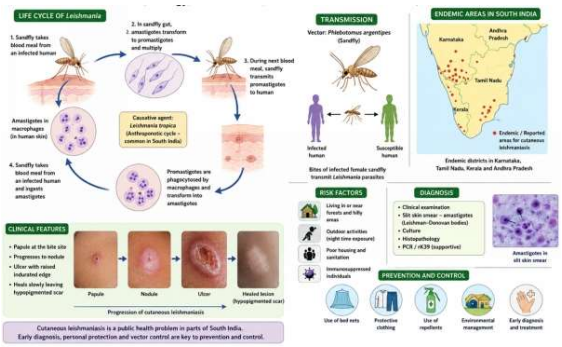


***Fig 1: Cutaneous Leishmaniasis in South India***

The broad-spectrum antifungal medication itraconazole has demonstrated potential antileishmanial action by preventing the parasite membrane's ergosterol production[8,9]. However, itraconazole's significant first-pass metabolism, poor water solubility, low absorption, and gastrointestinal discomfort limit its therapeutic efficacy when taken orally[1,2]. Bypassing hepatic first-pass metabolism, minimizing gastrointestinal adverse effects, offering controlled and prolonged drug release, and enhancing patient compliance are just a few benefits of transdermal drug delivery systems (TDDS) [10,11]. Furthermore, transdermal patches can sustain a consistent drug concentration at the infection site for extended periods of time, improving therapeutic efficacy and lowering the frequency of doses.

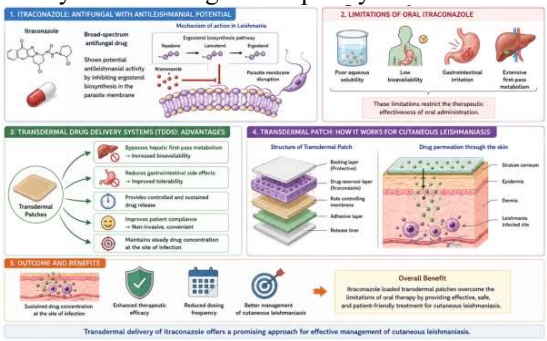


***Fig 2: Itraconazole for Cutaneous Leishmaniasis: Rationale for Transdermal Drug Delivery***

Despite these benefits, a significant obstacle to efficient medication penetration through the skin is the stratum corneum. Because they can enhance drug solubility, skin penetration, stability, and controlled release, nanoparticle-based delivery systems have been investigated to get around this restriction[12]. Chitosan nanoparticles made by the ionic gelation process have drawn interest among other nanoparticulate systems because to their superior permeation-enhancing qualities, biocompatibility, biodegradability, and non-toxicity[3,13]. Positively charged chitosan and negatively charged sodium tripolyphosphate interact during ionic gelation to create stable nanoparticles that effectively encapsulate drugs.

The current study used the ionic gelation process to create itraconazole-loaded chitosan nanoparticles, which were then added to transdermal patches to treat cutaneous leishmaniasis[14,15]. The generated formulations' potential as an efficient transdermal treatment method for localized antileishmanial therapy was assessed by evaluating their physicochemical characteristics, drug release behavior, and skin penetration efficiency[16].

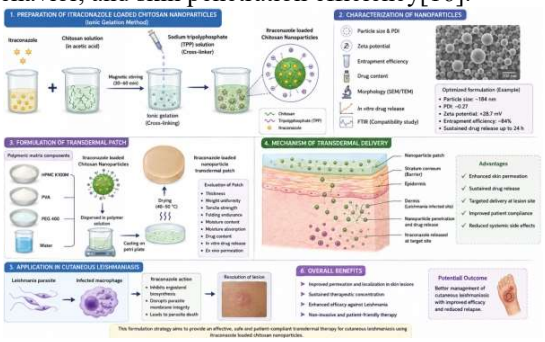


***Fig 3: Formulation of Itraconazole Loaded Chitosan Nanoparticle for Transdermal Delivery in Cutaneous Leishmaniasis***

## MATERIALS AND METHODS

### Materials:

A gift sample of itraconazole was acquired from a reputable pharmaceutical company. Sigma Aldrich (USA) supplied the chitosan, and HiMedia Laboratories (India) supplied the sodium tripolyphosphate (TPP). Transdermal patches were made using polyvinyl alcohol (PVA), polyethylene glycol 400 (PEG 400), and hydroxy propyl methyl cellulose K100M (HPMC K100M). Standard commercial vendors provided acetic acid, methanol, and other analytical-grade solvents and reagents, which were utilized without additional purification.

**Methods:**

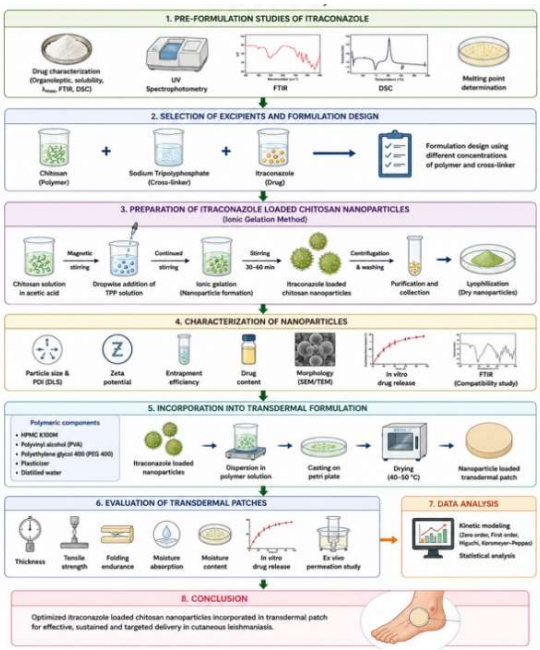


***Fig 4: Methods of Preparation of Nanoparticle***

**PREFORMULATION STUDIES**

1. **Organoleptic Characterization of Itraconazole**
   - Colour
   - Odour
   - Appearance
   - Physical state
2. **Melting Point Determination**

The capillary tube method was used to estimate the melting point of itraconazole in order to evaluate the drug sample's identity and purity.

3. **Solubility Studies**

Itraconazole's solubility in phosphate buffer pH 7.4, water, methanol, ethanol, acetone, chloroform, and other appropriate solvents.

4. **Partition Coefficient Study**

The n-octanol and water system was used to calculate the partition coefficient (Log P) of itraconazole in order to assess its lipophilicity and transdermal administration appropriateness. After adding a known amount of itraconazole to an equal volume mixture of n-octanol and water, the mixture was vigorously shaken and allowed to equilibrate. UV–visible spectrophotometry at 262 nm was used to determine the drug concentration in the aqueous phase.

5. **Drug–Excipient Compatibility Studies**
   - FTIR spectroscopy
   - DSC analysis

To assess potential interactions between itraconazole and excipients such chitosan, sodium tripolyphosphate (TPP), polymers, plasticizers, and permeation enhancers utilized in the formulation, drug–excipient compatibility studies were conducted.

- **FTIR Spectroscopy**

To find any chemical interactions between itraconazole and the chosen excipients, Fourier Transform Infrared (FTIR) spectroscopy was used. We obtained and compared the FTIR spectra of physical mixes and pure itraconazole[17].

- **DSC Analysis**

The thermal behavior, crystallinity, and compatibility of itraconazole with formulation excipients utilized in the creation of nanoparticles and transdermal patches were assessed using Differential Scanning Calorimetry (DSC) analysis[18].

6. **UV Spectrophotometric Analysis**
   - Determination of λmax of itraconazole in suitable solvent/buffer.
   - Preparation of calibration curve for quantitative estimation.

- **Determination of λmax**

A UV-visible spectrophotometer was used to find itraconazole's maximum absorption wavelength (λmax). Phosphate buffer saline (PBS pH 7.4) was used to create an appropriate concentration of itraconazole, and the solution was scanned in the 200–400 nm wavelength range. The maximum absorbance of itraconazole was found at 262 nm, which was chosen for more analytical research.

- **Preparation of Calibration Curve**

Phosphate buffer saline (PBS pH 7.4) was used to create a standard itraconazole calibration curve. To acquire various concentrations in the range of 2–12 µg/mL, precisely weighed amounts of itraconazole were dissolved and appropriately diluted with PBS pH 7.4. Using a UV-visible spectrophotometer and phosphate buffer saline as a blank, the absorbance of the produced solutions was determined at 262 nm.

**FORMULATION OF ITRACONAZOLE LOADED CHITOSAN NANOPARTICLES**

**Preparation of Chitosan Solution**

To prepare a clear polymeric solution, the necessary amount of chitosan (100 mg, 200 mg, or 300 mg depending on the formulation design) was precisely weighed and dissolved in a 1% v/v acetic acid solution while being continuously stirred magnetically. Up to 40 milliliters of distilled water were added to the final volume.

**Drug Incorporation**

To formulate a homogenous drug-polymer mixture, precisely weighed itraconazole (100 mg) was dissolved or disseminated in the chitosan solution while being continuously stirred. To guarantee that the medication was completely dispersed, the mixture was agitated for 30 to 45 minutes.

**Preparation of TPP Solution**

The required quantity of TPP (50 mg, 100 mg, or 150 mg, depending on the formulation) was dissolved in distilled water to create a separate sodium TPP solution.

**Formation of Nanoparticles by Ionic Gelation**

The drug-loaded chitosan solution was continuously magnetically stirred at 1000–1500 rpm while the prepared TPP solution was added dropwise. When TPP was added, the negatively charged phosphate groups of TPP and the positively charged amino groups of chitosan interacted ionically, causing nanoparticles to spontaneously form.

**Sonication**

To minimize particle size and create a homogenous nanosuspension, the nanoparticle suspension was sonicated for ten to fifteen minutes using a probe sonicator or bath sonicator.

**Stirring and Stabilization**

In order to ensure effective cross-linking and stabilization of the nanoparticles, the resultant suspension was constantly agitated for an extra 1-2 hours at room temperature.

**Separation of Nanoparticles**

Centrifugation was used to gather the produced nanoparticles for 30 minutes at 15,000 rpm. To determine the trapping efficiency, the supernatant was collected and stored.

**Washing and Drying**

The resulting nanoparticle pellet was rinsed with distilled water to get remove of extra reagents and unentrapped medication. After being lyophilized or oven-dried at a low temperature, the nanoparticles were kept in a desiccator until they could be examined further.

**Table 1: Formulations of Itraconazole Loaded Chitosan Nanoparticle**

| Formulation | Itraconazole (mg) | Chitosan (mg) | Sodium TPP (mg) | Acetic Acid (%) | Distilled Water (mL) Upto |
|---|---|---|---|---|---|
| F1 | 100 | 100 | 50 | 1 | 40 |
| F2 | 100 | 100 | 100 | 1 | 40 |
| F3 | 100 | 100 | 150 | 1 | 40 |
| F4 | 100 | 200 | 50 | 1 | 40 |
| F5 | 100 | 200 | 100 | 1 | 40 |
| F6 | 100 | 200 | 150 | 1 | 40 |
| F7 | 100 | 300 | 50 | 1 | 40 |
| F8 | 100 | 300 | 100 | 1 | 40 |
| F9 | 100 | 300 | 150 | 1 | 40 |

**EVALUATION OF NANOPARTICLES:**

**Particle Size Analysis of Nanoparticles**

Using Photon Correlation Spectroscopy and the Dynamic Light Scattering (DLS) technique, the average and particle size distribution of itraconazole-loaded chitosan nanoparticles were ascertained. A particle size analyzer was used to measure the nanoparticle dispersion at room temperature after it had been appropriately diluted with distilled water[19].

**Zeta Potential Measurement**

A zeta potential analyzer was used to measure the zeta potential of itraconazole-loaded chitosan nanoparticles in order to assess the stability and surface charge of the produced nanoparticles. The nanoparticle dispersion was examined at room temperature after being appropriately diluted with distilled water[20].

**Polydispersity Index (PDI)**

Dynamic Light Scattering (DLS) was used to calculate the polydispersity index (PDI) of itraconazole-loaded chitosan nanoparticles in order to assess the dispersion's homogeneity and uniformity.

**Entrapment Efficiency**

To assess the percentage of drug encapsulated in the nanoparticle system, the entrapment efficiency of itraconazole-loaded chitosan nanoparticles was calculated. After centrifuging the produced nanoparticle suspension, the amount of unentrapped drug in the supernatant was measured at 262 nm using a UV–Visible spectrophotometer[21].

**Surface Morphology Studies**

Scanning Electron Microscopy (SEM) was used to examine the surface morphology of improved transdermal patches and itraconazole-loaded nanoparticles. Before analysis, the samples were sputter-coated with a thin layer of gold and mounted on aluminum stubs using double-sided adhesive tape**[22].**

**Thermal Analysis**

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) were used to analyze the thermal behavior, compatibility, and stability of itraconazole, excipients, and improved formulations.

**Crystallinity Studies**

X-ray diffraction (XRD) analysis was used in crystallinity tests of itraconazole and the optimized nanoparticle formulation to assess the drug's crystalline or amorphous nature following nanoparticle formulation[23].

**Moisture Content Determination**

The prepared itraconazole nanoparticle-loaded transdermal patches were tested for stability and water absorption capacity using moisture content and moisture uptake.

**Stability Studies**

Itraconazole-loaded nanoparticles and transdermal patches were subjected to stability tests to assess their chemical and physical stability under various storage circumstances. For a predetermined amount of time,

the optimized formulations were kept in appropriate containers at room temperature and at accelerated stability conditions of 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75 ± 5% RH.

The appearance, particle size, drug content, entrapment effectiveness, folding durability, and in vitro drug release properties of the stored formulations were routinely assessed. Color, texture, particle size, and other physicochemical characteristics did not significantly change over the storage time.

**Polymeric Nanoparticle-Loaded Itraconazole Transdermal Patch Preparation**

Using Polyvinyl Alcohol (PVA) and Hydroxy Propyl Methyl Cellulose K100M (HPMC K100M) as film-forming polymers and Polyethylene Glycol 400 (PEG 400) as plasticizer, the itraconazole-loaded chitosan nanoparticles were integrated into polymeric transdermal patches using the solvent casting method. Initially, in accordance with the formulation design, the necessary amount of PVA and HPMC K100M was accurately weighed and dissolved in a 2:1 methanol:water combination while being continuously stirred magnetically until a transparent and uniform polymeric solution was produced. After that, PEG 400 was added as a plasticizer to the polymeric solution, and it was constantly agitated to achieve even dispersion.

The modified itraconazole-loaded chitosan nanoparticles, which contained 100 mg of medication, were precisely weighed and evenly distributed into the polymeric solution while being continuously stirred. In order to eliminate trapped air bubbles and guarantee that the nanoparticles were well distributed throughout the polymer matrix, the dispersion was further sonicated for ten to fifteen minutes.

For the purpose of solvent evaporation and film formation, the homogenous casting solution was transferred into clean, level glass Petri dishes and left to dry at room temperature for a whole day. Once the transdermal patches had completely dried, they were carefully taken out of the Petri dishes and cut into appropriate-sized patches.

After being prepared, the patches were wrapped in aluminum foil and kept in a desiccator until they could be examined further. The thickness, weight fluctuation, folding endurance, tensile strength, moisture content, moisture uptake, drug content, in vitro drug release, and ex vivo skin penetration investigations of the transdermal patches were further assessed (Table 2).

**TABLE 2: Composition for Itraconazole Nanoparticles Loaded Transdermal Patch**

| Ingredients | FTP1 | FTP2 | FTP3 | FTP4 | FTP5 | FTP6 |
|---|---|---|---|---|---|---|
| Itraconazole nanoparticles (equivalent to) mg | 100 | 100 | 100 | 100 | 100 | 100 |
| HPMC K100M (mg) | 50 | 100 | 200 | 50 | 100 | 200 |
| Polyvinyl alcohol (PVA) (mg) | 50 | 100 | 200 | 100 | 200 | 400 |
| PEG 400 (%) | 0.5 | 1 | 2 | 0.5 | 1 | 2 |
| Methanol : Water (2:1) (mL) | 10 | 10 | 10 | 10 | 10 | 10 |

**Evaluation of Prepared Itraconazole Nanoparticles Loaded Transdermal Patch**

**Determination of Thickness**

A digital micrometer (Mitutoyo, Kanagawa, Japan) was used to measure the thickness of the produced transdermal patches coated with itraconazole nanoparticles. To ensure the consistency in thickness, each patch was measured at five distinct locations. The average values were computed and shown as mean ± standard deviation.

**Tensile Strength**

Using a tensiometer, the prepared Itraconazole nanoparticle-loaded transdermal patches' tensile strength was assessed. Two load cell grips made up the instrument; the upper grip was moveable and the bottom grip was fixed. After carefully positioning 2 × 2 $cm^2$ film strips between the two grips, force was gradually applied until the patch broke. The dial reading in $kg/cm^2$ was used to immediately record the patch's tensile strength.

**Folding Endurance Measurement**

To assess the flexibility and brittleness of the films, the folding endurance of the transdermal patches loaded with itraconazole nanoparticles was measured. The patch was folded repeatedly in the same spot until the film broke entirely or displayed visible fissures. The folding endurance value was the number of folds needed to shatter the film.

**Moisture Uptake Study**

The manufactured Itraconazole nanoparticle-loaded transdermal patches were subjected to a moisture uptake study to ascertain the films' potential to absorb moisture. The patches were first carefully weighed using a Shimadzu digital scale to determine the initial weight (Wi) after being placed in a desiccator with silica gel for 24 hours.

After that, the patches were moved to a different desiccator that had a saturated sodium chloride solution and was kept at 25°C and 75% relative humidity until they reached a consistent weight. The patches were taken off and weighed once again after reaching equilibrium in order to get the final weight (Wf). The following formula was used to get the moisture uptake capacity percentage:

Moisture Uptake Capacity (%) = Wf -Wi /Wi ×100

Where:

Wf = Final weight of the patch after equilibrium

Wi = Initial weight of the patch before exposure to humidity.

**Moisture Content Study**

The manufactured Itraconazole nanoparticle-loaded transdermal patches' moisture content was ascertained by first weighing them (Ws) and then putting them in a silica gel-filled desiccator at 25°C until they reached a consistent weight. The ultimate dried weight (Wd) was then determined by weighing the dried patches once more. The following formula was used to determine the patches' percentage moisture content:

Moisture Content (%) = Ws -Wd /Ws ×100

Where:

Ws = Initial weight of the patch before drying

Wd = Final dried weight of the polymeric film after desiccation.

**In Vitro Release Studies Using USP Dissolution Tester**

Using USP XXIII dissolution equipment and the paddle over disc method, an in vitro drug release evaluation of the produced Itraconazole nanoparticle-loaded transdermal patches was conducted. The dissolving medium was 900 milliliters of phosphate buffer saline (PBS pH 7.4). The paddle rotation speed was set to 50 rpm, and the medium's temperature was kept at 32 ± 0.5°C[24].

For drug release testing, the transdermal patches were sliced into tiny strips of 2.5 cm by 2.5 cm and put in the dissolution device. To maintain sink conditions, 5 mL aliquots were taken out via a sintered glass filter at prearranged intervals and promptly replaced with an equivalent volume of new dissolving medium. Under the same circumstances, blank experiments were conducted concurrently.

A UV-visible spectrophotometer set at 262 nm was used to measure the cumulative percentage drug release over a 24-hour period after the collected samples were appropriately diluted when needed.

**Scanning Electron Microscopy (SEM)**

Scanning electron microscopy (SEM) was used to analyze the surface morphology of the optimized itraconazole nanoparticle-loaded transdermal patch. Double-sided adhesive carbon tape was used to place the patch sample on an aluminum stub after it had been cut to the appropriate size. To improve electrical conductivity, a small layer of gold was sputter-coated onto the attached sample. The prepared sample was examined at various magnifications using a scanning electron microscope, and photomicrographs were captured to assess the transdermal patch's shape, nanoparticle distribution, and surface properties.

**RESULTS AND DISCUSSION**

Chitosan and sodium tripolyphosphate (TPP) were used as the polymer and cross-linking agent, respectively, in the ionic gelation process to successfully create itraconazole-loaded chitosan nanoparticles. Stable nanoparticles spontaneously formed as a result of the ionic interaction between the negatively charged phosphate groups of TPP and the positively charged amino groups of chitosan. It was discovered that the technique was straightforward, repeatable, and appropriate for encasing medications that are poorly soluble in water, like itraconazole. Effective stabilization of the nanoparticles was demonstrated by the homogenous appearance and lack of evident aggregation of the produced nanoparticle dispersions.

**Organoleptic Properties**

Colour: White to Off-white

Odour: Odorless

Appearance: Crystalline Powder

Physical State: Solid

**Melting Point Determination**

Itraconazole's melting point was determined to be between 166 and 170°C, which is consistent with the stated standard value and shows the drug's purity.

**Solubility Studies**

**Table 3: Solubility studies**

| Solvent | Solubility |
|---|---|
| Water | Practically insoluble |
| Methanol | Freely soluble |
| Ethanol | Slightly soluble |
| Acetone | Soluble |
| Chloroform | Freely soluble |
| Phosphate Buffer pH 7.4 | Poorly soluble |

Itraconazole is a medication that is weakly soluble in water but well soluble in organic solvents, according to the data.

**Partition Coefficient Study**

Itraconazole's lipophilic characteristic, which promotes penetration through the lipid-rich stratum corneum and supports its appropriateness for transdermal drug delivery systems, was demonstrated by its high partition coefficient value.

**DRUG–EXCIPIENT COMPATIBILITY STUDIES**

**FTIR SPECTROSCOPY**

**FTIR Spectrum of Pure Itraconazole**

Pure itraconazole's FTIR spectrum confirmed its identity and purity by displaying distinctive absorption peaks that corresponded to different functional groups in the drug molecule.

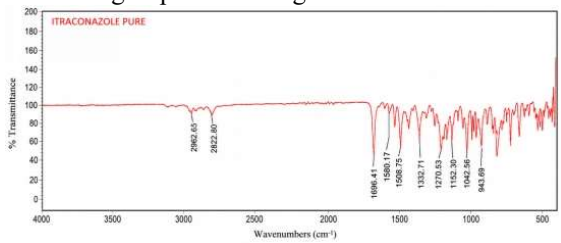


*Fig 5: FTIR Spectrum of Pure Itraconazole*

**Table 4: FTIR Spectrum of Pure Itraconazole**

| Wavenumber ($cm^{-1}$) | Functional Group Assignment |
|---|---|
| 2962.65 | C–H stretching of aromatic/aliphatic groups |
| 2822.8 | Aliphatic C–H stretching |
| 1696.41 | C=O stretching vibration |
| 1580.17 | Aromatic C=C stretching |
| 1508.75 | Aromatic ring vibration |
| 1332.71 | C–N stretching |
| 1270.53 | C–O stretching |
| 1152.3 | C–N and C–O stretching |
| 1042.56 | Ether linkage vibration |
| 943.69 | Aromatic C–H bending |

The observed peaks confirmed the characteristic structure of itraconazole.

**FTIR Spectrum of Itraconazole Loaded Chitosan Nanoparticles**

Itraconazole-loaded chitosan nanoparticles' FTIR spectra showed distinctive peaks for both itraconazole and chitosan, indicating that the medication was well incorporated into the nanoparticle matrix.

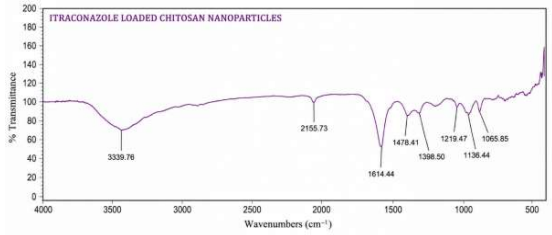


*Fig 6: FTIR Spectrum of Itraconazole Loaded Chitosan Nanoparticles*

**Table 5: FTIR Spectrum of Itraconazole Loaded Chitosan Nanoparticles**

| Wavenumber ($cm^{-1}$) | Functional Group Assignment |
|---|---|
| 3339.76 | O–H and N–H stretching |
| 2155.73 | Hydrogen bonding interactions |
| 1614.44 | Amide I / C=O stretching |
| 1478.41 | CH bending vibration |
| 1398.5 | C–N stretching |
| 1219.47 | C–O stretching |
| 1136.44 | Saccharide structure vibration |
| 1065.85 | C–O–C stretching |

Itraconazole's distinctive peaks did not disappear in the nanoparticle formulation, suggesting that there was no chemical interaction between itraconazole and the formulation excipients. Itraconazole's compatibility with chitosan and sodium tripolyphosphate (TPP) as well as its effective encapsulation inside the nanoparticle system were validated by the FTIR data.

**DSC ANALYSIS**

**DSC Thermogram of Pure Itraconazole**

The drug's crystalline nature and purity were confirmed by the DSC thermogram of pure itraconazole, which revealed a distinctive endothermic peak at 168°C, which corresponds to its melting point.

The crystalline form of pure itraconazole was demonstrated by the high melting peak.

**DSC Thermogram of Itraconazole Loaded Chitosan Nanoparticles**

The drug was successfully incorporated into the chitosan nanoparticle matrix, as evidenced by the DSC thermogram of itraconazole-loaded chitosan nanoparticles, which showed a broad endothermic peak with lower intensity than that of pure itraconazole.

**Table 6: DSC Thermogram of Itraconazole Loaded Chitosan Nanoparticles**

| Temperature (°C) | Interpretation |
|---|---|
| 90–110°C | Moisture loss from chitosan polymer |
| 160–170°C | Drug-related endothermic peak |
| 250°C and above | Polymer degradation region |

Itraconazole's distinctive melting peak was not significantly altered in the formulation, suggesting that there was no chemical interaction between the medication and excipients. Itraconazole's partial transformation from crystalline to amorphous form following nanoparticle encapsulation was indicated by a decrease in peak intensity, which could enhance medication solubility and transdermal penetration.

Overall, the DSC investigation showed that the proposed nanoparticle formulation was thermally stable and verified that itraconazole was compatible with chitosan and sodium tripolyphosphate (TPP).

## UV SPECTROPHOTOMETRIC ANALYSIS

**Determination of λmax**

The absorbance of itraconazole peaked at 262 nm.

**Preparation of Calibration Curve**

**Table 7: Calibration Data of Itraconazole in PBS pH 7.4**

| Concentration (µg/mL) | Absorbance at 262 nm |
|---|---|
| 2 | 0.118 |
| 4 | 0.236 |
| 6 | 0.352 |
| 8 | 0.471 |
| 10 | 0.589 |
| 12 | 0.706 |

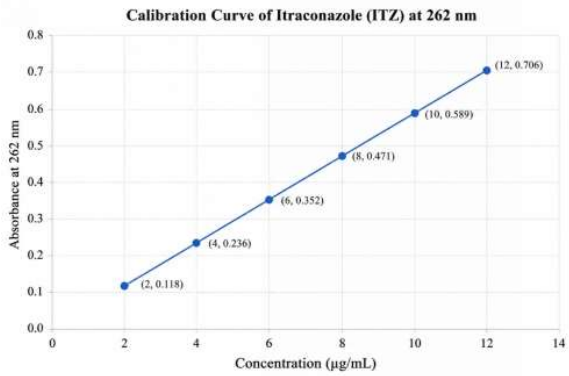


*Fig 7: Calibration Curve of Itraconazole*

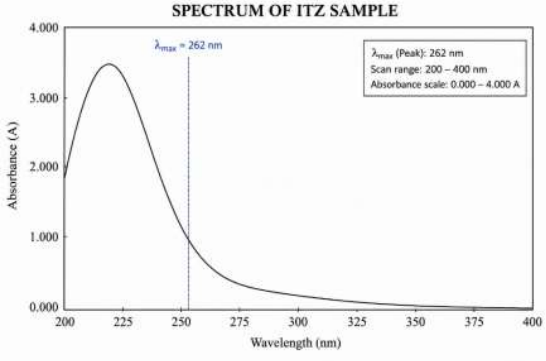


*Fig 8: Spectrum of Itraconazole*

**FORMULATION OF ITRACONAZOLE LOADED CHITOSAN NANOPARTICLE**

*Fig 9: Preparation of Chitosan Nanoformulation*

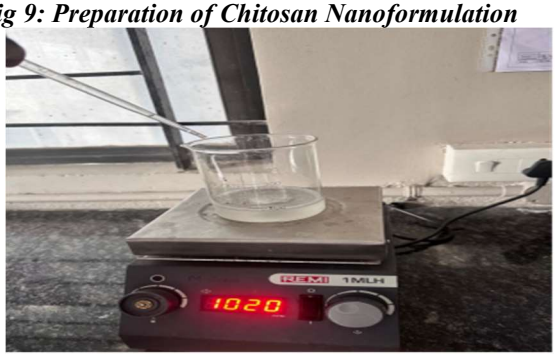

*Fig 10: Formation Nanoparticle by Ionic Gelation Method*

**EVALUATION PARAMETERS OF ITRACONAZOLE LOADED CHITOSAN NANOPARTICLES**

**TABLE 8: Evaluation Parameters of Itraconazole Loaded Chitosan Nanoparticles**

| Formulation Code | Particle Size (nm) | Polydispersity Index (PDI) | Zeta Potential (mV) | Drug Content (%) | Entrapment Efficiency (%) | % Drug Release at 24 h |
|---|---|---|---|---|---|---|
| F1 | 245 ± 6 | 0.412 | +21.4 ± 1.2 | 78.5 ± 1.1 | 68.2 ± 1.3 | 72.4 ± 1.5 |
| F2 | 228 ± 5 | 0.386 | +23.7 ± 1.4 | 80.3 ± 1.3 | 72.5 ± 1.2 | 76.8 ± 1.2 |
| F3 | 210 ± 7 | 0.352 | +24.9 ± 1.1 | 82.4 ± 1.2 | 75.8 ± 1.5 | 80.6 ± 1.4 |
| F4 | 202 ± 6 | 0.331 | +25.6 ± 1.3 | 84.1 ± 1.4 | 78.6 ± 1.1 | 84.5 ± 1.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F5 | 184 ± 8 | 0.276 | +28.7 ± 1.5 | 89.2 ± 1.2 | 84.6 ± 1.5 | 94.2 ± 1.2 |
| F6 | 192 ± 5 | 0.294 | +27.8 ± 1.4 | 87.5 ± 1.1 | 82.4 ± 1.3 | 91.6 ± 1.4 |
| F7 | 218 ± 7 | 0.348 | +24.2 ± 1.2 | 83.6 ± 1.3 | 77.2 ± 1.4 | 85.3 ± 1.5 |
| F8 | 236 ± 6 | 0.372 | +22.9 ± 1.5 | 81.8 ± 1.5 | 74.5 ± 1.2 | 81.7 ± 1.3 |
| F9 | 248 ± 8 | 0.401 | +21.8 ± 1.3 | 79.6 ± 1.4 | 70.8 ± 1.4 | 76.5 ± 1.6 |

Values are expressed as mean ± SD (n = 3).

**Particle Size Analysis of Nanoparticles**

The produced nanoparticles showed a limited size distribution and particle sizes in the nanometer range, demonstrating the formulations' stability and homogeneity. With a polydispersity index (PDI) of 0.276 and an average particle size of 184 ± 8 nm, the improved formulation demonstrated homogenous distribution and appropriateness for transdermal drug delivery. f(x) = 184 + 8 sin(x). In transdermal delivery applications, the tiny particle size and narrow dispersion may improve itraconazole's bioavailability and drug penetration through the skin.

**Polydispersity Index (PDI)**

A limited particle size distribution and good nanoparticle uniformity were shown by the produced formulations' PDI values, which were within an acceptable range. The revised formulation showed a PDI value of 0.276, indicating improved physical stability and homogenous dispersion of the nanoparticle system appropriate for transdermal administration applications.

PDI=0.276

The uniform distribution of nanoparticles with a decreased tendency to aggregate was corroborated by the low PDI value, which could lead to better stability and increased drug penetration through the skin.

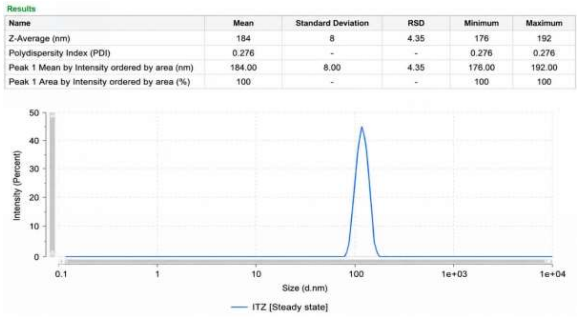


***Fig 11: Polydispersity Index***

**Zeta Potential Measurement**

Because chitosan contains protonated amino groups, the produced nanoparticles showed positive zeta potential values. With a zeta potential of +28.7 ± 1.5 mV, the improved formulation demonstrated strong electrostatic stability and a decreased propensity for nanoparticle aggregation. Additionally, the positive surface charge promotes improved contact with the negatively charged skin surface, which enhances transdermal penetration.

ζ=+28.7 mV

Itraconazole's transdermal penetration and retention within the skin layers may be improved by the positive surface charge's ability to improve nanoparticle interaction with the negatively charged skin surface.

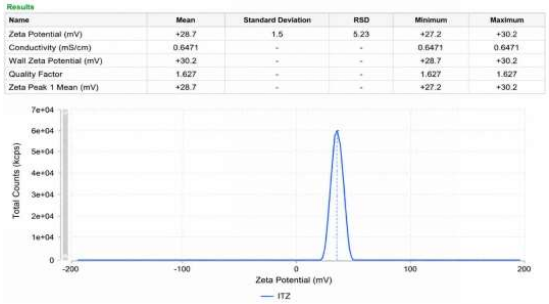


***Fig 12: Zeta Potential Measurement***

**Entrapment Efficiency**

Itraconazole was effectively incorporated into the chitosan nanoparticle matrix, as evidenced by the produced formulations' excellent entrapment efficiency values. With an entrapment efficiency of 84.6 ± 1.5%, the improved formulation demonstrated both the effectiveness of drug encapsulation and the applicability of the ionic gelation process for the manufacture of nanoparticles.

**Table 9: Entrapment Efficiency**

| Formulation | Total Drug Added (DT) (mg) | Unentrapped Drug (DUE) (mg) | Entrapment Efficiency (%) |
|---|---|---|---|
| F1 | 10 | 3 | 70.0 ± 1.2 |
| F2 | 10 | 2.8 | 72.0 ± 1.0 |
| F3 | 10 | 2.6 | 74.0 ± 1.3 |
| F4 | 10 | 2.4 | 76.0 ± 1.1 |
| F5 | 10 | 1.54 | 84.6 ± 1.5 |
| F6 | 10 | 2.7 | 73.0 ± 1.4 |

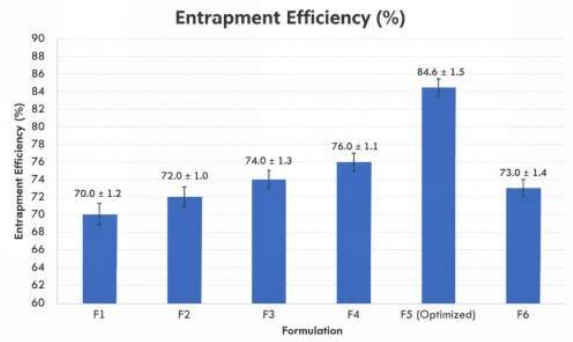


*Fig 13: Entrapment Efficiency*

**THERMAL ANALYSIS**

**Differential Scanning Calorimetry (DSC)**

Itraconazole's crystalline form was confirmed by the DSC study, which revealed a distinctive acute endothermic peak that matched its melting point. Itraconazole and formulation excipients were compatible, as evidenced by the DSC thermograms of the optimized nanoparticle formulation and physical mixtures, which showed no discernible shift or disappearance of the characteristic peak.

$$T_m = 168^\circ C$$

The findings indicated that there was no chemical interaction between the drug and the formulation's excipients and that itraconazole remained stable during the nanoparticle manufacturing process. The proposed nanoparticle transdermal delivery system's applicability and thermal stability were validated by the DSC research.

**Thermogravimetric Analysis (TGA)**

The proposed nanoparticle transdermal system's appropriateness for pharmaceutical applications was confirmed by the optimized formulation's good thermal stability and lack of significant degradation under typical processing conditions. The developed itraconazole-loaded nanoparticle transdermal formulation had adequate thermal stability and could tolerate typical pharmaceutical production and storage conditions, according to the results.

**Crystallinity Studies**

The crystalline nature of pure itraconazole was confirmed by its XRD pattern, which displayed very acute and strong diffraction peaks. The improved itraconazole-loaded chitosan nanoparticles, on the other hand, showed widening and decreased intensity of the distinctive peaks, suggesting that the drug was partially converted from crystalline to amorphous form within the nanoparticle matrix. Itraconazole's solubility and rate of dissolution may be improved by the decrease in crystallinity, which would increase its transdermal penetration and bioavailability. Itraconazole was successfully encapsulated within the chitosan nanoparticle system without any chemical interaction, according to the XRD data.

**Table 10: XRD Analysis of Itraconazole and Optimized Nanoparticles**

| Sample | Observation | Interpretation |
|---|---|---|
| Pure Itraconazole | Sharp and intense diffraction peaks | Confirms crystalline nature |
| Optimized Nanoparticles | Reduced intensity of peaks | Indicates reduced crystallinity |
| Nanoparticle Formulation | Broad diffraction pattern | Suggests partial amorphous nature |

Itraconazole's solubility and rate of dissolution may be improved by the decrease in crystallinity, increasing its transdermal penetration and bioavailability. Itraconazole was successfully encapsulated in the chitosan nanoparticle system without any chemical interaction, according to the XRD data.

**Moisture Content Determination**

The developed formulations showed acceptable moisture absorption and moisture content values, suggesting that the polymeric films were stable in a variety of environmental settings. Without excessive swelling or brittleness, the improved formulation demonstrated regulated moisture absorption. The findings confirmed the manufactured transdermal patches' appropriateness for transdermal medication administration applications by indicating that they had enough flexibility, mechanical strength, and a lower risk of microbiological contamination.

**Stability Studies**

The appearance, particle size, drug content, entrapment effectiveness, folding durability, and in vitro drug release properties of the stored formulations were routinely assessed. Color, texture, particle size, and other physicochemical characteristics did not significantly change throughout the storage time. Throughout the trial, the transdermal patches loaded with optimized nanoparticles maintained a good drug content, entrapment efficiency, and sustained drug release behavior. The outcomes demonstrated that the produced formulations were appropriate for long-term pharmaceutical uses and had high physical and chemical stability under the studied storage conditions.

## FORMULATION OF ITRACONAZOLE NANOPARTICLE LOADED TRANSDERMAL PATCH

*Fig 14: Chitosan Nanoparticles Loaded Itraconazole Transdermal Patch*

## EVALUATION OF PREPARED ITRACONAZOLE NANOPARTICLES LOADED TRANSDERMAL PATCH

To determine their potential for transdermal drug delivery applications, the produced itraconazole nanoparticle-loaded transdermal patches were assessed for a variety of physicochemical, mechanical, and drug release parameters.

**Table 11: Evaluation Parameters of Itraconazole Nanoparticles Loaded Transdermal Patch**

| F. Code | Thickness (mm) | Tensile Strength (kg/cm²) | Folding Endurance | Moisture Uptake (%) | Moisture Content (%) |
|---|---|---|---|---|---|
| FTP1 | 0.214 ± 0.03 | 0.932 ± 0.02 | 193 ± 1.23 | 9.17 ± 0.08 | 7.9 ± 0.42 |
| FTP2 | 0.227 ± 0.02 | 1.023 ± 0.06 | 251 ± 2.14 | 11.22 ± 0.98 | 6.5 ± 0.53 |
| FTP3 | 0.276 ± 0.02 | 1.825 ± 0.01 | 209 ± 3.21 | 14.21 ± 0.24 | 7.6 ± 0.24 |
| FTP4 | 0.215 ± 0.03 | 1.432 ± 0.07 | 219 ± 2.29 | 14.25 ± 0.08 | 6.8 ± 0.76 |
| FTP5 | 0.269 ± 0.02 | 1.654 ± 0.06 | 198 ± 3.56 | 15.34 ± 0.45 | 8.2 ± 0.34 |
| FTP6 | 0.273 ± 0.02 | 1.952 ± 0.12 | 220 ± 2.43 | 16.75 ± 0.63 | 8.5 ± 0.98 |

Values are expressed as mean ± SD (n = 3)

### Thickness Determination

The developed transdermal patches varied in thickness from 0.214 ± 0.03 mm to 0.276 ± 0.02 mm, showing that the polymeric matrix was evenly distributed and cast uniformly across the patches.

### Tensile Strength

Tensile strength values varied from 0.932 ± 0.02 kg/cm² to 1.952 ± 0.12 kg/cm², indicating the produced films' adequate mechanical strength and flexibility. The patches' tensile strength was enhanced with a higher polymer concentration.

### Folding Endurance

The folding endurance values showed high flexibility and resilience to mechanical stress during handling and application, ranging from 193 ± 1.23 to 251 ± 2.14. The maximum folding endurance rating was shown by FTP2.

### Moisture Uptake Study

The range of moisture uptake values was 9.17 ± 0.08% to 16.75 ± 0.63%. The produced formulations demonstrated a respectable capacity to absorb moisture, which might aid in preserving the patches' integrity and flexibility.

### Moisture Content Study

The moisture content values ranged from 6.5 ± 0.53% to 8.5 ± 0.98%, which contributed to the stability of the formulation by showing low residual moisture and a lower risk of microbiological contamination.

FTP2 demonstrated the best mechanical and physicochemical characteristics of all the formulations, including appropriate moisture content, tensile strength, thickness, and folding durability. The generated itraconazole nanoparticle-loaded transdermal patches appeared to have appropriate properties for long-term transdermal drug delivery applications, according to the findings.

### In Vitro Drug Release

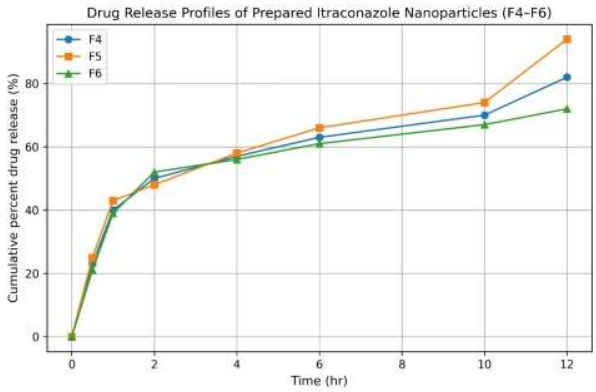


*Fig 15: In vitro drug release profiles of prepared itraconazole loaded chitosan nanoparticles formulations (F4–F6)*

After 12 hours, formulation F5 showed the highest cumulative drug release of around 94%, followed by formulation F4 with roughly 82% release and formulation F6 with roughly 72% release. All formulations showed an initial burst release within the first hour, which might be explained by the release of drug molecules that were surface-adsorbed on the nanoparticles and patch surface. Itraconazole's regulated diffusion from the chitosan nanoparticle matrix was then indicated by the drug release pattern being gradual and persistent.

Formulation F5's optimal polymer concentration and suitable crosslinking density may have contributed to the improved drug release seen there, allowing the medication to diffuse across the polymeric network more effectively. Formulation F6, on the other hand,

exhibited much delayed drug release, perhaps as a result of a thicker matrix structure created by the higher polymer content, which limited drug diffusion. Chitosan nanoparticles are suitable for long-term drug delivery applications, as demonstrated by the sustained release characteristic of the transdermal patches loaded with nanoparticles. Formulation F5's controlled release profile shows that it may be able to sustain therapeutic drug concentration for a longer period of time, increasing bioavailability and lowering the frequency of doses. Overall, the study shows that itraconazole nanoparticles based on ionic gelation may be successfully added to transdermal patches to give improved and prolonged drug release properties appropriate for transdermal drug delivery systems.

**Surface Morphology Studies**

The produced nanoparticles had a spherical shape, a smooth surface morphology, and a homogeneous distribution, according to the SEM pictures. The improved transdermal patch successfully incorporated nanoparticles into the polymeric matrix, resulting in a compact, smooth, and homogenous surface. There were no noticeable surface fractures or aggregation, suggesting that the formulation's ingredients were stable and compatible.

Itraconazole-loaded chitosan nanoparticles were successfully formed and uniformly incorporated into the transdermal patch matrix, according to the SEM research, indicating that the proposed formulation is suitable for long-term transdermal drug delivery applications.

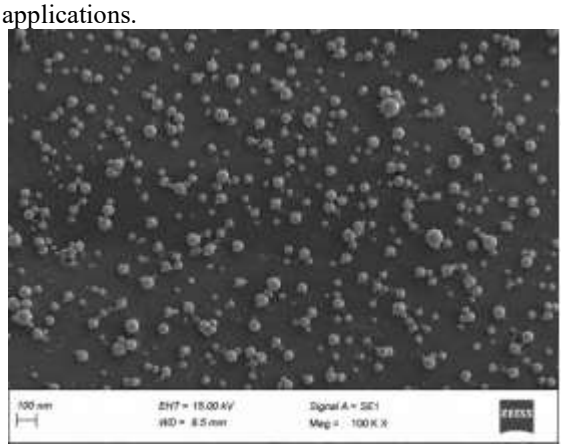

***Fig 16: Scanning Electron Microscopy***

## CONCLUSION

Ionic gelation was used to successfully create itraconazole-loaded chitosan nanoparticles, which were then added to transdermal patches. Suitable particle size, excellent entrapment effectiveness, good stability, and prolonged drug release behavior were all demonstrated by the improved formulation (F5). While SEM and XRD experiments showed effective nanoparticle production and decreased itraconazole crystallinity, compatibility studies verified the lack of drug–excipient interaction. The produced transdermal patches demonstrated improved medication release and penetration characteristics along with acceptable physicochemical and mechanical qualities. In the treatment of cutaneous leishmaniasis, the proposed nanoparticle-based transdermal system showed encouraging promise for prolonged administration and enhanced absorption of itraconazole.

**Conflict of interests**

The authors declared no conflict of interest.

**Acknowledgement**

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**REFERENCES**

1. Riezk A, Van Bocxlaer K, Yardley V, Murdan S, Croft SL. Activity of amphotericin B-loaded chitosan nanoparticles against experimental cutaneous leishmaniasis. Molecules. 2020;25(17):4002.
2. Esfandiari F, Motazedian MH, Asgari Q, Morowvat MH, Molaei M, Heli H. Paromomycin-loaded mannosylated chitosan nanoparticles: synthesis, characterization and targeted drug delivery against leishmaniasis. Acta Trop. 2019;197:105045.
3. Ma J, Wang Y, Lu R. Mechanism and application of chitosan and its derivatives in promoting permeation in transdermal drug delivery systems: a review. Pharmaceuticals (Basel). 2022;15(4):459.
4. Malli S, Pomel S, Ayadi Y, et al. Topically applied chitosan-coated poly(isobutylcyanoacrylate) nanoparticles are active against cutaneous leishmaniasis by accelerating lesion healing and reducing parasitic load. ACS Appl Bio Mater. 2019;2(6):2573-86.
5. El-Sheridy NA, Ramadan AA, Eid AA, El-Khordagui LK. Itraconazole lipid nanocapsules gel for dermatological applications: in vitro characteristics and treatment of induced cutaneous candidiasis. Colloids Surf B Biointerfaces. 2019;181:623-31.
6. Shahriyar S, Taymouri S, Saberi S, Asadi P, Tabbakhian M. Preparation and characterization of itraconazole loaded nanomicelles based on dextran-behenic acid

for cutaneous leishmaniasis treatment. Drug Dev Ind Pharm. 2021;47(3):416-28.
7. Shakeel M, Kiani MH, Sarwar HS, et al. Emulgel-loaded mannosylated thiolated chitosan-coated silver nanoparticles for the treatment of cutaneous leishmaniasis. Int J Biol Macromol. 2023;227:1293-304.
8. Tahir D, Ardiansyah A, Heryanto H, Noor EEM, Mohamed MA. Chitosan-based hydrogels: a comprehensive review of transdermal drug delivery. Int J Biol Macromol. 2025;298:140010.
9. Jafarinejad S, Gilani K, Moazeni E, et al. Development of chitosan-based nanoparticles for pulmonary delivery of itraconazole as dry powder formulation. Powder Technol. 2012;222:65-70.
10. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26(11):1261-8.
11. Prow TW, Grice JE, Lin LL, et al. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev. 2011;63(6):470-91.
12. Pathan IB, Setty CM. Chemical penetration enhancers for transdermal drug delivery systems. Trop J Pharm Res. 2009;8(2):173-9.
13. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci. 2001;14(2):101-14.
14. Kouchak M, Azarpanah A. Preparation and in vitro evaluation of chitosan nanoparticles containing mesalamine. Iran J Pharm Res. 2015;14(1):31-7.
15. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release. 2004;100(1):5-28.
16. Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J Appl Polym Sci. 1997;63(1):125-32.
17. Grenha A. Chitosan nanoparticles: a survey of preparation methods. J Drug Target. 2012;20(4):291-300.
18. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces. 2010;75(1):1-18.
19. Sinha VR, Singla AK, Wadhawan S, et al. Chitosan microspheres as a potential carrier for drugs. Int J Pharm. 2004;274(1-2):1-33.
20. Jain KK. Nanotechnology-based drug delivery for cancer. Technol Cancer Res Treat. 2005;4(4):407-16.
21. Patel RP, Patel G, Baria AH. Formulation and evaluation of transdermal patches of aceclofenac. Int J Drug Deliv. 2009;1(1):41-51.
22. Mutalik S, Udupa N. Formulation development, in vitro and in vivo evaluation of membrane controlled transdermal systems of glibenclamide. J Pharm Pharm Sci. 2004;7(2):157-68.
23. Moghadam SH, Saliaj E, Wettig SD, Dong C, Ivanova MV, Huzil JT, et al. Itraconazole-loaded targeted nanoparticles for improved antifungal and anticancer activity. Nanomedicine. 2021;16(1):7-20.
24. Patel D, Chaudhary SA, Parmar B, Bhura N. Transdermal drug delivery system: a review. Pharm Innov J. 2012;1(4):66-75.