Design, Synthesis and Characterization of Nanosponges for Controlled Release Application

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Abstract—**This study focuses on the design, synthesis, and characterization of Itraconazole-loaded nanosponges for controlled drug release in the treatment of fungal infections. Itraconazole is a broad-spectrum antifungal drug with poor aqueous solubility and limited bioavailability, restricting its effectiveness in topical applications. To overcome these limitations, nanosponges were prepared using the emulsion solvent evaporation technique with Eudragit S100 as the polymer and Polyvinyl Alcohol (PVA) as the surfactant. A Central Composite Design (CCD) was employed to optimize formulation variables, including polymer concentration, surfactant concentration, and stirring speed. The prepared nanosponges were evaluated for particle size, zeta potential, polydispersity index (PDI), and entrapment efficiency. The optimized formulation showed a particle size of approximately 131.95 nm, zeta potential of −22.3 mV, PDI of 0.421, and entrapment efficiency of 93%. The optimized nanosponge formulation was incorporated into a Carbopol 934 hydrogel and assessed for pH, viscosity, spreadability, swelling index, and drug release characteristics. In-vitro studies demonstrated sustained drug release of approximately 77.86% over 6 hours, while ex-vivo studies showed effective permeation through biological membranes. The developed nanosponge-loaded hydrogel exhibited enhanced solubility, improved drug penetration, and controlled release behavior, suggesting its potential as an effective topical drug delivery system for fungal infections with reduced dosing frequency and improved therapeutic outcomes.**

I. INTRODCUTION

The delivery of medications to achieve a therapeutic effect in humans and animals is a regular practice [1]. There is a variety of promising drug delivery systems, including topical, controlled, and conventional ways that can be employed to distribute a few medications and provide a desired therapeutic activity with a minimum of side effects. Targeted drug delivery is a novel method for administering medications at a specific site at a particular time with limiting side effects [2,3]. Modern medicine extensively uses nanotechnology to enhance drug delivery and prevent overdose. Nano sponge technology is one of the emerging strategies used to deliver various medications to a particular target site and then release the drug substance in a controlled manner. The preferred method of medicine delivery for local administration is topical. It provides benefits such as simple delivery, avoiding the first-pass effect, and improved patient compliance. Various topical formulations, including ointments, gels, creams, pastes, lotions, and foams, are produced by combining excipients and active medicinal ingredients [4,5]. Topical gels are typically regarded as the safest and most efficient medications for dermatological ailments. A cross-linked polymer structure called a gel has swelling capabilities and is comprised of a three-dimensional network of dispersed phase particles. Gels are a better choice than ointments and creams for topical drug administration because they are easier to apply and remove [6].

Itraconazole is a broad-spectrum antifungal medication effective against dermatophytosis, blastomycosis,tineapedis caused by Tinearubrum, and tineacruris [7]. Itraconazole is antriazole class of drug that works by obstructing ergosterol production. It inhibits the cytochrome P450, a 14-demethylase enzyme that blocks the formation of cell walls in fungi [8]. Itraconazole is a BCS class-II medication with restricted permeability, limited solubility, and bioavailability that is a barrier to topical delivery. Traditional topical cream formulations have various limitations, including limited penetration from the stratum corneum and low retention at the point of application. The poor solubility limits medication absorption through the skin during topical

administration. It can be avoided by entrapping the drug in nano-carriers such as nanoparticles, and nano-emulsions have gained significance for delivering Itraconazole. One of the crucial ways to produce Itraconazole topical gel employs nanosponge technology, which has the advantages such as lower toxicity, better solubility, and permeation. The current research aims to design and develop the nanosponge formulations of Itraconazole applying central composite design using eudragitS100 as a polymer to improve drug release properties and enhanced stability. The formulation was optimized for particle size, entrapment efficiency (%EE), and drug release in experimental design[9]. The final formulation was produced using a variety of gelling agent [10], including HPMC, Sodium CMC, and Carbopol 934, and evaluated for various parameters, including viscosity, swelling, drug diffusion, zone of inhibition (mm), skin irritation, etc.

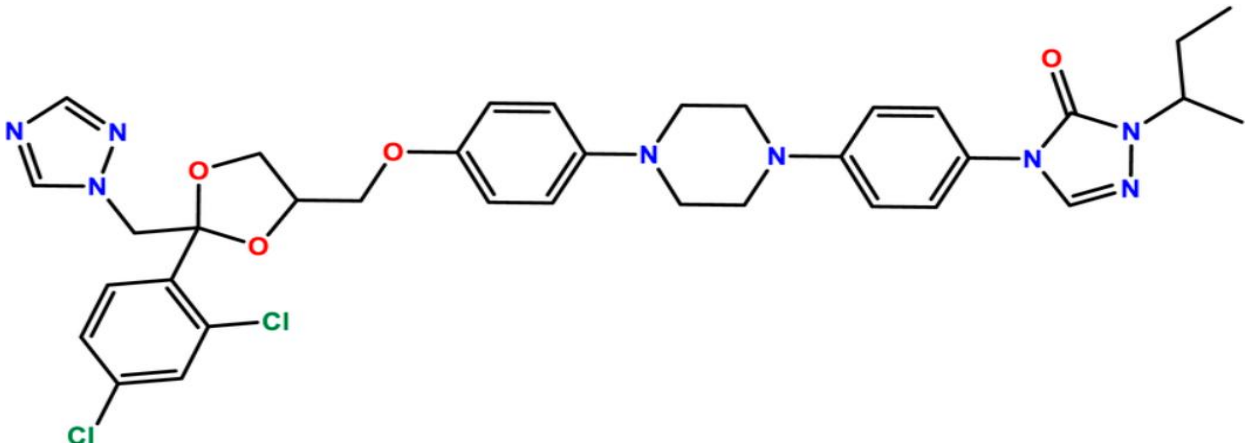


Fig. 1.Chemical structure of Itraconazole.

As these nanoparticles move through the body, they eventually get into interaction with the face of a tumor cell, where they cleave and begin to release the medication in a predictable and regulated way [30]. These kinds of targeted medication delivery systems offer a number of initial benefits. It should be more effective for a particular lozenge since the medication is delivered at the excrescence location rather than spreading widely throughout the body. They should also have less harmful side effects because healthy towels get into touch with less medication. The Nanosponge patches' ability to withstand water is an additional benefit. By re-creating the anticancer medication in Nanosponge, hydrophobic medications that are difficult to dissolve in water can be used. Later, these medicines must be mixed with adjuvant reagents, which potentially can reduce the toxicity of the medicine or adverse effects .

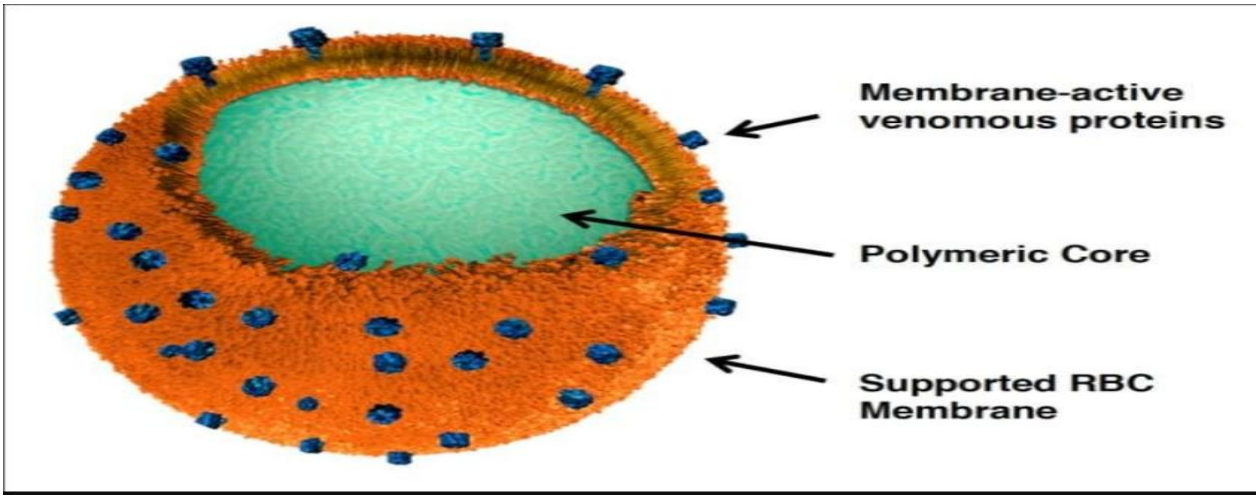


Fig 2 Nanosponges

II. MATERIALS AND METHOD

Chemical and solvents

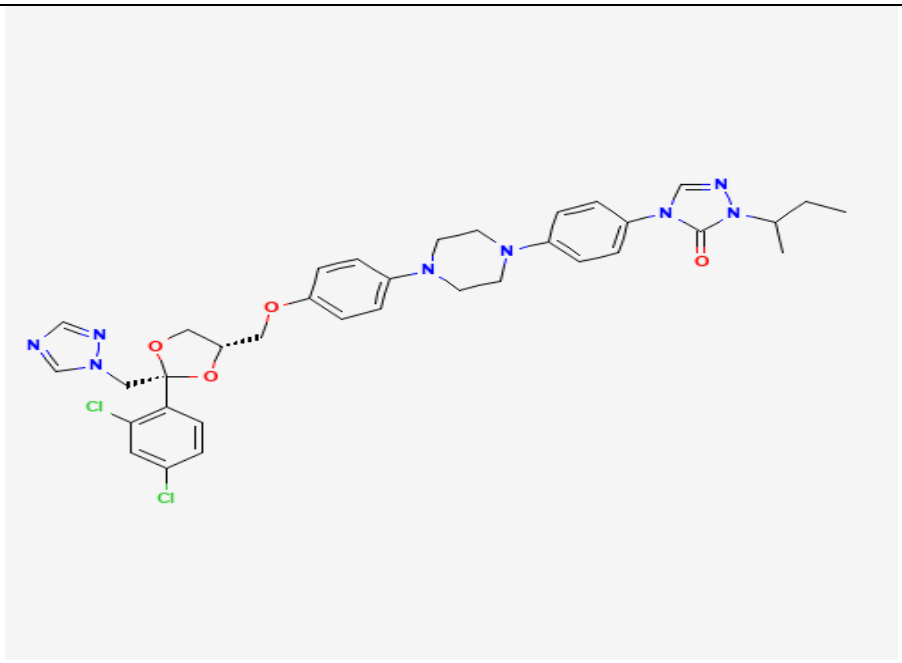
Itraconazole was purchased from Dr. Reddy Laboratory,Hydrabad as a gift sample, Eudragit S100, Polyvinyl Alcohol as a gift sample purchased from Research Lab Fine Chemicals Industry, Mumbai and Dichloromethane, Carbopol, propyl paraben, triethanolamine, Sodium Hydroxide, Potassium Di-hydrogen Orthophosphate purchased from LobaChemi, Mumbai as a gift sample. Deionized water (18.2 MX 25 C) were collected from Millipore Mill-Q Gradi-ent A-10 water-purification systems.

Analysis

Analysis of BTF and their prepared formulation samples was carried out using RP-HPLC according to reported method [11]. Briefly, RP-HPLC equipped with UV detector using C18 column, mobile phase consisting of methanol and water (90:10, v/v) at flow rate 1 mL/min. Detection was per formed at 254 nm. The calibration curve was plotted using concentration (50, 75, 100, 150 and 200 mg/mL) Vs peak area. The regression coefficient of correlation was measured (R2) 0.9973.

III. DRUG PROFILE

Table 1: ITRACONAZOLE

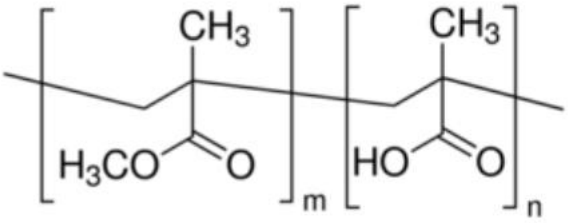
Category	Anti-infective agent Antifungal
Chemical Structure	
Chemical Name	2-butan-2-yl-4[4-[4-[[(2R,4S)-2-(2,4-dichloromethane)-2-(1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl-1,2,4-triazol-3-one
CAS Number	84625-61-6
Brand Name	Soprano, Tolsura
Chemical Formula	• $C_{35}H_{38}Cl_2N_8O_4$
Molecular Weight	705.6 g/ mol
Description	White Powder
Melting point	165^0C

Pharmacodynamics	The antifungal medication itraconazole inhibits the growth of fungal cells and promotes their death. It has shown effect against histoplasmacapsulatum, the histoplasmaduboisii, as well as Aspergillusfumigatus The bacterium dermatities, and the Trichophyton species in vitro.
Mechanism of action	Itraconazole works against fungi by blocking the fungus cytochrome P450, or enzyme called antioxidant 14α-demethylase from transforming lanosterol to ergosterol, which is a crucial part of fungal cell membranes. The molecule that forms between the two azole nitrogen tittles in the molecular structure of Itraconazole slows down the fungal enzyme's active point, or haem iron. Lanosterol and 14-methylated sterol accumulation results in dysregulated chitin production, increased permeability of the fungal cell membrane, and changed membrane-bound enzyme activity. Additionally, it has been proposed that itraconazole functions by blocking the oxidative and peroxidative enzymes of fungal cytochrome c, which results in the dislocation of fungal cell membranes.
Absorption	After being taken orally, itraconazole is quickly absorbed. Oral capsules of itraconazole reach their peak tube attention in two to five hours. Itraconazole's experimentally reported arbitrary bioavailability is approximately 55. The oral form of itraconazole is more exposed than the capsule form when the same medication is taken. Medication immersion is achieved with a minimal amount of stomach acidity.
Volume of distribution	Adults have distribution volumes exceeding 700 L. Lipophilic and extensively dispersed into apkins is itraconazole. Compared to the lungs, order, and liver, the bone, intestines, spleen, and muscle, the breakdown into keratinous apkins, notably the skin, was found to be up to four times more advanced. The amount of attention given to cerebrospinal fluid is far less than in tubes.
Protein Binding	The majority of itraconazole in the tube is bound to protein (99.8), with albumin serving as the primary list element (99.6 for the hydroxy-metabolite). Additionally, it exhibits a strong attraction for lipids. There is only 0.2 of the free medication itraconazole in the tube.
Metabolism	Itraconazole is significantly metabolized in the liver. Based on in vitro experiments, CYP3A4 is the key enzyme involved in the metabolism of itraconazole. Though there are more than 30 distinct metabolites of itraconazole, hydroxyitraconazole is the main one. In vitro, itraconazole and hydroxyitraconazole both have similar antifungal activity; the metabolite's dip tube attention is about twice that of the parent emulsion.
Route of Elimination	Itraconazole is substantially removed as inert compounds in both urine (35), as well as feces (54), within the first week following an oral result cure. The reason for less than one intravenous cure is the active ingredient in hydroxyitraconazole and the renal removal of itraconazole. Between three and eighteen percent of the oral radiolabeled treatment is eliminated in the stool as unchanged medication. Since it tends to diffuse very little from keratinous apkins, the removal of itraconazole from these apkins is associated with epidermal rejuvenescence.

Half Life	Itraconazole usually has a terminal half-life of 16–28 hours following a single cure; with repeated dosing, this period increases to 34–42 hours. The parent emulsion's excretion is longer than that of the this drug metabolite from the tube.
Toxicity	Rats had oral LD50 values of >320 mg/kg and intraperitoneal LD50 values of 100 mg/kg. Clinical information regarding itraconazole overdose is minimal. The threshold for poisonous trough circumstances is more than 3 mcg/mL. In the event of an overdose, prophylactic measures should be initiated because dialysis does not remove itraconazole. The treatment for itraconazole intoxication is unknown.
Solubility	It has a solubility of 1 µg/mL or less in water, 300 µg/mL in alcohol, and 239 mg/mL in methylene chloride, which makes it very soluble.
Storage	9^0F to 77^0F (15^0C to 25^0C).
Dose	200 mg/ml
Adverse Effect	▪ Hepatotoxicity ▪ Nausea ▪ Diarrhea ▪ Dizziness ▪ Headache ▪ Itching ▪ Tiredness ▪ Double Vision ▪ Impotence

IV. EXCIPIENTS PROFILE

Table 2: EUDRAGIT S100

Synonyms	Methyl acrylic acid, Methyl acrylate Co-polymer
Chemical structure	
Chemical name	Methyl Acrylate
IUPAC name	Poly(methyl methacrylate-co-metharylic acid
Density	1 g/cm^3
CAS NO	25086-15-1
Appearance	White Powder
Molecular Formula	$C_8H_{12}O_4$

Molecular Weight	125,000 g/mol
Melting point	Around 163°C
Solubility	Its solubility is dependent on pH; it dissolves in alkaline environments (above 7) but stays intact in acidic ones.
Stability and Storage condition	The pH-responsive polymer Eudragit S100 remains stable in dry powder form for at least three years when stored tightly closed below 300C and remains dispersed for at least eighteen months between 8 and 250C.
Safety	Eudragit S100, an anionic methacrylate copolymer, has a solid track record of more than 60 years and is supported by monographs from major pharmaceutical companies. It is generally regarded as safe for its intended application in oral solid dosage form, especially for colon-targeted drug administration.

V. METHOD OF PREPARATION

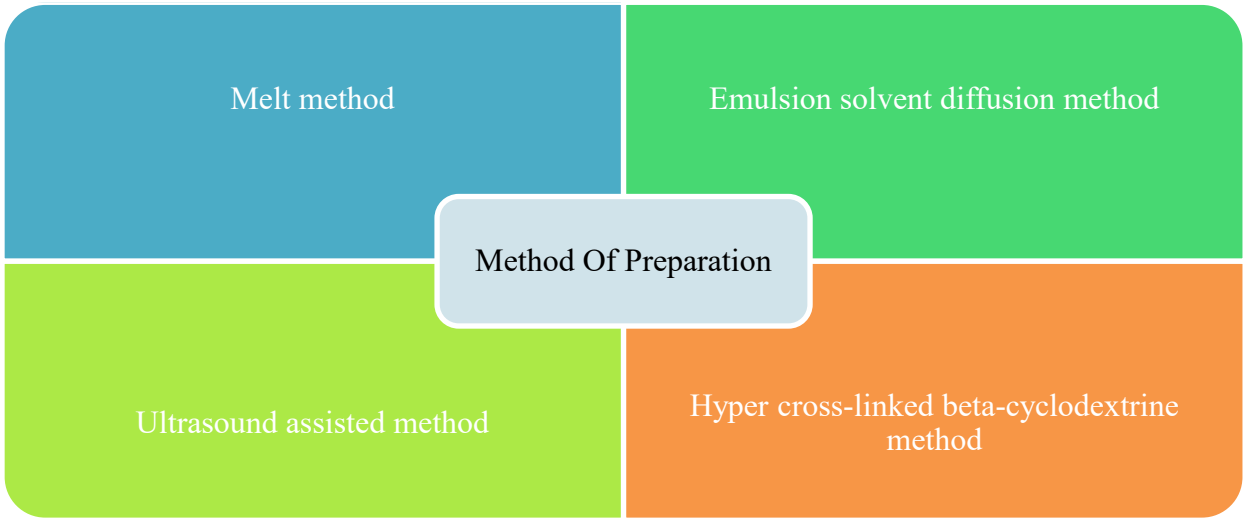


Fig 3 Method of Preparation

3. EXPERIMENTAL DESIGN

The surface response curve method (RSM) in central composite design (CCD) was used to design nanosponge formulations of Itraconazole. This approach is frequently used to assess the impact of the independent variables' optimal variable concentration. It enables the assessment of multiple-factor interactions and effects on multiple response variables [12,13]. The current study analyses the impact of independent factors such as polymer (Eudragit S100) (X1), surfactant (PVA) (X2), and string speed (RPM)(X3) concentrations on particle size (Y1), %EE (Y2), and drug release (Y3). To achieve an optimized formulation that meets all the parameters and critical quality attributes, all the combinations are considered utilizing levels -1 and +1 for three independent variables [14].

4. FORMULATION OF NANOSPONGES

Thirteen formulations (NS1-NS13) were prepared using various polymer ratios of Eudragit S100 and PVA and various stirring speeds (1000 to 1500 rpm) by emulsion solvent evaporation technique. The organic phase was prepared by dissolving the drug and

rate retardant polymer (Eudragit S100) in DCM (20 mL). The organic phase was added dropwise to the aqueous phase (varying quantities of polyvinyl alcohol in distilled water (100 mL), followed by evaporation at room temperature using a magnetic stirrer for 2h. Filtered nanosponges, washed with distilled water, dried (at 40^0C), and stored in a desiccator [15].

Table3. Composition of Itraconazole loaded nano sponges using different solvents

Sr. No.	Ingredients	Formulation Code								
		NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9
1	Itraconazole (mg)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2	Eudragit S100 (gm)	200	200	600	600	117.157	682.84	400	400	400
3	Polyvinyl Alcohol (gm)	0.4	0.4	0.3	0.45	0.5	0.45	0.4	0.5	0.45
4	Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
5	Distilled Water (ml)	150	150	150	150	150	150	150	150	150

5. FORMULATION CONTAINING OF CARBOPOL ITRACONAZOLE GEL LOADED NANOSPONGES

500 mg of Carbopol 934 was dispersed in 5 ml of distilled water and allowed for swelling overnight. The swelled Carbopol was stirred for 60 minutes at 800 rpm. The previously prepared required Itraconazole equivalent nanosuspensions ,methylparaben and propylparaben were incorporated into the polymer dispersion with stirring at 500 rpm, by a magnetic stirrer for 1 h. The PH of above mixture was adjusted to 4.5 with tri ethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10ml with distilled water [16].

Table 4.Formulation of Itraconazole Nanosponge loaded hydrogel

Ingredients	Quantity
Itraconazole nano sponges (gm)	3.61 gm
Carbopol (gm)	4 gm
Polyethylene glycol 400 (ml)	5 ml
Triethanolamine %v/v	2% v/v
propyl paraben (ml)	0.01
Distilled water (q. s.)	35 ml

CHARACTERIZATION OF NANOSPONGES

1. Drug polymer interaction studies

We used FT-IR, DSC, and XRD spectroscopy to study the drug-polymer relationships. Using the Perkin Elmer Spectrum IR Version 10.6.0 device, the spectra were obtained. The polymers hydroxypropyl methylcellulose (HPMC), polymethyl methacrylate (PMMA), and ethyl cellulose (EC) were present in the nanosponges that we collected their spectra of [17].

2. Solubility studies

This technique is the most commonly used for assaying the addition complexes of nanosponges. To ascertain the level of completion, utilize the phase solubility plot. The pH of the drug, its solubilization profile, and the factors influencing its solubility are all determined by solubility studies [18].

3. Zeta potential determination

The zeta potential, the main crucial index of the colloidal dispersion equilibrium, is the potential discrepancy between two fluid layers tied up with dispersion particles, the dispersed medium, and the immobile subcases[19].

4. X-ray diffractometry

The detection of inclusion complexation in the solid state can be accomplished by powder X-ray diffractometry. An combination of substances'

complex development and chemical breakdown can be ascertained using diffraction peaks. The complex creation causes many new peaks to emerge, some old peaks to get sharper, and some peaks to shift [20,21].

5. Thin layer chromatography(TLC)
A method for separating evaporative or non-volatile mixtures is one way to characterize TLC. The identification of the complex conformation between the drug and nanosponges is made easier by the examination of a drug particle's Rf values in thin layer chromatography [22].

6. Microscopy studies
SEM and TEM (Transmission Electron Microscopy) can be used to examine the microscopic properties of some medications, nanosponges, and other products (nanosponge/medicine complexes). Inclusion complex formation is suggested by an electron microscope. Utilizing the co-precipitation method, products and raw materials were obtained [23].

7. Infrared(IR) spectroscopy
IR spectroscopy is used to estimate the interaction between solid-state pharmacological molecules and nanosponges. The immersion band becomes more intense and moves to a lower frequency as a result. Widening of the bands is caused by the stretching vibration of the group that forms hydrogen bonds [24].

8. Loading effectiveness
To determine how well the nanosponge complex is loaded, it should be dissolved in an appropriate solvent, sonicated to break up the complex, diluted properly, then examined by UV spectroscopy and HPLC [25].

9. Drug entrapment effectiveness
Water-suspended powder nanosponges were sonicated for 20 minutes. After shaking for an additional twenty minutes, the medication was completely removed from the nanosponges. For this combination, a 0.45 m membrane sludge was employed for filtering. Set to 263 nm, a UV visible spectrophotometer was used to measure the drug content. The following formula was used to determine how effective trapping was:

$$\text{Entrapment efficiency} = \frac{\text{Actual weight of Drug in sample}}{\text{Weight of Microsphere sample} * 100}$$

CHARACTERIZATION OF NANOSPONGE GEL
The pH of the gel formulations was measured using a Labtronics pH meter. The pH electrode was dipped directly into the gel formulation and allowed to stabilize for 2-3 min; after equilibrium, measure the pH using calibrated pH meter. Samples were analyzed in replicate, and the deviated values, if any, were adjusted to the skin using triethanolamine. The viscometer using a T-bar spindle (Rheometer Merlin VR) was used to measure the rheological properties of the developed gel at room temperature. Viscosity was measured in Cp[26]. Two hundred mg of nanosponge gel was taken in a diffusion bag previously soaked in distilled water for 24 h. Then, allowed the gel to soak in 100 mL of distilled water for 3 h and observe the swelling capacity of the gel.

In vitro drug diffusion study
Nanosponge gel was evaluated for drug diffusion studies to determine the amount of drug diffused through the membrane. The cellophane membrane was used as a diffusion membrane. Franz diffusion apparatus was used to assess drug diffusion with buffer. 2 g of nanosponge gel was placed carefully on the cellophane membrane attached to donor and receptor compartments. The receptor compartment was at a temperature of 37±0.5°C and under constant stirring at 50 rpm using a magnetic stirrer. Regular samples were withdrawn from the receptor compartment, and equal buffer volumes were replaced to maintain the persistent volume. Collected samples were analyzed using UV-visible spectroscopy at 299 nm [27].

In vitro drug release kinetics
Drug release kinetics was used to find the mechanism of drug release. All the drug release results were fitted in Zero-order, First-order, Higuchi, and Peppas equations and observed drug release patterns.
Ex vivo permeation study
A freshly cleaned rat skin membrane was used as a diffusion membrane. It was procured from the experimental animals from VIPER. IAEC committee approval was granted for the completion of the (06/IAEC/VIPER/Ph.D./2021-22/II). study Franz

diffusion apparatus was used to determine drug diffusion with buffer. 2g of nanosponge gel was placed carefully on the membrane between the donor and receptor compartments. The receptor compartment was at a temperature of 37±0.5oC and under constant stirring at 50 rpm using a magnetic stirrer. Regular samples withdrawn from the receptor compartment and equal buffer volume should replace to maintain the persistent volume. Collected samples were analyzed using UV-visible spectroscopy at 299 nm [28].

Skin irritation study

Skin irritation studies were performed to identify the skin reaction at the site of the application of nanosponge gel. Six Wistar rats were selected and isolated before 24 h of the test, maintained at a constant temperature of 25±0.5°C and under humid conditions. The hair on the dorsal part of the rat skin was shaved and cleaned with a saline solution. The rats are divided into groups: Group-I for control, Group-I for the optimized formulation, and Group III for the marketed product. The sample was applied to hair-free rat skin homogeneously covering 4 cm in three groups of rats and observed for skin reaction for 24, 48, and 72 h [29].

Table 5: Skin irritation Study

Ingredients Eudragit L100 (mg) 378nm±0.25	OLF Predicted 444	Responses Particle size	Observed 355nm
PVA (mg) 84.64%±0.45	399	%EE	85.4%
Stirring speed 96.13%±0.54	1500	Drug release	88

VI. RESULTS AND DISCUSSION

Physical Properties:

Itraconazole's sensory qualities, including color, odor, and general appearance, were estimated. Table 8.1 provides specifics of the assessment's results.

Table 6 Description Itraconazole

Characteristics properties	Specifications	Results
Appearance	White crystalline powder	White crystalline powder
Colour	White	White
Odour	Odourless	Odourless

8.1.2 Melting Point:

Itraconazole's melting point was determined by utilizing a Digital Melting Point Apparatus to introduce a little amount of the drug into a tiny capillary tube. Itraconazole's melting point is between 162°C and 165°C, according to reported data. In Table 8.2, this value is recorded.

Table no.7 Melting point of Itraconazole

Sr. No	Reported Value	Observed Value	Conclusion
	162°C to 165°C	162±3^{0}C	Matches with USP N.F.

The determined melting point was found to be 162 ± 3^{0}C. This value closely aligns with the value specified in the I.P. The deviation of ± 3^{0}C can be attributed to experimental error. Therefore, the selected drug compiles with the USP N. F. Standard.

B. Development of Calibration Curve of Itraconazole:

1. Calibration curve of Itraconazole in PBS 7.4

Table no. 8Calibration curve data of Itraconazole

Sr. No.	Concentration (µg/ml)	Absorbance
1.	5	0.165
2.	10	0.320
3.	15	0.433
4.	20	0.579
5.	25	0.748
6.	30	0.870

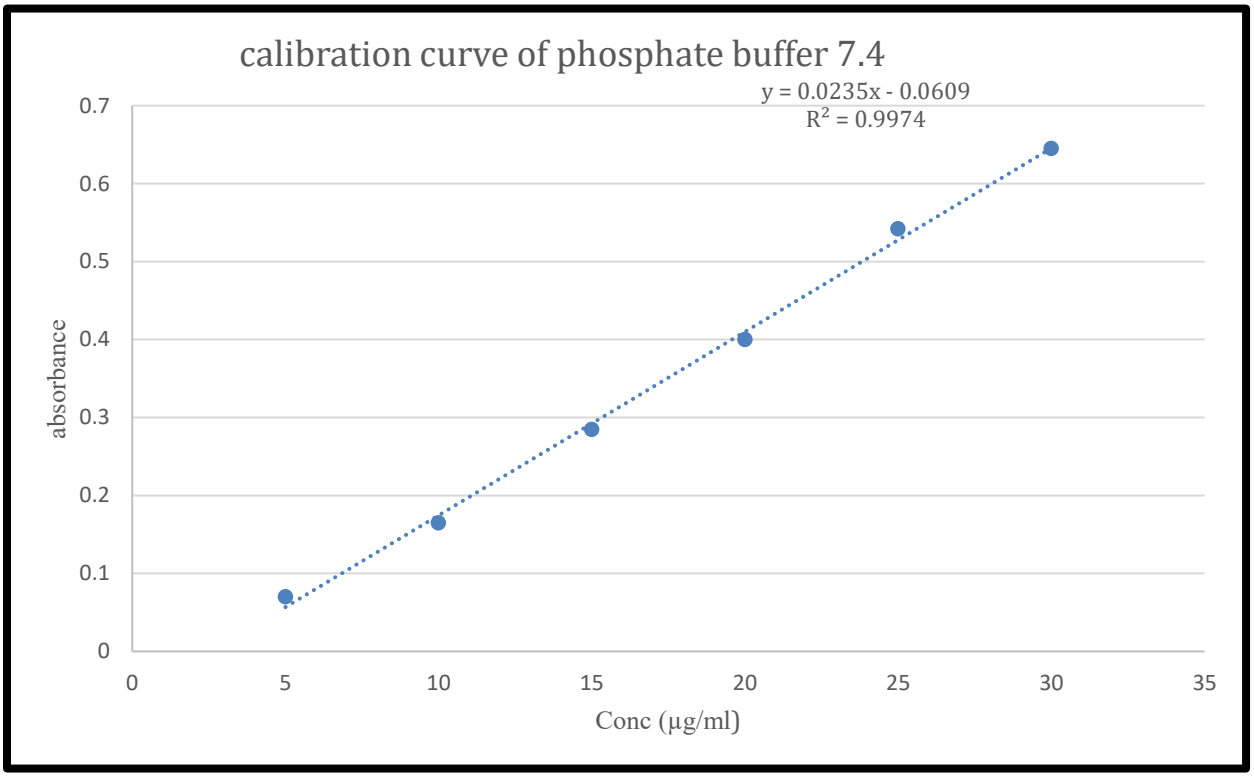


Fig.4 Calibration curve of Itraconazole in PBS 7.4

Discussion –

In the concentration range of 10 to 60 µg/ml, the istraconazole calibration curve (CC) in distilled water exhibits linearity. A high degree of linearity is shown by the regression coefficient value of 0.9990, which is close to the optimal value of 1. This suggests that the correlation with concentration and absorbance is very predictable and complies well with Beer-Lambert's rule.

Itraconazole's lambda max (λ max) in a phosphate buffer with a pH of 7.4 was found to be 262 nm based on the experimental results. This wavelength was used in order to build the calibration curve.

2. Calibration Curve of Itraconazole in phosphate buffer pH 6.8:

Table No: 9 Absorbance values in buffer 6.8

Sr. No.	Concentration (µg/ml)	Absorbance
1.	5	0.70
2.	10	0.165
3.	15	0.285
4.	20	0.400
5.	25	0.542
6.	30	0.645

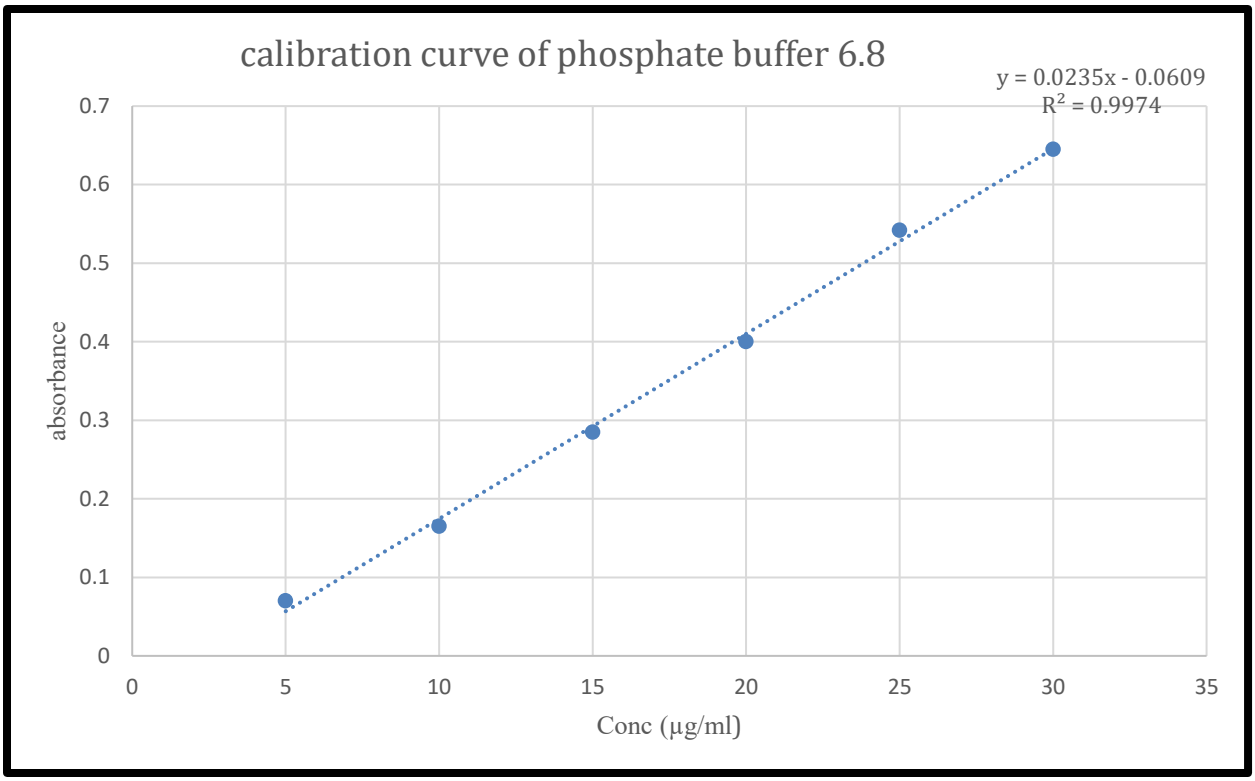


Fig No:5 Calibration curve in phosphate buffer 6.8

Itraconazole's wavelength in 6.8 pH phosphate buffer was found to be 262 nm, according to the experimental investigation. For the calibration curve's construction, this particular wavelength was used. Below are the calibration curve and the related absorbance data.

Discussion

The calibration curve was created by plotting the absorbance-concentration data, and then a linear regression analysis was performed. It has been determined that the linear concentration spread of itraconazole is between 10 and 60 µg/ml. Significantly, 262 nm was found to have the highest absorption. Additionally, the range of 10–60 µg/ml was used according to Beer-Lambert's law. The regression line equation y = 0.0228x + 0.0107 was found, along with a correlation coefficient of 0.9990. The best fit of the line is indicated by the R2 value's closeness to 1.

Solubility Test:

Drug Solubility:

Table No 10: Solubility of Itraconazole

Sr. No.	Solvent	Reported conc. (mg/ml)	Observed cnoc. (mg/ml)
1.	Phosphate buffer pH 6.8	0.00702	0.00672
2.	Water	0.0017±0.005	0.0014±0.007
3.	Dichloromethane	5.94±0.0074	5.5±0.0076

8.1.3 FT-IR Spectroscopy

FT-IR of Itraconazole

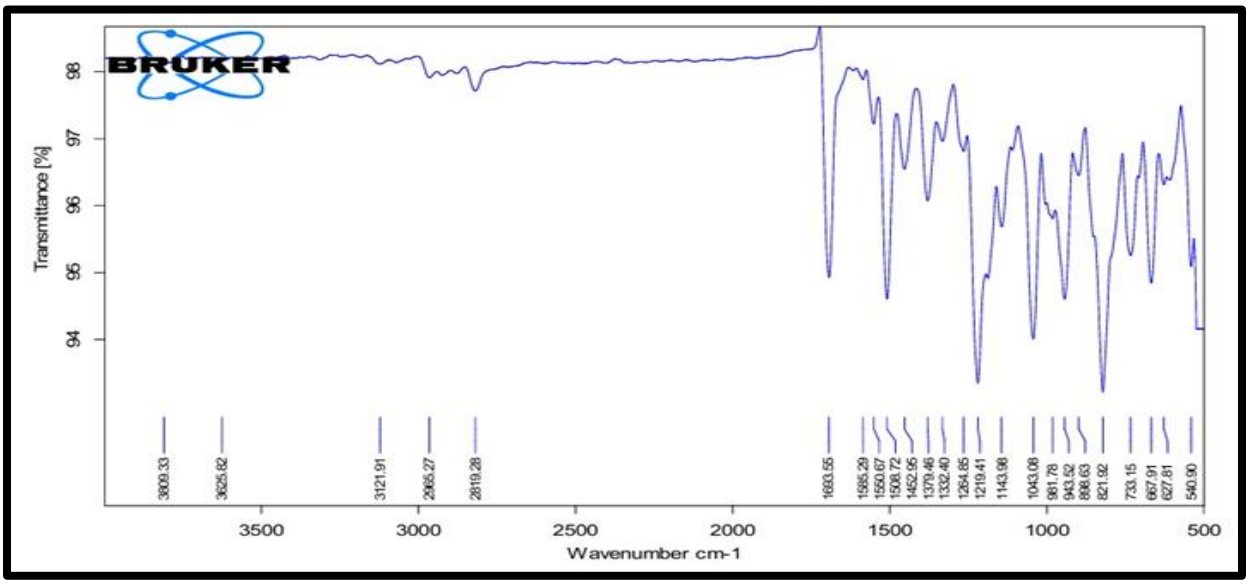


Fig No 6: FT- IR Spectrum of ITZ

Table No 11: Observation of ITZ

Functional groups	Standard ranges wave numbers in cm^{-1}	Observed wave number cm^{-1}
C-N	2210-1300	1693.55
C-Cl	600-800	733.15
C-C	1600-1650	1585.29
C-O-C	1200-1275	1264.85
C=O	1600-1900	1693.55
C-O	1200-1300	1264.85

Discussion

The unique peaks in the drug's spectral properties match the date of publication, indicating that it is itraconazole. Peaks at 2210-1300 cm-1 (C=N stretching), 600-800 cm-1 (C-Clstretching), 1600-1650 cm-1 (C=C stretching), 1200-1285 cm-1 (C-O-C stretching), 1600-1900 cm_1 (C=O stretching), and 1200-1300 cm-1 (C-O stretching) are in this group.

8.1.4 DSC of Itraconazole (Differential scanning calorimeter)

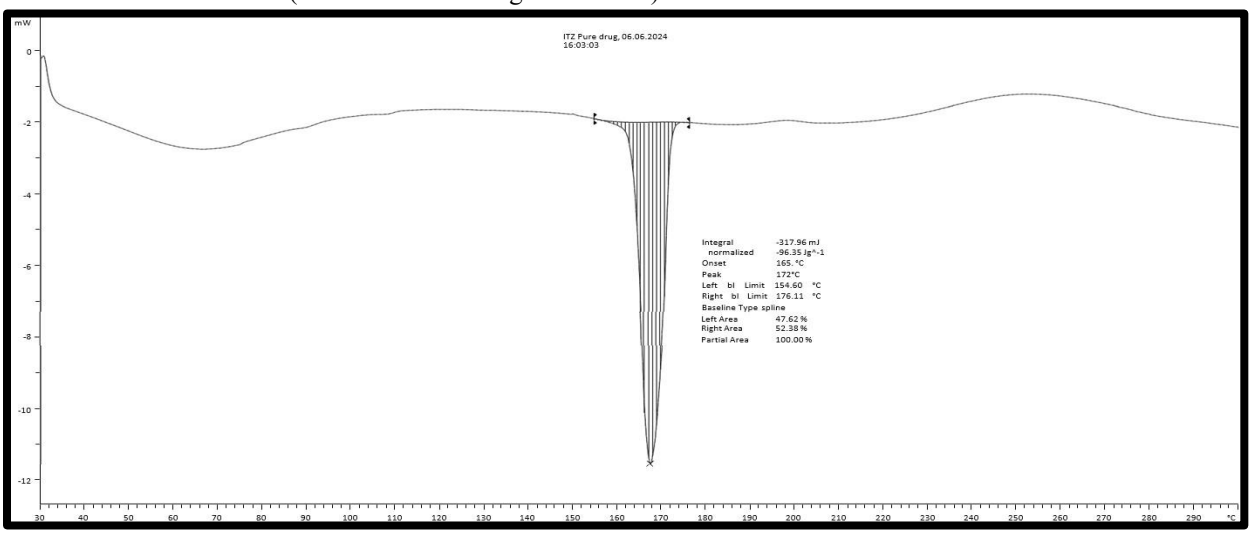


Fig no 7: DSC Thermogram of Itraconazole

Discussion

At 172^{0}C, the drug's strong endothermic peak indicates that itraconazole has a melting point of 1650C. This suggests the presence of the chemical in crystalline form. DSC was used to assess the medicines' thermodynamic properties and physical state (crystalline or amorphous).

8.2 Authentication of Polymer:

8.2.1 Physical Properties:

Visual inspection was used to analyse the polymers' physical properties, including color and appearance. The note is noted in Table No. 8.7.

1.Eudragit S100:

Tableno.12:PhysicalPropertiesofEudragit S 100

Characteristics	Reported	Observed
Color	White	White
Nature	Brittle powder	Brittle powder
Odour	Faint characteristic	Faint characteristic
	odor	odour

2. Polyvinyl Alcohol:

Table no.13: Physical Properties of PVA

Characteristics	Reported	Observed
Color	White to creamy	White
Nature	Granular powder	Granular powder
Odour	Odorless	Odorless

8.2.1 Fourier Transform Infrared spectroscopy analysis:

Examining the FT-IR spectroscopy results allowed for the verification of polymers. Tables 8.9 and 8.10 provide the interpretation of the FTIR spectrum, which is shown in Figures 8.5 and 8.6

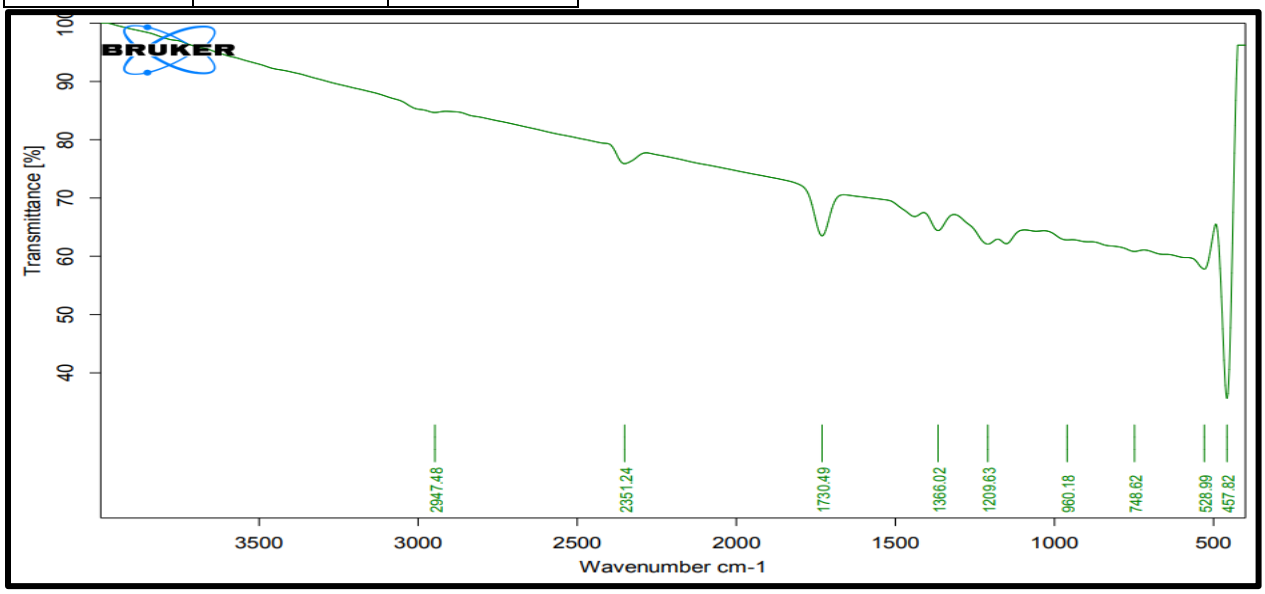


Fig.no. 8: FT-IR spectrum of Eudragit S 100

Table no 14: Interpretation of FTIR Spectrum of Eudragit S 100

Sr. no.	Functional group	Standard frequencies cm^{-1}	Observed frequencies cm^{-1}
1	O-H Stretching	3200-3600	3100
2	C-H Stretching	2900-3000	2947.48
3	C=O Stretching	1660-1780	1730.49
4	=C-H in plane bending	1330-1560	1366.02
5	C-O Stretching	900-1300	1209.63
6	C-O-C Stretching	100-1100	748.62

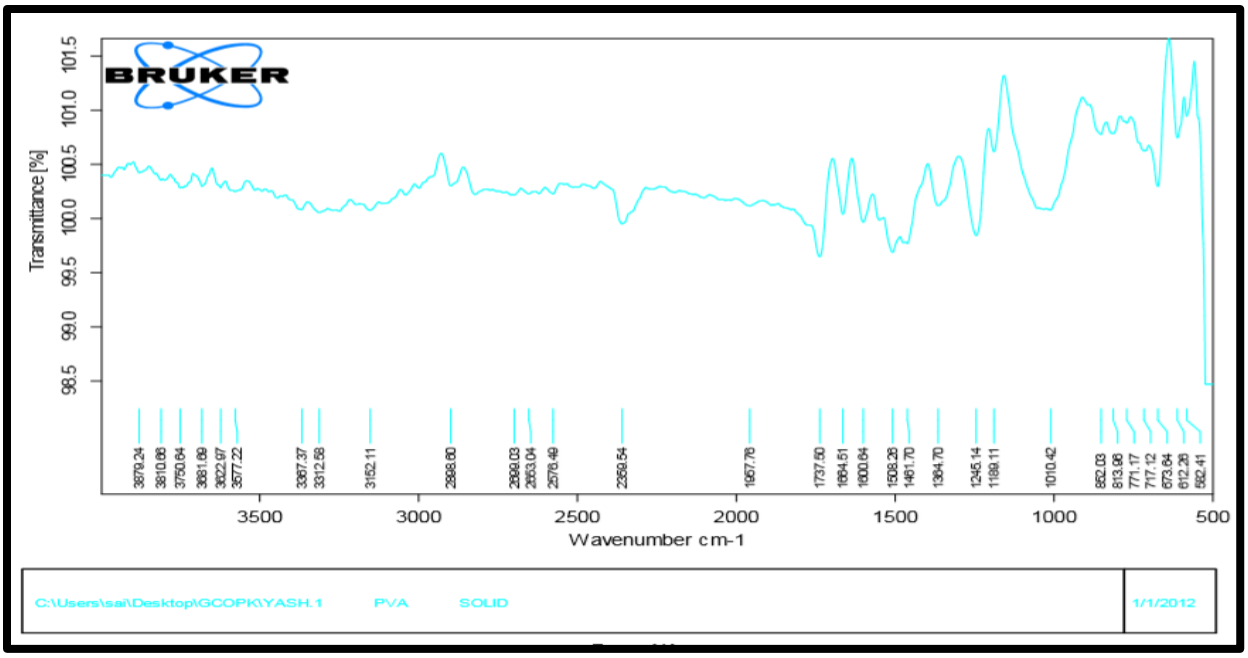


Fig.no. 9 FT-IR spectrum of PVA

Table no.15: Elucidation of FT-IR Extent of PVA

Sr. No.	Functional group	Standard frequencies cm-1	Observed frequencies cm-1
1.	O-H Stretching	3200-3400	3312.58
2.	COOH Stretching	1500-1760	1737.50
3.	=C-H in plane bending	1300-1500	1461.70
4.	C-O Stretching	900-1300	1245.14
5.	C-H out plane bending	800-830	813.96
6.	C-C Stretching	900-1000	1010.42

Discussion:
According to the literature, these peaks supported the use of polyvinyl alcohol and Eudragit S100 as the excipient.

Characterization of Itraconazole loaded Nano sponge:
Evaluation of CCD DOE batches of Nano sponges:

Table 16: CCD DOE batches of Nano sponges with factors and responses.

DOE Exp No.	Factor1	Factor 2	Response 1	Response 2	Response 3	Response 4
	Eudragit S100 (mg)	Polyvinyl Alcohol (mg)	Particle Size (nm)	Zeta Potential (mV)	PDI	EE %
NS1	200	400	123.6	-13	0.43	84
NS2	600	400	138.04	-17.5	0.426	72.5

NS3	200	600	145.45	-22	0.397	95
NS4	600	600	148.57	-23.7	0.471	93
NS5	117.157	500	138.8	-13	0.4	92
NS6	682.843	500	141.53	-14.3	0.432	82
NS7	400	358.579	127.03	-6.8	0.296	83.46
NS8	400	641.421	146.28	-23.7	0.421	93
NS9	400	500	131.95	-22.3	0.221	85
NS10	400	500	133.6	-15.8	0.376	92.2
NS11	400	500	136.03	-20	0.221	81
NS12	400	500	135.03	-15.3	0.217	87.75
NS13	400	500	134.69	-25	0.214	92

Effect of DOE Variables in the formulation of formulation:
Using an ANOVA quadratic model, statistical significance was demonstrated for itraconazole nanosponges based on the response of particle size, zeta potential, PDI, and % entrapment efficiency (Table 17–18).

Response 1: Particle Size of Nanosponges

Table 17: Response 1- Particle size of Nanosponges

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	605.42	5	121.08	22.92	0.0003	significant
A-Eudragit S100	57.36	1	57.36	10.86	0.0132	
B-PVA	444.07	1	444.07	84.07	< 0.0001	
AB	32.04	1	32.04	6.06	0.0433	
A^2	65.94	1	65.94	12.48	0.0096	
B^2	12.19	1	12.19	2.31	0.1725	
Residual	36.97	7	5.28			
Lack of Fit	27.29	3	9.10	3.76	0.1167	not significant
Pure Error	9.68	4	2.42			
Cor Total	642.40	12				

Factor coding is Coded .
Sum of squares is Type III - Partial
The Model F-value of 22.92 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2 are significant model terms.

The factor coefficients can be compared to determine the relative importance of each factor, and the equation with coded factors enables predictions of the responses for specified factor values. According to the design of experiments (DOE), response curves for nanosopnges particle size are shown in Figures 10 and 11.

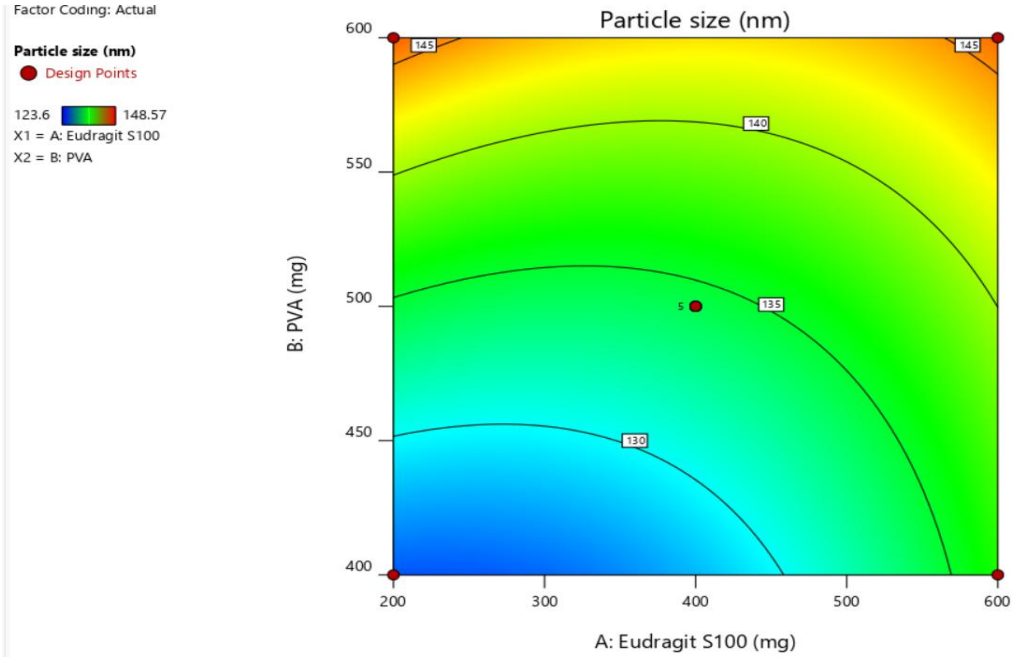


Fig no. 10 Contour plot for Particle Size

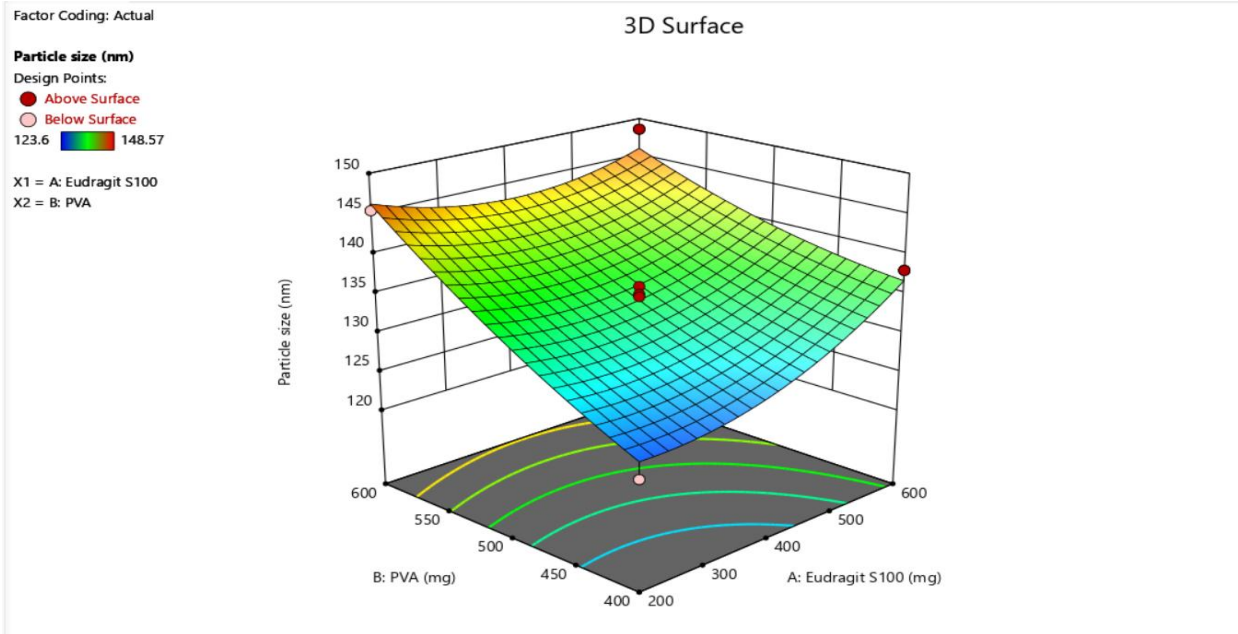


Fig no.11: 3D plot for Particle Size

Conclusion: The size of the particles was determined to be between 123.6 and 148.57 nm. The particle size of batch NS1 was the lowest, measuring 123.6 nm. The 3D Surface graph, ANOVA analysis, and the statistical data obtained from the design expert tool revealed that the particle size first falls and then progressively grows as the concentration of Eudragit S100 and Polyvinyl alcohol is raised.

Response 2: Zeta Potential of Nanosponges:

Table 18: Response 2- Zeta Potential of Nanosponges

Model	199.18	2	99.59	6.44	0.0159	significant
A-Eudragit S100	8.08	1	8.08	0.5224	0.4864	
B-PVA	191.10	1	191.10	12.36	0.0056	
Residual	154.60	10	15.46			
Lack of Fit	85.09	6	14.18	0.8162	0.6084	not significant
Pure Error	69.51	4	17.38			
Cor Total	353.78	12				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 6.44 implies the model is significant. There is only a 1.59% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term.

For certain amounts of each element, predictions regarding the reaction can be made using the equation expressed in terms of coded factors. The factors' respective impacts can be determined by comparing their factor coefficients. Nanosponges' zeta potential DOE response curves are shown in Graph No.12–13.

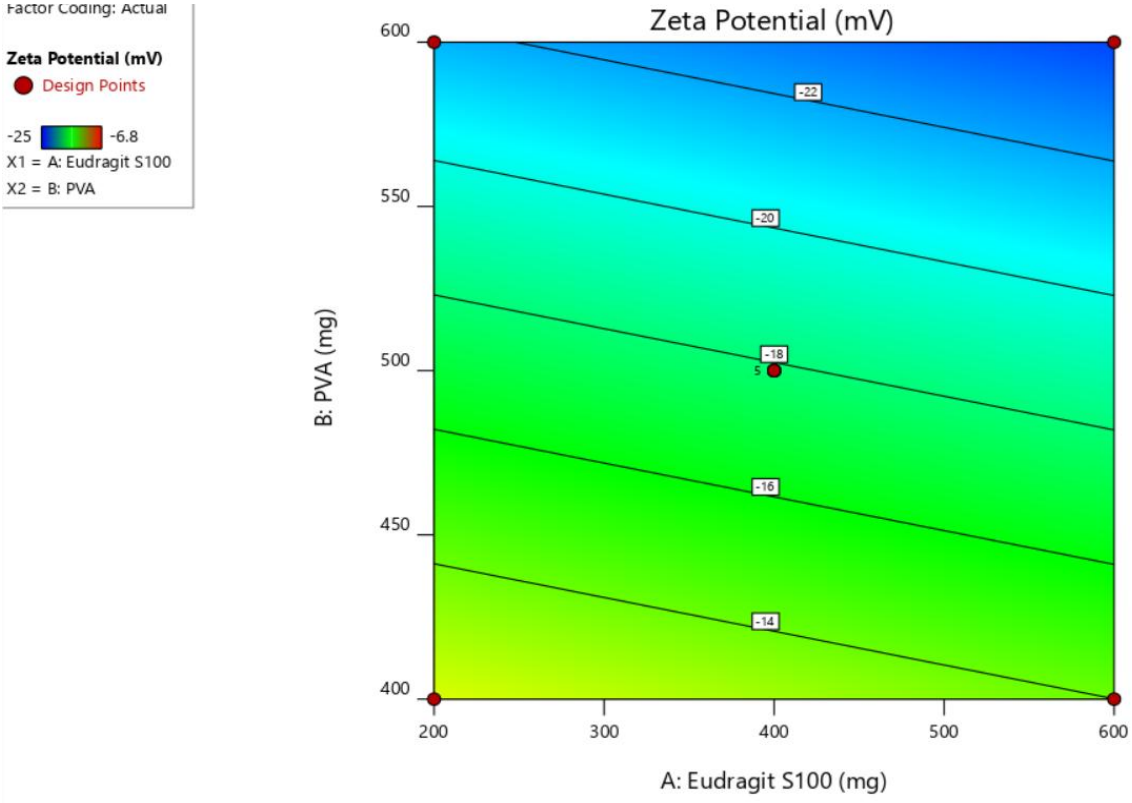


Fig no 12 Contour plot for zeta potential

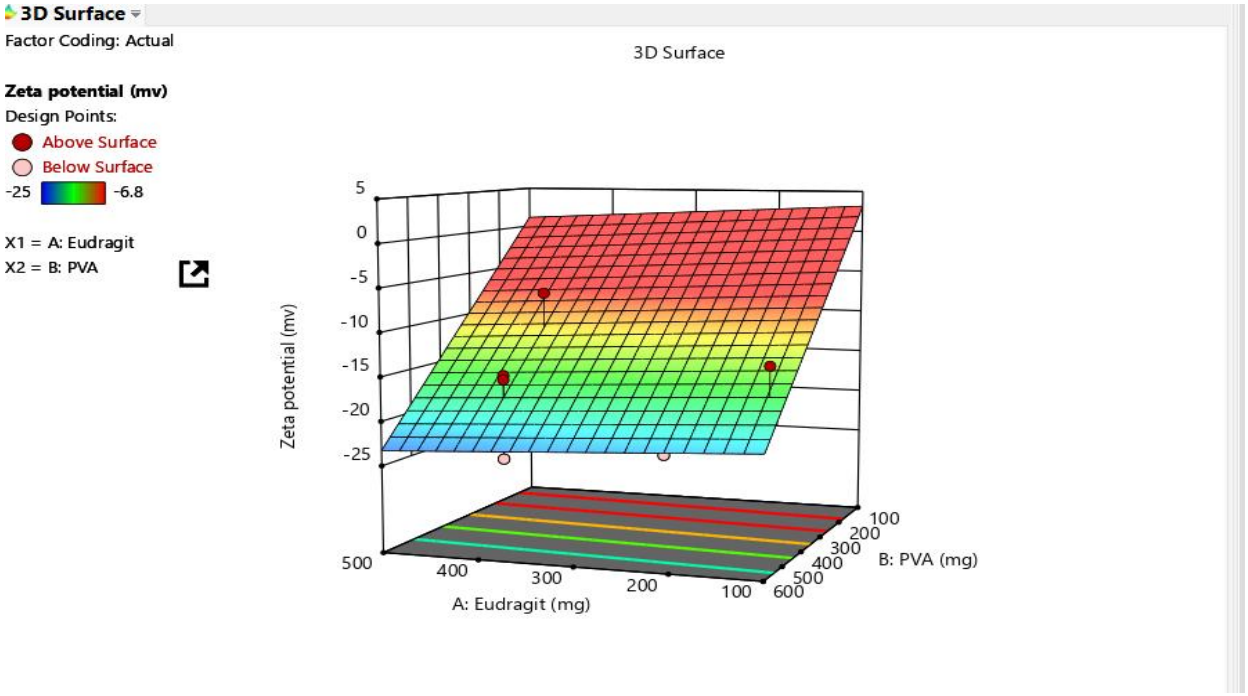


Fig 13: 3D plot for Zeta Potential

Discussion: It is the distinction between the outermost layer of a liquid and that of a particle. All batches had zeta potentials ranging from -6.8 to -23.7 mV. The zeta potential of batches NS4 and NS8 is the highest, measuring -27.7 mV. based on the statistical information obtained from the tool for design experts. The 3D surface graph and ANOVA analysis revealed that zeta potential rose in tandem with a rise in Eudragit S100 concentration. In contrast, a higher PVA concentration first reduced the zeta potential before raising it.

Response 3: Poly-dispersability Index of Nanosponges:

Table 19: Response 3- Polydispersibility Index (PDI) of Nanosponges

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0890	5	0.0178	4.58	0.0357	significant
A-Eudragit S100	0.0017	1	0.0017	0.4267	0.5345	
B-PVA	0.0045	1	0.0045	1.14	0.3202	
AB	0.0015	1	0.0015	0.3908	0.5517	
A^2	0.0615	1	0.0615	15.81	0.0054	
B^2	0.0297	1	0.0297	7.62	0.0281	
Residual	0.0272	7	0.0039			
Lack of Fit	0.0073	3	0.0024	0.4880	0.7090	not significant
Pure Error	0.0199	4	0.0050			
Cor Total	0.1163	12				

Factor coding is Coded.Sum of squares is Type III – Partial. The Model F-value of 4.58 implies the model is significant. There is only a 3.57% chance that an F-value this large could occur due to noise.P-values less than 0.0500 indicate model terms are significant. In this case A^2, B^2 are significant model terms.

DOE response curves of Nanosponges Polydispersability Index (PDI) are presented in Fig 14&15.

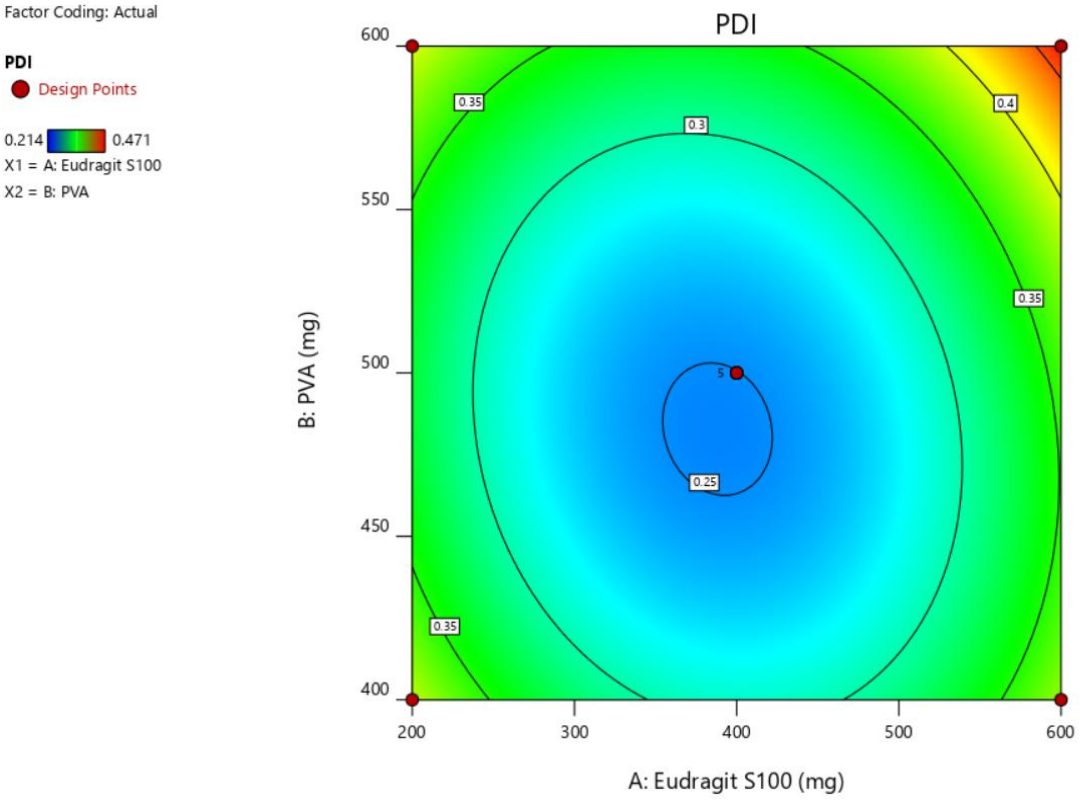


Fig no. 14 : Contour Plot for PDI

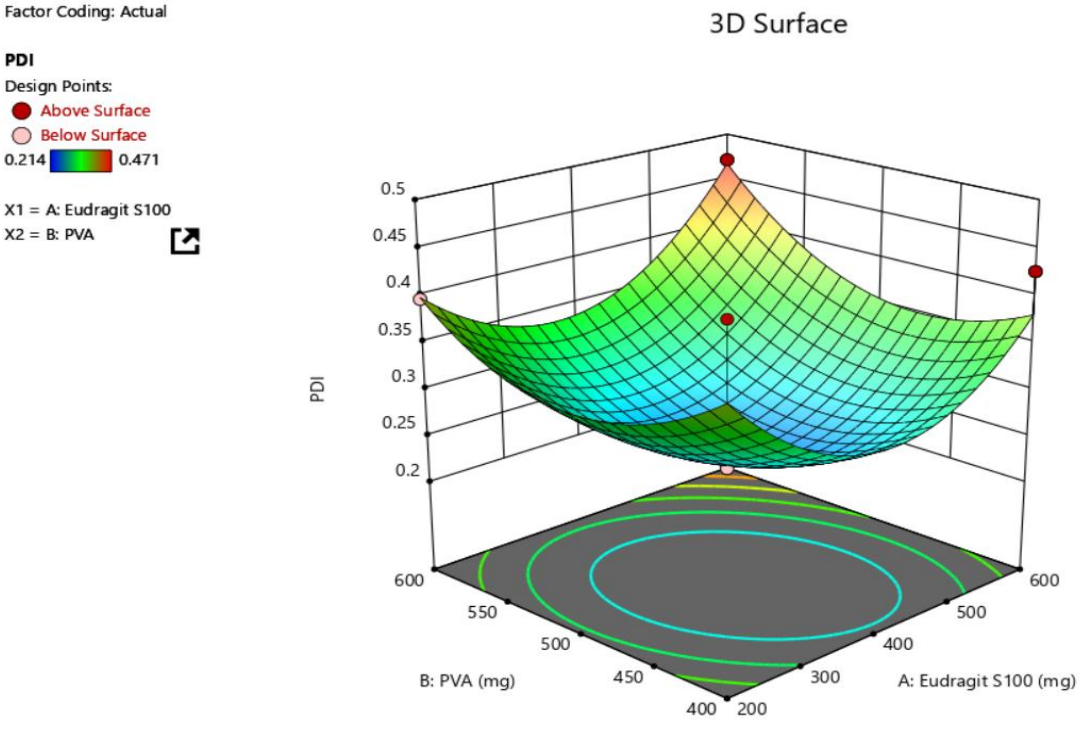


Fig no.15: 3D plot for PDI

Response 4: Entrapment Efficiency of Nanosponges

Table 20: Response 4- Entrapment Efficiency of Nanosponges

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	348.54	2	174.27	10.79	0.0032	significant
A-Eudragit S100	95.51	1	95.51	5.91	0.0354	
B-PVA	253.03	1	253.03	15.66	0.0027	
Residual	161.56	10	16.16			
Lack of Fit	70.70	6	11.78	0.5187	0.7748	not significant
Pure Error	90.86	4	22.72			
Cor Total	510.10	12				

Factor coding is Coded.
Sum of squares is Type III - Partial
The Model F-value of 10.79 implies the model is significant. There is only a 0.32% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.
The DOE response curves for the entrapment efficiency of nanosponges are shown in Figures 16 and 17.

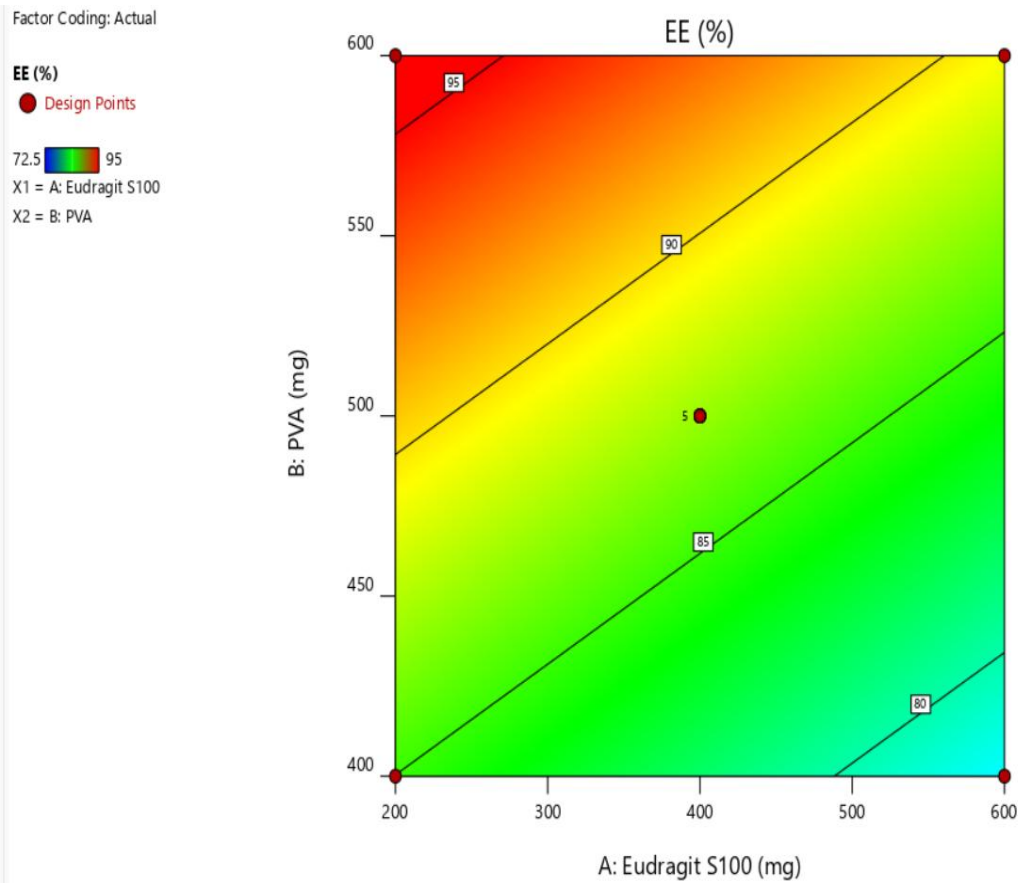


Fig no. 16: Contour plot for Entrapment Efficiency

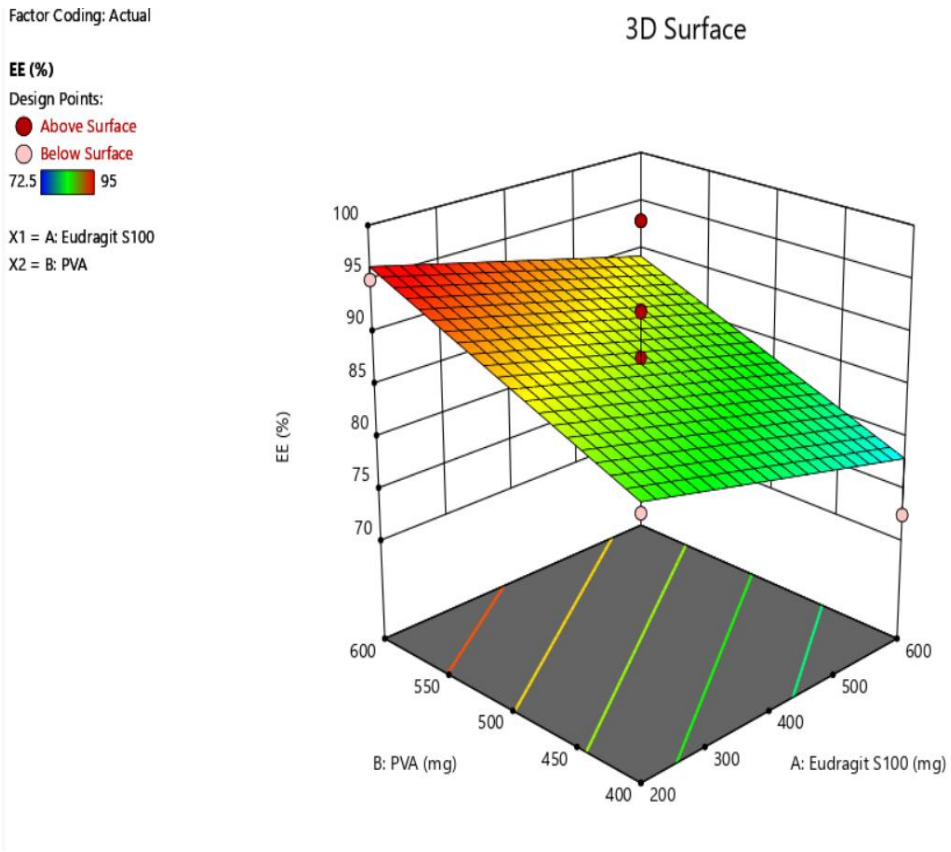


Fig.no.17: 3D plot for Entrapment Efficiency

Discussion: The %EE computation was done using an indirect method. The range is between 72.5 and 95. The amount of the medication that was not entrapped in the aqueous solution was measured using this method. We computed the Entrapment Efficiency for every batch of itraconazole nanosponges. PVA and Eudragit S100 nanosponges with increasing concentrations demonstrated better drug entrapment efficiency.

Point of Prediction:

Software called Design Expert 12 was used to statistically analyze the outcomes of the several runs. Based on the variables and reactions of DOE batches, Central Composite Design recommended optimal concentrations of Ethyl Cellulose and Polyvinyl Alcohol to achieve the most favorable result for Particles size, Zeta Potential, Polydispersability Index (PDI), and Entrapment Efficiency (%EE).

The following is the particle size (R1) polynomial equation:

$+134.26+2.68*A+7.45*B*-2.83AB*+3.08A^2+1.32B^2$ ..... (1)

The Polynomial equation for Zeta Potential (R2) is as follows:

-17.88-1.00*A-4.89*B ..... (2)

The Polynomial equation for Polydispersability Index (PDI) (R3) is as follows:

$+0.2498-0.0144*A+0.0236*B+0.0195*AB+0.0940*A^2+0.0653*B^2$ ..... (3)

The Polynomial equation for Entrapment Efficiency (%EE) (R4) is as follows:

+87.15-3.46*A+5.62B ..... (4)

A formulation was proposed by the software and chosen for additional assessment in order to produce nanosponges with the smallest particle size, within the range of zeta potential, Polydispersability Index (PDI), and highest Entrapment Efficiency (%EE). A comparison between the expected and observed values was made.

Table 21: Point Prediction

Solution 1of 1 Response	Particle Size (nm)	Zeta Potential(mV)	Polydispersability Index(PDI)	% Entrapment Efiiciency
Predicted Mean	134.26	-17.8769	0.2498	87.1469
Predicted Median	134.26	-17.8769	0.2498	87.1469
Observed	131.95	-22.3	0.421	93
Standard Deviation	2.29828	3.93195	0.0623829	4.01989
SE Mean	1.02782	1.09053	0.0278985	1.1148
95% CI low for Mean	131.83	-20.3068	0.183831	84.663
95% CI high for Mean	136.69	-15.4471	0.315769	89.6309
95% CI low for 99% Pop	121.674	-35.9882	-0.0918359	68.6325
95% CI high for 99% Pop	146.846	-0.234318	0.591436	105.661

Predicted value:
Zeta Potential, Particle Size, Polydispersability Index, and Entrapment Efficiency:

Table 22: Predicted value of Optimized Batch

Sr No.	Optimized Batch No.	Particle Size (nm)	Polydispersability Index(PDI)	Zeta Potential (mV)	Entrapment Efficiency (%EE)
1	Predicted Values	134.26	0.2498	-17.8769	87.1469
2	Observed Values	131.95	0.421	-22.3	93

Calculation Results

Peak No.	S.P. Area Ratio	Mean	S.D.	Mode
1	1.00	128.6 nm	13.4 nm	130.5nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	134.26 nm	134.26 nm	130.5 nm

Cumulant Operations

Z- Average : 159.1 nm
PI : 0.421

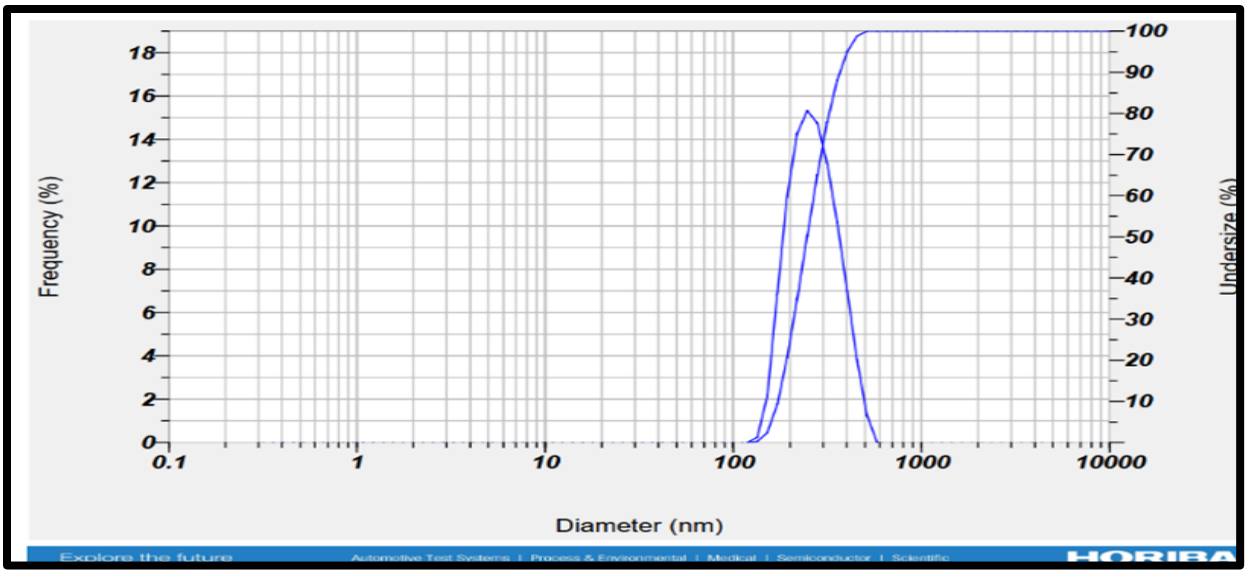


Fig No.18: Particle size for Optimized batch

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-17.8769mV	-0.000155 cm2/Vs
2	---mV	---cm2/Vs
3	---mV	---cm2/Vs

Zeta Potential (Mean) : -17.8769 mV

Electrophoretic Mobility Mean : -0.000155 cm^2/Vs

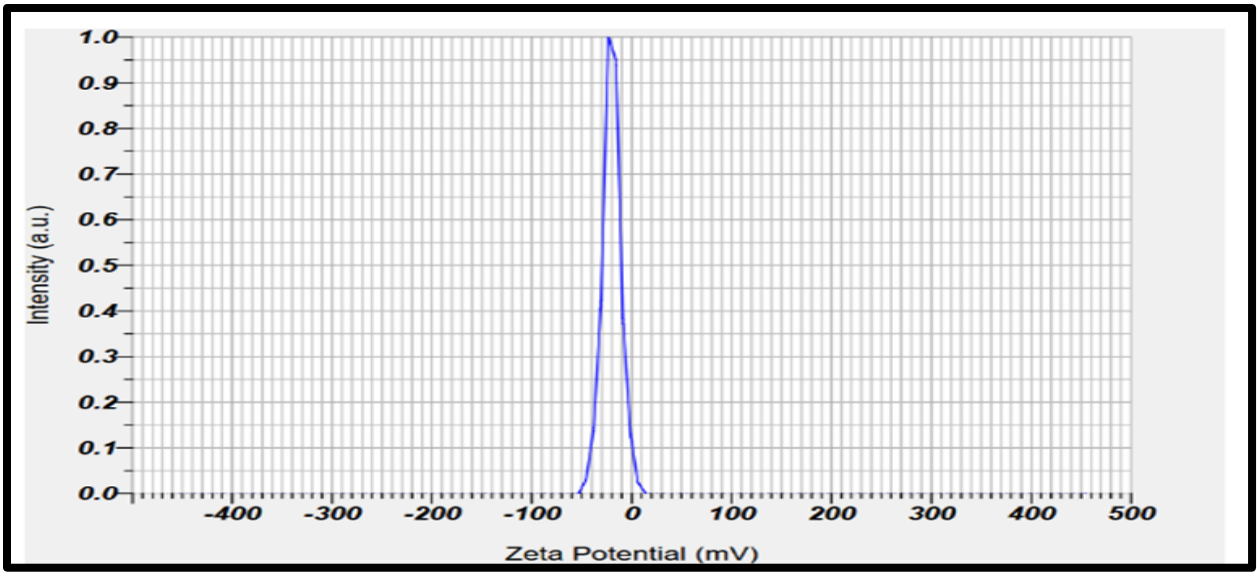


Fig No.19: Zeta potential of Optimized batch

Vesicle Morphology:

SEM: The optimized batch of nanosponges' SEM image showed that, at a 1µm size, the nanosponges were spherical.

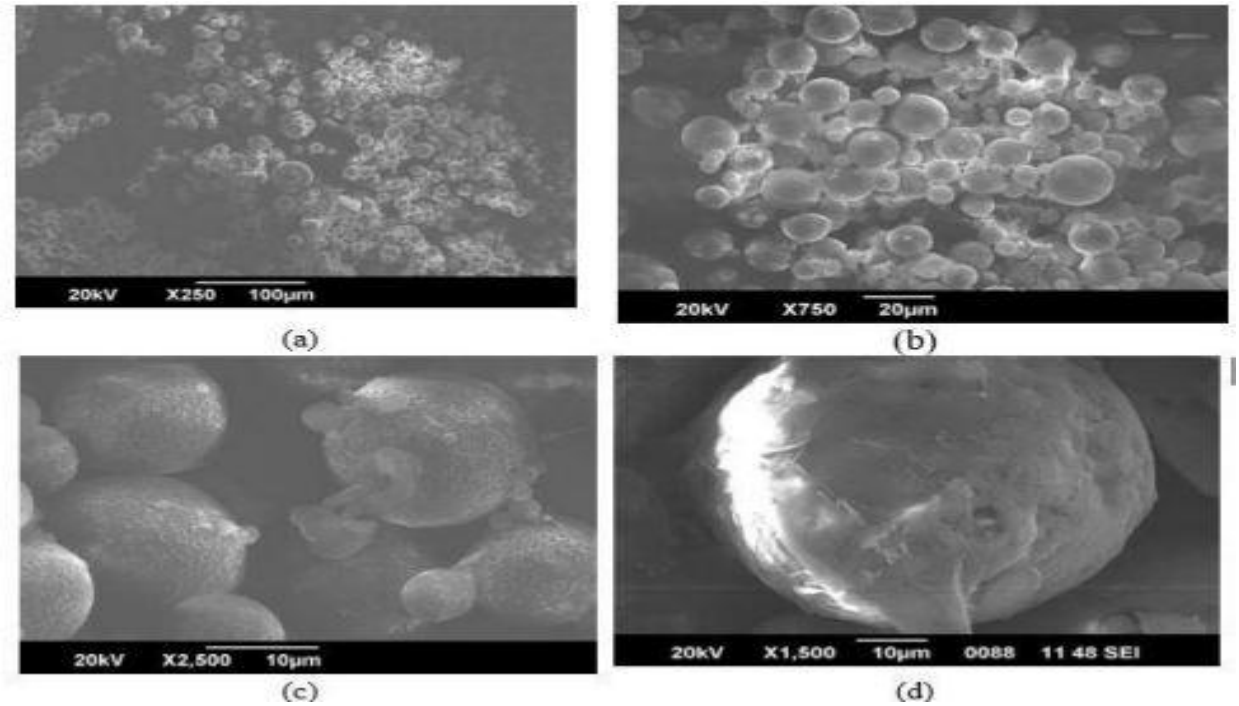


Fig 21:The optimized nanosponges in SEM

3. Entrapment Efficiency :

The outcomes of all the formulations are presented in the table. NS9 batch displayed 92.2 % EE. The entanglement of the polymer could contribute to the higher entrapment of the drug, resulting in improved entrapment efficiency.

4. FT-IR Spectroscopy Study:

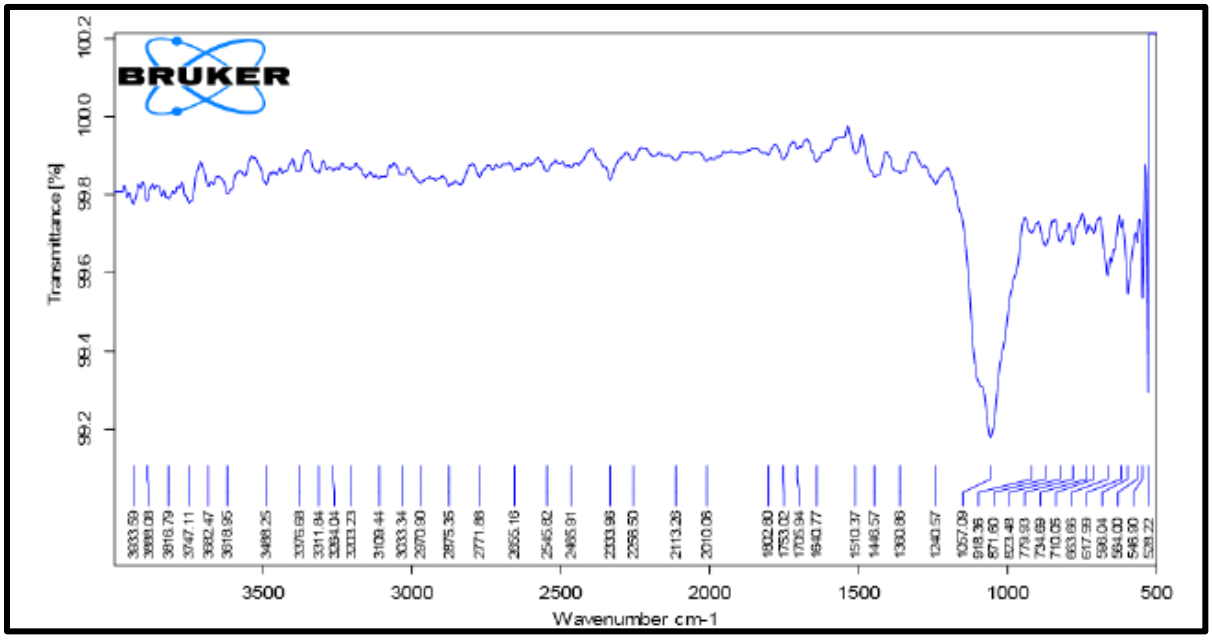


Fig no. : FT-IR spectrum of optimized batches NS9

Table no.: FT-IR data of optimized batches NS9

Material	Functional Groups	Standard frequencies cm-1	Observed frequencies cm-1
FTIR Spectrum of optimized batch NS9	N-H Stretch	3450 - 3260	3376.68
	C-H Stretch	3100 - 2800	2970.90
	C=O Stretch	1750 - 1700	1706.94
	C=C Stretch	1670 -1640	1640.77
	C-H Stretch	3100-2900	3033.34
	C-H Bend	900-675	734.69
	C-Cl Stretch	800-600	779.93

The peaks were compared and documented in Table 8.35. The peaks identified in the pure Itraconazole drug were also detected in the formulation, indicating the absence of any compatibility issues in the formulation.

5. Saturation Solubility:

Based on the closely matching solubility values observed in comparison to literature values, it can be concluded that the procured Itraconazole is likely in a pure state. This consistency suggests reliability in the composition of the compound.

Table no.: Saturation solubility data of batch NS9

Sr. No.	Formulation	Nature of solvents	Solubility reported (mg/ml)	Solubility observed (mg/ml)
1.	Itraconazole	Distilled water	0.062	0.048
2.	NS9 (optimized batch)		.....	0.412
3.	Itraconazole	pH 7.4 Phosphate Buffer	1.92	1.5
4.	NS9 (optimized batch)		....	8.3

Discussion:

The improved Nanosponge batch (NS9) demonstrated increased solubility compared to the pure drug. This enhanced solubility in both deionized water and pH 7.4 phosphate buffer is likely attributed to the presence of the hydrophilic carrier PVA. PVA facilitates wetting of drug molecules and enables localized solubilization. The saturation solubility values of both the pure drug and the optimized nanosponge batch (NS9) in deionized water and pH 7.4 phosphate buffer are presented in Table .

8.12.DEVELOPMENT OF ITRACONAZOLE LOADED HYDROGEL OF OPTIMIZED BATCHES:

8.12.1. Gelling agent selection and development :

In the initial investigation, the compatibility of Itraconazole nanosponges was evaluated with various gelling agents such as carbopol934, carbopol940, and HPMCK15. The outcomes of the gels formulated with these gel-forming agents are presented in the table provided.

Table no. : Assortment of gelling agent

Sr. No.	Gelling agent	Concentration (gm)	Problem faced
1.	Carbapol 940	0.40	Formation of drug precipitate
2.	Carbapol 934	0.40	NIL

3.	HPMC K14	0.25	It undergoes a reduction in viscosity over a 16 days period and fail to achieve uniform dispersion

In the preliminary study, Carbopol 934 was selected for the gel formulation due to its superior characteristics compared to other gelling agents. Carbopol 934 demonstrated consistent viscosity, an appealing appearance, and a pH level close to the skin's natural pH (pH 6.7).

8.12.2. Selection of preservatives:

According to findings from a literature review, propyl paraben has been used as a preservative at concentrations of 0.01%.

8.13. Evaluation Itraconazole loaded Nanosponges hydrogel:

Physical determination :

Table no.: Result of Physical determination

Formulation	Appearance	Consistency	Grittiness	Uniformity
Itraconazole-NS loaded Hydrogel	Whitish	Smooth	None	Good
Plain Itraconazole loaded Hydrogel	Whitish	Smooth	None	Good

pH Measurement :

Table no. pH of Hydrogel preparation

Skin pH range	Observed pH	Result
6.8-7.4	6.38	Within acceptable limit

Discussion

The pH of the Itraconazole loaded Nanosponges loaded hydro gel was found to be 6.38 which falls well within the acceptable range for skin application. This indicates that the formulation is non - irritating and suitable for administration through the skin route.

Viscosity determination:

Table no. : Viscosity of Hydrogel Preparation

Formulation	Viscosity (cps)
Itraconazole-NS loaded Hydrogel	9186
Plain Itraconazole loaded Hydrogel	6713

Drug content:

Drug Content of Itraconazole-NS based hydrogel was found to be 87.6 % ± 0.25.

Swelling index:

The investigation into swelling is crucial as it represents a primary mechanism for drug release in gel formulations. This process involves water permeating the gel, leading to drug dissolution within the water and subsequent release. In our study, we conducted swelling experiments using a pH 7.4 phosphate buffer. When the gel interacts with the buffer, water infiltrates the gel, causing it to swell and facilitating drug dissolution, thereby promoting drug release. Importantly, our formulations exhibited the highest percentage of swelling within a 10-hour period. This indicates a direct correlation between swelling percentage and drug release rate. Put simply, higher swelling percentages correspond to increased drug release levels.

Spredability study:

The spread ability of Itraconazole-NS based hydro gel was found to be 5.5 g cm/sec.

Fig no. : Spredability study of Hydrogel

VII. DIFFUSION STUDY

In-vitro Drug Release Studies:

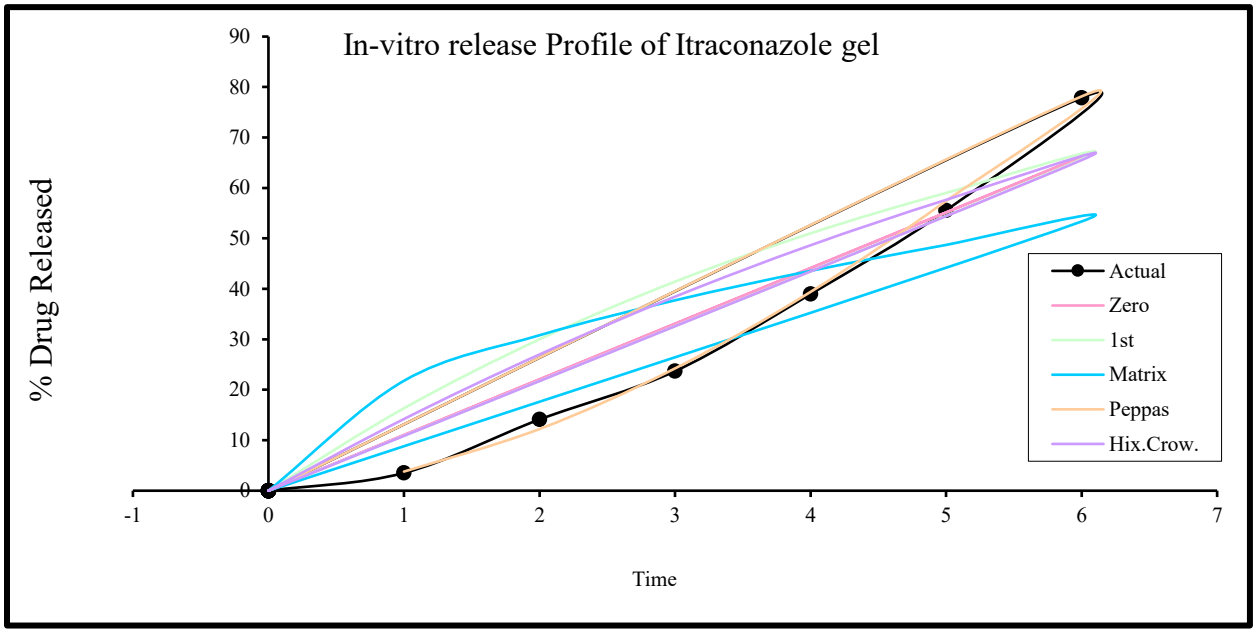
The in- vitro drug release profile of the formulated nasal gel was evaluated using a Delta diffusion cell apparatus with cellophane membrane as the barrier. The study was conducted over a 6 - hour period, and samples were collected at 1 - hour intervals. The cumulative percentage of drug released at each time point is presented

Average % Release Over the Period of 6 Hours

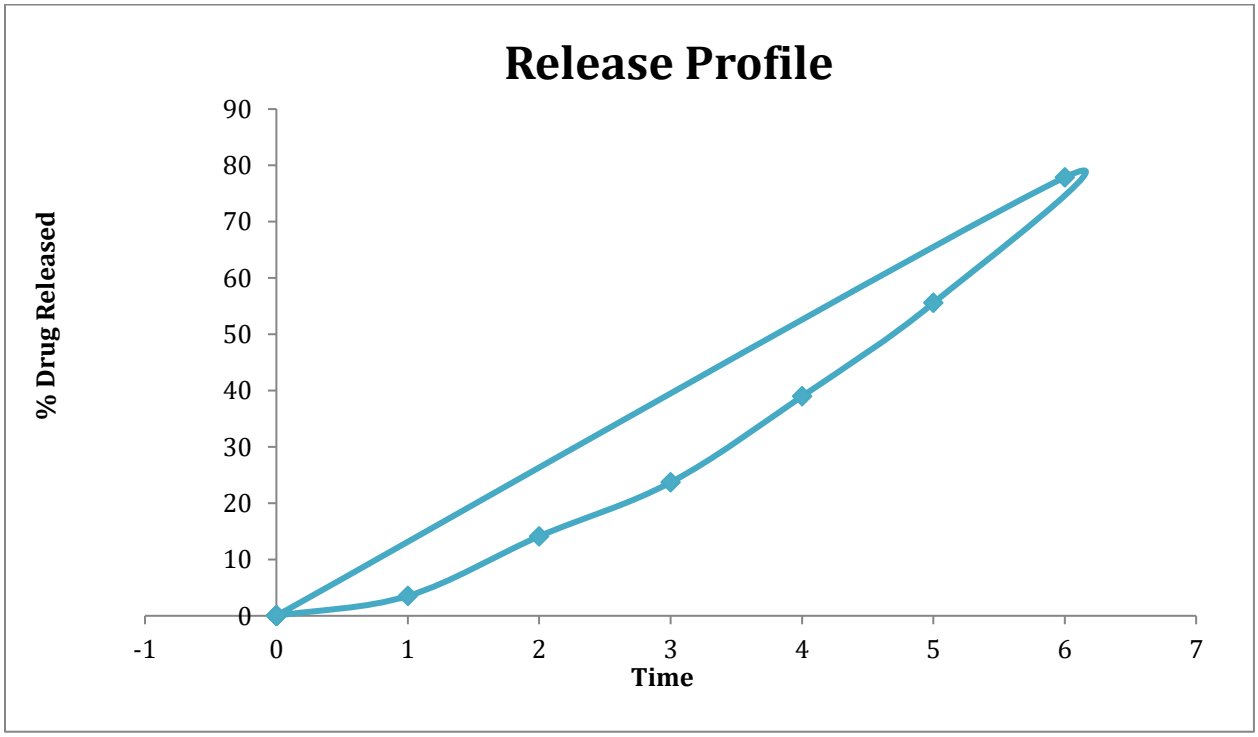
Fig no. : Delta Diffusion cell

❖ In-vitro release of Itraconazole gel

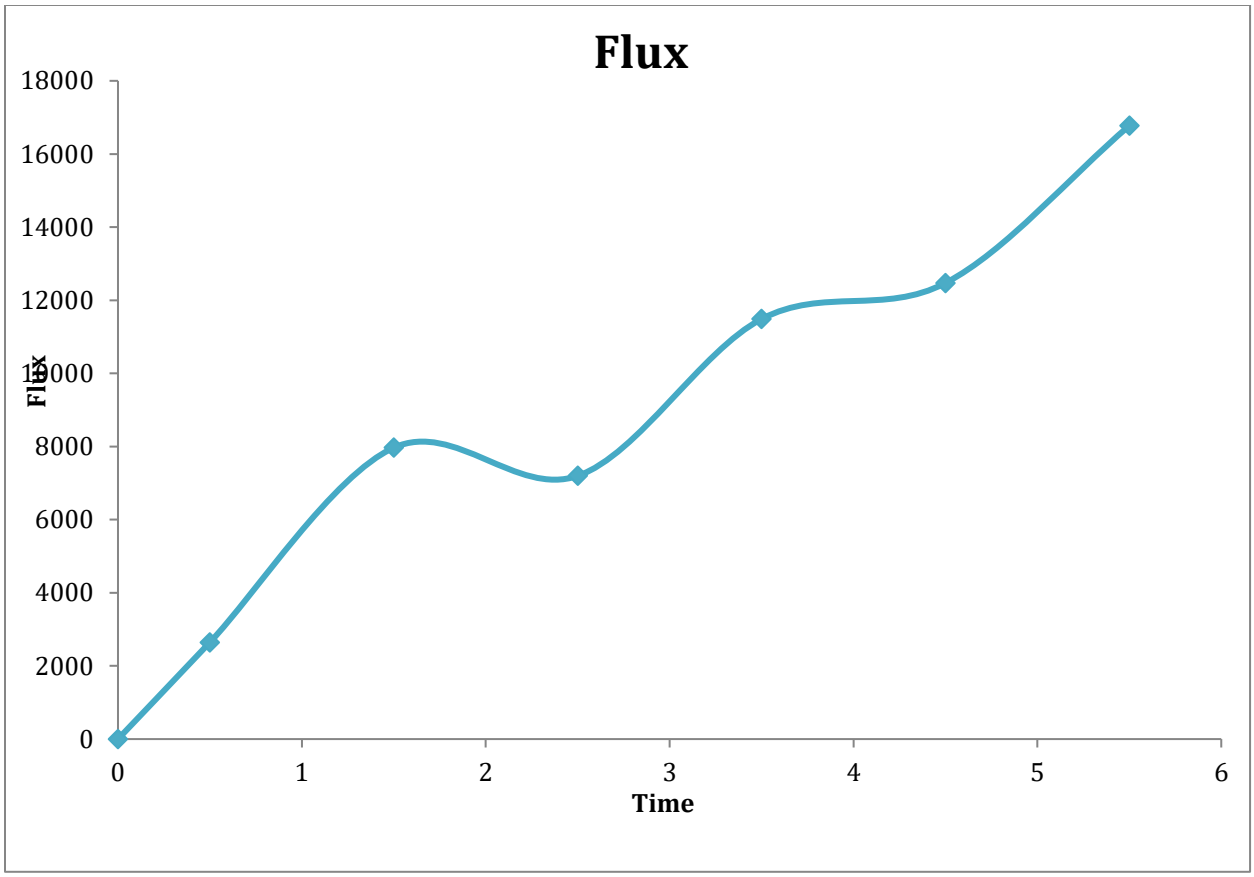
TIME	% CUMULATIVE RELEASE
0	0.000
1	3.520
2	14.117
3	23.690
4	38.968
5	55.555
6	77.864



Drug release profile (with model fitting) of optimized batch over 6 hours using cellophane membrane.



Release profile (Without model fitting) of optimized batch using cellophane membrane



Flux of optimized batch using cellophane membrane

Best fit model: Peppas model

n and k values for Korsmeyer-Peppas Equation

Parameter for Korsmeyer-Peppas Equation	
N	1.6890
k	3.7896

Discussion:

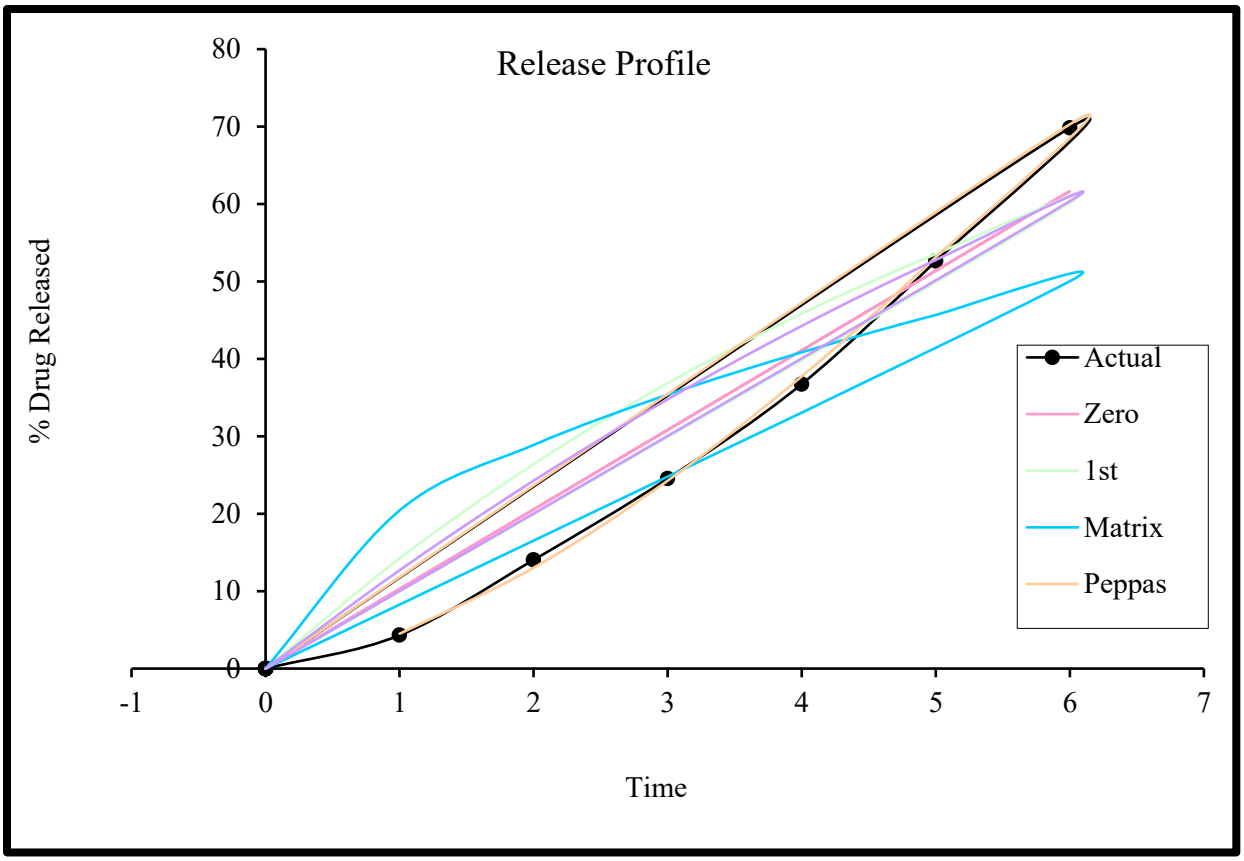
The release profile demonstrated a sustained and gradual release of the drug from the skin gel over a period of 6 hours. More than 77.86% of the drug was released by the end of the study (6 hours) , indicating effective diffusion through the formulation and cellophane membrane. Using a model- dependent approach, the PCP dissolution technique was used to analyse the drug release mechanism and rate. PCP disso V3 software used to study the drug release profile. Software suggests that peppas model is best fit for this

❖ Ex-vivo release of Itraconazole gel

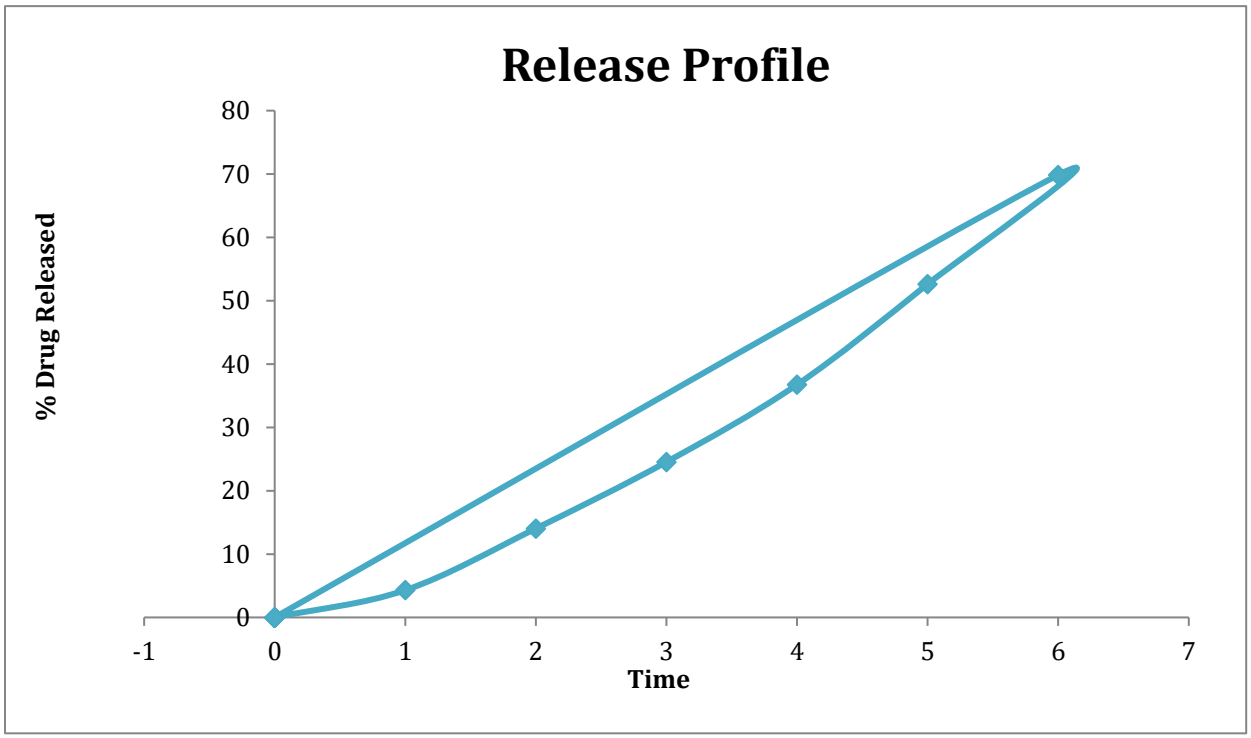
The ex-vivo permeation study was conducted using excised goat nasal mucosa mounted on a Delta diffusion cell apparatus. The cumulative percentage of drug permeated across the biological membrane was measured over a 6- hour period. The results indicate a time-dependent increase in drug permeation.

Average % Release Over the Period of 6 Hours (Optimized Batch)

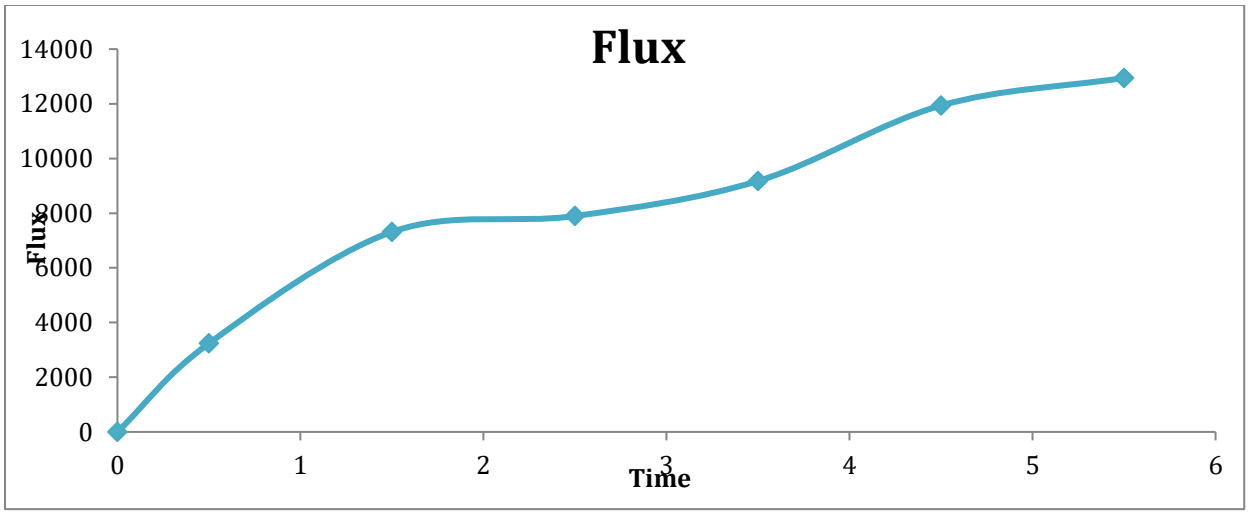
Time	% Cumulative release
0	0.000
1	4.308
2	14.038
3	24.541
4	36.746
5	52.624
6	69.841



Drug release profile (with model fitting) of optimized batch over 6 hours using Cadaver skin



Drug release profile (without model fitting) of optimized batch using cadaver skin



Flux of optimized batch using Cadaver skin

Best fit model: Peppas model

n and k values for Korsmeyer-Peppas Equation

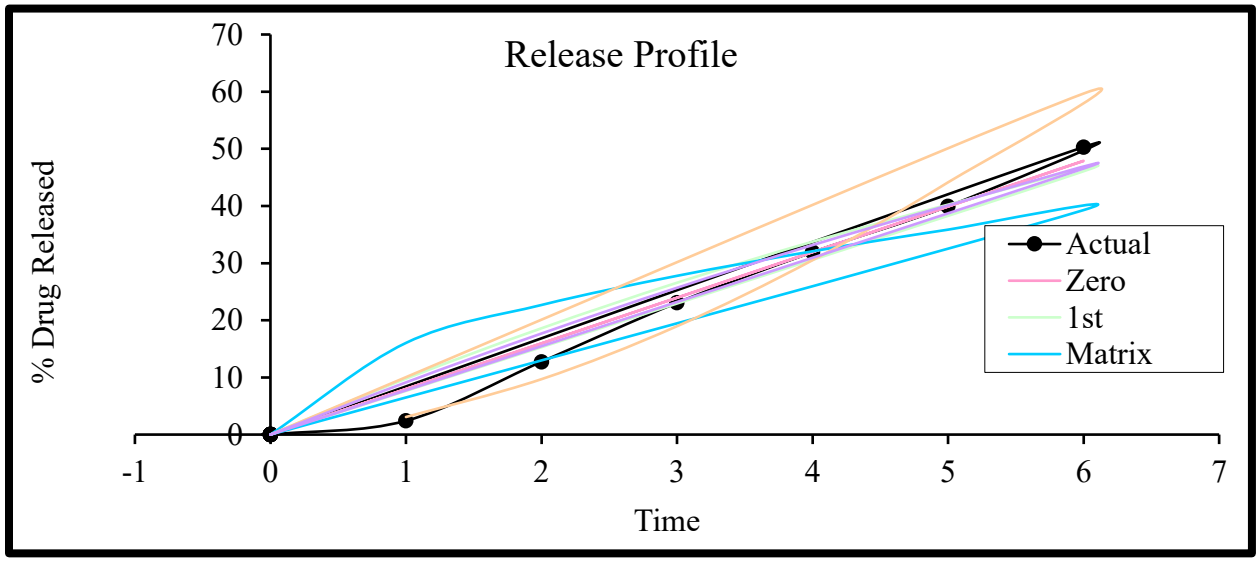
Parameter for Korsmeyer-Peppas Equation	
N	1.5343
k	4.4978

Discussion:

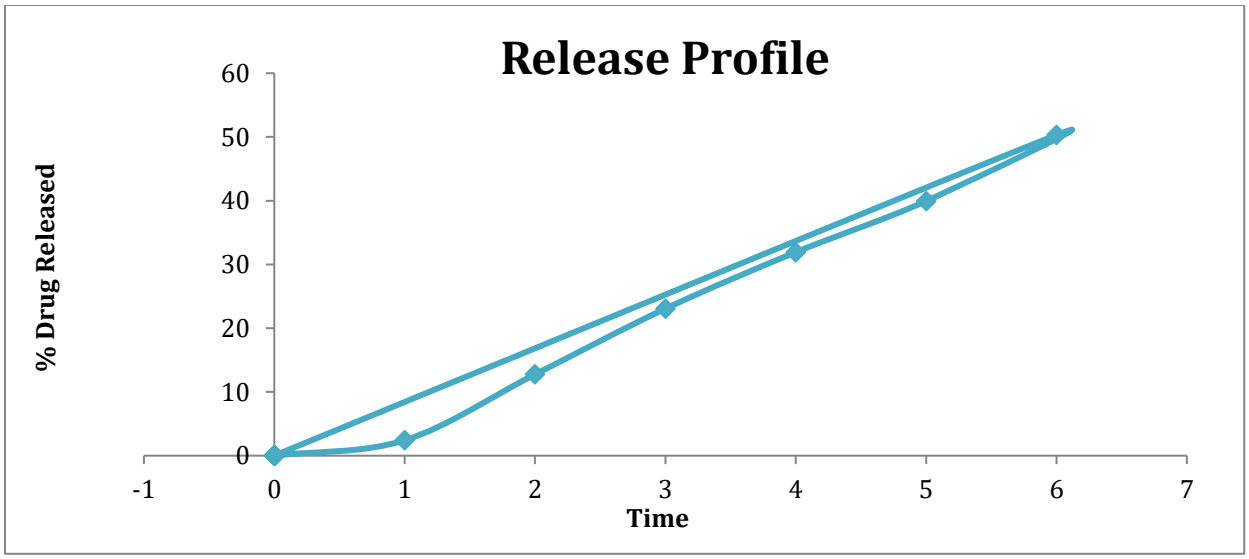
The ex-vivo drug diffusion study demonstrated a sustained and progressive permeation of the drug through the Cadaver skin. Approximately 69 % of the drug permeated the biological membrane by the end of 6 hours. The drug release profile suggests the formulation has potential for effective skin delivery, providing a sustained release over time. The consistent increase in drug permeation con firms the suitability of the skin for such delivery systems.

❖ Ex-Vivo Permeation Control release of Itraconazole gel

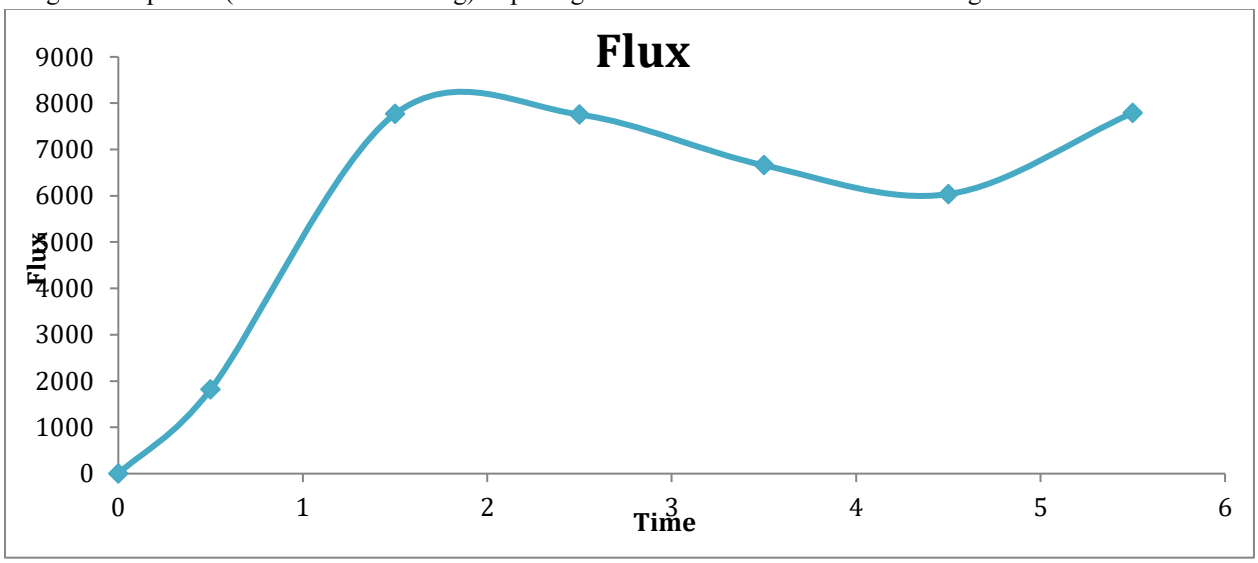
Time	% Cumulative release
0	0.000
1	2.416
2	12.746
3	23.059
4	31.915
5	39.943
6	65.34



Drug release profile (with model fitting) of plain gel of Itraconazole over 6 hours using Cadaver skin



Drug release profile (without model fitting) of plain gel of Itraconazole over 6 hours using Cadaver skin



Flux of Plain gel of Itraconazole using Cadaver skin

Discussion:

The ex-vivo diffusion study demonstrated a gradual increase in the amount of Itraconazole permeating through the Cadaver skin. By the end of 6 hours, approximately 65% of the drug had permeated the skin. The release profile was steady, but the diffusion rate was moderate, likely due to the lack of permeation enhancers in the plain gel formulation. While the drug was able to diffuse through the skin, it suggests that further optimization of the gel formulation (e.g., with permeation enhancers, skin permeable agents, or particle size reduction) could significantly improve the rate of drug permeation and bioavailability for skin.

VIII. CONCLUSION

The nano based gel formulation is ideal for the effective treatment of fungal infections, since the nanocarrier could permeate the drug deeper into skin layer where other topical semisolid preparations may not reach. Nanocarriers improve therapeutic efficacy by channelizing drug into target site deeper into skin layers for complete eradicated of fungal infections. The developed Itraconazole loaded NS impregnated Carbopol polymeric gel could be efficient drug delivery system (DDS) of antifungal agent for the effective treatment of fungal infections by sustaining the drug release, thereby reducing the dosing frequency and reoccurrence of SFI. Reoccurrence of fungal infection is very often in Candidiasis and

Aspergillosis, therefore Itraconazole loaded topical gel could be considered as a potential DDS in the treatment of skin-related fungal infections

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