

Formulation and Evaluation of Voriconazole Microsphere Loaded Gel for the Treatment of Dermatophytosis

Jadhav Himani Nitin^{1*}, Trusha P. Shangrapawar², Ashok Bhosale³

¹Department of Pharmaceutics,

^{1*} PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre Kharadi Pune.

Email : himanijadhav7276@gmail.com

²Assistant Professor, Department of Pharmaceutics, PDEA's

Shankarrao Ursal College of pharmaceutical sciences and research centre Kharadi Pune

³Principal, PDEA's Shankarrao Ursal College of pharmaceutical sciences and research centre Kharadi Pune

ABSTRACT

Dermatophytosis (ringworm) is a common superficial fungal infection caused by dermatophytes such as *Trichophyton*, *Microsporum*, and *Epidermophyton*, affecting the skin, hair, and nails. Although Voriconazole is an effective broad-spectrum antifungal agent, conventional topical formulations often suffer from poor skin retention and limited drug penetration, resulting in reduced therapeutic efficacy. The present study aims to formulate and evaluate a Voriconazole microsphere-loaded gel for the treatment of dermatophytosis. Voriconazole-loaded microspheres will be prepared using the ionotropic gelation method employing sodium alginate and Eudragit RS100 as polymers, followed by incorporation into a Carbopol 934P gel base. The prepared microspheres will be evaluated for particle size, zeta potential, entrapment efficiency, and surface morphology. The microsphere-loaded gel will be assessed for physicochemical properties such as appearance, pH, viscosity, spreadability, drug content, and in vitro drug diffusion. A comparative evaluation with a plain Voriconazole gel will be performed to determine the enhancement in sustained drug release and antifungal performance. Stability studies will also be conducted on the optimized formulation. The developed microsphere-loaded gel is expected to provide controlled drug release, improved skin retention, enhanced antifungal efficacy, and better patient compliance, making it a promising topical delivery system for the effective management of dermatophytosis.

Keywords: Dermatophytosis, Voriconazole, Microspheres, Microsphere-loaded gel, Ionotropic gelation, Controlled drug release, Topical drug delivery, Antifungal activity.

Conflict of Interest Statement: The author declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

INTRODUCTION

Fungal infections are among the most common dermatological disorders affecting millions of people worldwide. These infections are caused by various fungi, including dermatophytes, yeasts, and molds, and can affect the skin, nails, hair, and mucosal tissues. The increasing incidence of fungal infections, coupled with the emergence of antifungal resistance, has created a need for more effective and targeted drug delivery systems. [1,2]

Voriconazole is a broad-spectrum triazole antifungal agent widely used in the treatment of invasive and superficial fungal infections. It exhibits potent activity against a wide range of pathogenic fungi, including *Candida* and *Aspergillus* species. However, its conventional administration may be associated with systemic side effects, variable bioavailability, and frequent dosing requirements. Therefore, the development of topical delivery systems has gained considerable attention to improve localized drug action while minimizing systemic exposure. [3,4]

Microspheres are small spherical particles that can encapsulate drugs and provide controlled and sustained drug release. They offer several advantages, including enhanced drug stability, improved drug retention at the application site, reduced dosing frequency, and better patient compliance. Incorporation of drug-loaded microspheres into a gel base further improves the ease of application, spreadability, and residence time on the skin. [5,6]

A microsphere-based gel system combines the benefits of both microspheres and topical gels, enabling prolonged drug release and improved antifungal efficacy. Such systems can maintain therapeutic drug concentrations at the site of infection for an extended period while reducing systemic absorption and associated adverse effects. Therefore, the development of a Voriconazole-loaded microsphere gel represents a promising approach for effective topical antifungal therapy and enhanced treatment outcomes. [7,8]

MATERIALS AND METHODS:

Materials:

Voriconazole was obtained as a gift sample from Aarti Pharma. Sodium alginate was procured from Sentalab Industries and used as the matrix-forming polymer. Calcium chloride (CaCl_2), employed as a crosslinking agent, Eudragit RS 100 as a rate-controlling polymer, Carbopol 934 as a gelling agent, glycerin as a humectant, methyl paraben as a preservative, and triethanolamine as a pH-adjusting agent were purchased from Research Lab Fine Chem Industry, Mumbai.

Methods:

Characterization of Voriconazole

Organoleptic Properties

The received sample of Voriconazole was evaluated for its organoleptic characteristics, including appearance, color, and odor. The observed properties were recorded and compared with the reported specifications.[9]

Melting Point Determination

The melting point of Voriconazole was determined using the capillary tube method. A small quantity of the drug was filled into a capillary tube and attached to a thermometer. The assembly was immersed in a paraffin oil bath and heated gradually. The temperature at which the drug completely melted was recorded as the melting point. [10]

Solubility Study

The solubility of Voriconazole was determined in water, methanol, and phosphate buffer (pH 7.4). An excess amount of drug was added separately to 2 mL of each solvent, sonicated for 10 min, and shaken mechanically for 24 h using an orbital shaker. The samples were centrifuged at 2000 rpm for 10 min and filtered through a 0.45 μm membrane filter. The filtrates were suitably diluted and analyzed spectrophotometrically at 256 nm to determine drug concentration.[11]

Spectrophotometric Analysis of Voriconazole

Determination of λ_{max}

Voriconazole solutions were prepared separately in methanol, water, and phosphate buffer (pH 7.4). The solutions were scanned using a UV-Visible spectrophotometer over the wavelength range of 200–800 nm for methanol and 200–400 nm for water and phosphate buffer. The absorption maximum (λ_{max}) was determined and compared with the reported standard spectrum.[12]

Calibration Curve of Voriconazole

Calibration Curve in Methanol

A standard solution was prepared by dissolving 25 mg of Voriconazole in methanol and making the volume up to 25 mL. From this solution, 10 mL was diluted to 100 mL with methanol to obtain a stock solution (100 $\mu\text{g/mL}$). Aliquots of 1–5 mL were transferred into separate 10 mL volumetric flasks and diluted with methanol

to obtain concentrations of 10–50 µg/mL. The absorbance of each solution was measured at 256 nm against methanol as blank, and a calibration curve was constructed.

Calibration Curve in Water

A standard solution containing 25 mg of Voriconazole was prepared in water and diluted to 25 mL. Subsequently, 10 mL of this solution was diluted to 100 mL with water to obtain a stock solution (100 µg/mL). Appropriate aliquots were further diluted to obtain concentrations ranging from 10–50 µg/mL. Absorbance was measured at 256 nm using water as blank, and a calibration curve was plotted.

Calibration Curve in Phosphate Buffer (pH 7.4)

A standard solution was prepared by dissolving 25 mg of Voriconazole in phosphate buffer (pH 7.4) and making the volume up to 25 mL. A stock solution of 100 µg/mL was prepared by diluting 10 mL of the standard solution to 100 mL. Further dilutions (10–50 µg/mL) were prepared and analyzed spectrophotometrically at 256 nm using phosphate buffer (pH 7.4) as blank. A calibration curve was plotted between concentration and absorbance.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Analysis of Voriconazole

The FTIR spectrum of Voriconazole was recorded using an FTIR spectrophotometer (Jasco FT/IR-4600, Japan) to confirm the identity of the drug and characterize its functional groups. The obtained spectrum was compared with reported reference spectra for authentication.[13]

Drug–Excipient Compatibility Study

Compatibility studies between Voriconazole and selected excipients were performed using FTIR spectroscopy. Physical mixtures of the drug and excipients were analyzed, and the spectra obtained were compared with that of the pure drug to identify any potential interactions or chemical incompatibilities.[14]

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry analysis was carried out to evaluate the thermal behavior, crystallinity, and purity of Voriconazole. The sample was subjected to controlled heating, and the thermogram obtained was analyzed to determine the characteristic melting endotherm of the drug.[15]

Preparation of Voriconazole Microspheres -Loaded by Ionotropic Gelation Method

Voriconazole-loaded microspheres were prepared using the ionotropic gelation technique. Sodium alginate and Eudragit RS 100 were dispersed in 10 mL of purified water to obtain a homogeneous polymeric solution. Voriconazole (100 mg) was then incorporated into the polymer mixture and stirred continuously to form a uniform viscous dispersion.

The resulting dispersion was extruded dropwise through a 22-gauge needle into an aqueous calcium chloride solution under continuous stirring at 200 rpm. Upon contact with the cross-linking solution, [16,17] the droplets underwent ionic gelation and formed microspheres. The formed microspheres were allowed to remain in the calcium chloride solution for 30 min to ensure complete curing and hardening.

The microspheres were subsequently collected by decantation, washed repeatedly with purified water to remove residual calcium chloride from their surface, and dried at room temperature or in a hot air oven until a constant weight was obtained.[18]

Table No.2 Formulation Table of Microspheres

Batches	Sodium Alginate (g)	Eudragit RS100 (g)	Voriconazole (Drug) (g)	Calcium Chloride (%)	Distilled Water
F1	0.5	0.2	0.1	5	25 mL

F2	0.5	0.4	0.1	5	25 mL
F3	0.75	0.2	0.1	5	25 mL
F4	0.75	0.4	0.1	5	25 mL
F5	1.0	0.2	0.1	5	25 mL
F6	1.0	0.4	0.1	5	25 mL

Preparation of Voriconazole Microsphere-Loaded Gel

The microsphere-loaded gel was prepared using Carbopol 934P as the gelling agent. Accurately weighed Carbopol 934P was dispersed in distilled water containing glycerol and stirred using a homogenizer until a uniform dispersion was obtained. Methyl paraben was incorporated as a preservative. The resulting mixture was sonicated for 20 min to eliminate entrapped air bubbles and facilitate complete hydration of the polymer. The pH of the formulation was adjusted by the dropwise addition of 50% w/w triethanolamine until a clear and translucent gel was formed.[19]

Incorporation of Microspheres into Gel

The Voriconazole-loaded microspheres prepared by the ionotropic gelation method were incorporated into the prepared Carbopol 934P gel base. The microspheres were added gradually and mixed uniformly using a magnetic stirrer at 25 rpm for 2 min to ensure homogeneous distribution throughout the gel matrix. The final microsphere-loaded gel was then stored in suitable containers for further evaluation.[19]

Formulation of Microsphere Loaded Gel: -

Table No.3 Formulation Table of Microsphere Loaded Gel Batches

Ingredients	G1	G2	G3
Drug-loaded microspheres	1 g	1 g	1 g
Carbopol 934	0.1g	0.2g	0.3g
Propylene glycol	2 g	2g	2g
Methyl paraben	0.02 g	0.02g	0.02g
Triethanolamine	q.s.	q.s	q.s
Distilled water	q.s	q.s	q.s

EVALUATIONS OF PREPARED VORICONAZOLE-LOADED MICROSPHERES:

Determination of Particle Size and Zeta Potential

The particle size and zeta potential of the prepared Voriconazole-loaded microspheres were determined using a particle size analyzer. The microsphere suspension was appropriately diluted with distilled water and analyzed at room temperature. The average particle size, and zeta potential were recorded.

Percentage Yield

The percentage yield of the prepared microspheres was determined by comparing the practical yield obtained after drying with the theoretical yield of the formulation components. The microspheres were collected, dried to constant weight, and weighed accurately. The percentage yield was calculated using the following equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Practical Yield}}{\text{Theoretical yield}} \times 100$$

Where:

- **Practical Yield** = Actual weight of microspheres obtained after preparation and drying.
- **Theoretical Yield** = Total weight of drug and polymer initially used in the formulation.

Drug Entrapment Efficiency

The drug entrapment efficiency of the prepared microspheres was determined by estimating the amount of Voriconazole entrapped within the polymeric matrix. An accurately weighed quantity of microspheres was crushed and dissolved in a suitable solvent. The solution was filtered, suitably diluted, and analyzed using a

UV-Visible spectrophotometer at 256 nm. The drug entrapment efficiency was calculated using the following equation:

$$\text{Drug Entrapment Efficiency (\%)} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

In Vitro Drug Release Study

The in vitro drug release study of Voriconazole-loaded microspheres was carried out using a USP dissolution apparatus. Microspheres equivalent to a predetermined amount of drug were placed in the dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$ with continuous stirring. At predetermined time intervals, aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The samples were filtered, suitably diluted, and analyzed spectrophotometrically at 256 nm. The cumulative percentage drug release was calculated and plotted against time using the following equation:

$$\text{Cumulative Drug Release (\%)} = \frac{\text{Amount of Drug Released}}{\text{Total Amount of Drug Loaded}} \times 100$$

Swelling Index

The swelling behavior of the prepared microspheres was evaluated by immersing a known weight of dried microspheres in phosphate buffer pH 7.4. After a specified time interval, the swollen microspheres were removed, blotted gently to remove excess surface liquid, and weighed. The swelling index was calculated using the following equation:

$$\text{Swelling Index (\%)} = \frac{W_s - W_d}{W_d} \times 100$$

Where

- W_s = Weight of swollen microspheres
- W_d = Weight of dried microspheres

Drug Content

The drug content of the prepared Voriconazole-loaded microspheres was determined by accurately weighing a known quantity of microspheres and crushing them into a fine powder. The powdered microspheres were dissolved in a suitable solvent, filtered, and suitably diluted. The absorbance of the resulting solution was measured using a UV-Visible spectrophotometer at 256 nm. The amount of Voriconazole present in the microspheres was calculated using the previously prepared calibration curve. The drug content was calculated using the following equation:

$$\text{Drug Content (\%)} = \frac{\text{Amount of Drug Present}}{\text{Total Weight of Microspheres}} \times 100$$

Where: **Amount of Drug Present** = Quantity of Voriconazole determined experimentally.

Total Weight of Microspheres = Weight of microspheres taken for analysis.

➤ Evaluation Parameters of Prepared Voriconazole-Loaded Microsphere Gel

1. Physical Appearance

The prepared Voriconazole-loaded microsphere gel was visually inspected for color, clarity, homogeneity, consistency, grittiness, and the presence of any phase separation. The formulation was examined for its overall appearance and suitability for topical application.

2. pH Determination

The pH of the prepared microsphere-loaded gel was determined using a calibrated digital pH meter. Approximately 1 g of gel was dispersed in distilled water and allowed to equilibrate. The electrode was immersed in the dispersion, and the pH was recorded after attaining a stable reading. The measurements were

performed in triplicate, and the average value was reported.

3. Viscosity

The viscosity of the prepared gel was determined using a Brookfield viscometer. The spindle was immersed in the gel sample, and the viscosity was measured at a controlled temperature of $25 \pm 1^\circ\text{C}$ after attaining a constant reading. The measurements were performed in triplicate, and the average viscosity was reported in centipoise (cP).

4. Drug Content

The drug content of the microsphere-loaded gel was determined by accurately weighing a specified quantity of gel and dissolving it in a suitable solvent. The solution was sonicated, filtered, and suitably diluted. The absorbance was measured at 256 nm using a UV-Visible spectrophotometer. The drug content was calculated from the calibration curve of Voriconazole.

5. In Vitro Diffusion Study

The in vitro diffusion study of the microsphere-loaded gel was carried out using a Franz diffusion cell. A known quantity of gel was placed in the donor compartment over a suitable diffusion membrane. The receptor compartment was filled with phosphate buffer (pH 7.4) maintained at $37 \pm 0.5^\circ\text{C}$ under continuous stirring. At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were analyzed using a UV-Visible spectrophotometer at 256 nm, and the cumulative percentage drug release was calculated.

6. Stability Study

The stability study of the optimized formulation was carried out according to ICH guidelines. The gel was packed in suitable airtight containers and stored under accelerated conditions of $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for a period of three months. Samples were withdrawn at predetermined intervals and evaluated for physical appearance, pH, viscosity, drug content, and in vitro drug release.

7. Comparative Study

A comparative evaluation was performed between the optimized Voriconazole-loaded microsphere gel and a plain Voriconazole gel formulation. Both formulations were assessed for pH, viscosity, spreadability, drug content, and in vitro drug release characteristics to determine the effect of microsphere incorporation on formulation performance and drug release behavior.

8. Antifungal Activity

The antifungal activity of the prepared microsphere-loaded gel was evaluated by the agar well diffusion method. Sterile potato dextrose agar plates inoculated with a suitable fungal strain were prepared, and wells were made in the agar medium. A measured quantity of the gel formulation was placed into the wells, and the plates were incubated under suitable conditions. After incubation, the diameter of the zone of inhibition was measured in millimeters, and the antifungal activity of the formulation was assessed.

➤ Results and Discussion

• PREFORMULATION STUDIES

1. Organoleptic Properties

Received sample of voriconazole was studied for organoleptic characters such as color, odour and appearance. The results are presented in Table No.4 organoleptic properties of voriconazole sample were found to be complied as per IP.

Table No.4: Comparison of Organoleptic Properties of Voriconazole with the Reported Standards

Identification Test	Observation	Standard as per IP	Inference
---------------------	-------------	--------------------	-----------

Appearance	White powder	White or almost white powder	Complies as per IP
Colour	White	White or almost white	Complies as per IP
Odour	Odourless	Odourless	Complies as per IP

2. Melting Point:

The melting point was found to be in between $132^{\circ}\text{C} \pm 2^{\circ}\text{C}$ which is similar to melting point mention in IP which was $129\text{-}134^{\circ}\text{C}$ this study indicates purity of the sample and for further conformation more test is carried out. The results are presented in Table No.5

Table No.5: Melting point of voriconazole

Drug	Method	Observed melting point	Standard melting point
Voriconazole	Capillary Method	$132^{\circ}\text{C} \pm 2^{\circ}\text{C}$	$129\text{-}134^{\circ}\text{C}$

Results are mean of three determinations.

3. Solubility:

Solubility of Voriconazole was performed in methanol, water and buffer pH 6.8 solution. Drug stance belongs to BCS class II with low solubility and high permeability. Solubility is shown Table No.6. From this result was found that the Voriconazole has poor solubility in water.

Table No.6: Solubility of Voriconazole

Sr. No.	Media	Solubility (mg/ ml)	Interference
1	Methanol	9.2	Freely soluble
2	Water	0.9	Slightly soluble
3	Phosphate buffer 7.4	1.3	Slightly soluble

4. UV Spectroscopy Study

UV spectroscopic analysis of Voriconazole was carried out using a Jasco V-730 UV-Visible spectrophotometer. The maximum wavelength (λ_{max}) of Voriconazole in methanol was found to be 256 nm. This value was in agreement with the reported λ_{max} of Voriconazole (256 nm), confirming the identity and purity of the drug sample. The obtained λ_{max} was therefore selected for further quantitative analysis and preparation of the calibration curve.

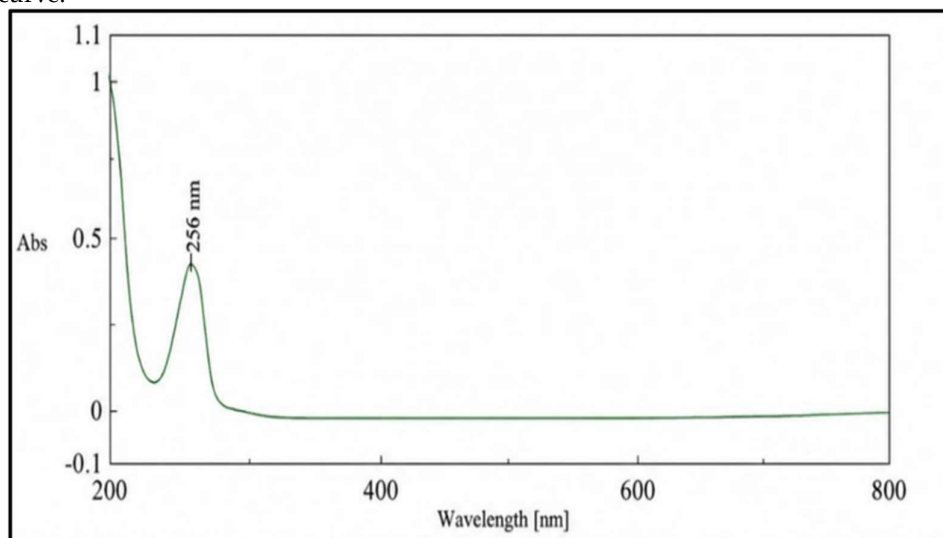


Figure 1: UV spectra of Voriconazole in Methanol

In UV spectroscopy study, the maximum wavelength (λ_{max}) of Voriconazole in Water was and to be 256.2 nm.

The reported λ max values of Voriconazole was 256 nm, so the given values similar with the reported values this indicates that the given sample of Voriconazole was in pure form.

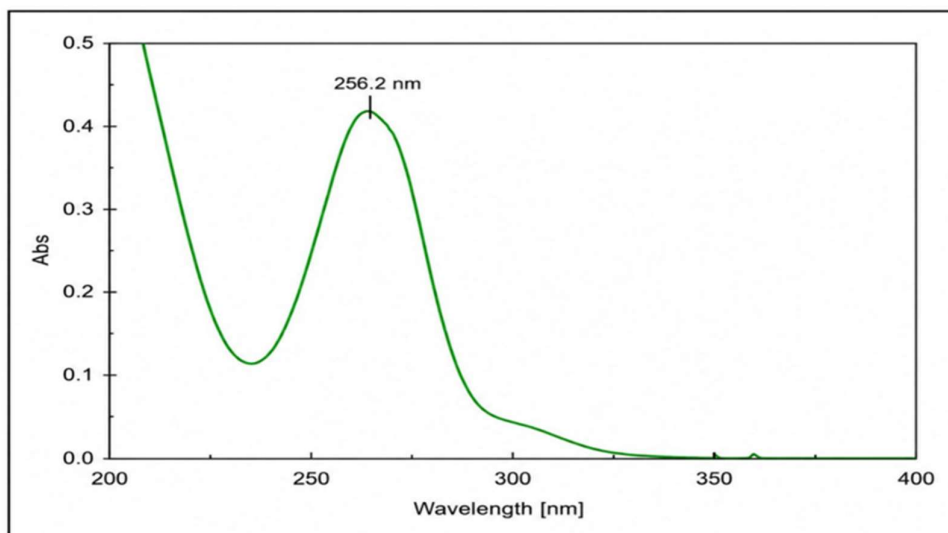


Figure 2: UV spectra of Voriconazole in Water

In UV spectroscopy study, the maximum wavelength (λ max) Voriconazole in Phosphate buffer pH 7.4 was found to be 256 nm. The reported λ max values of Voriconazole was 256 nm, so the given values similar with the reported values this indicates that the given sample of Voriconazole was in pure form.

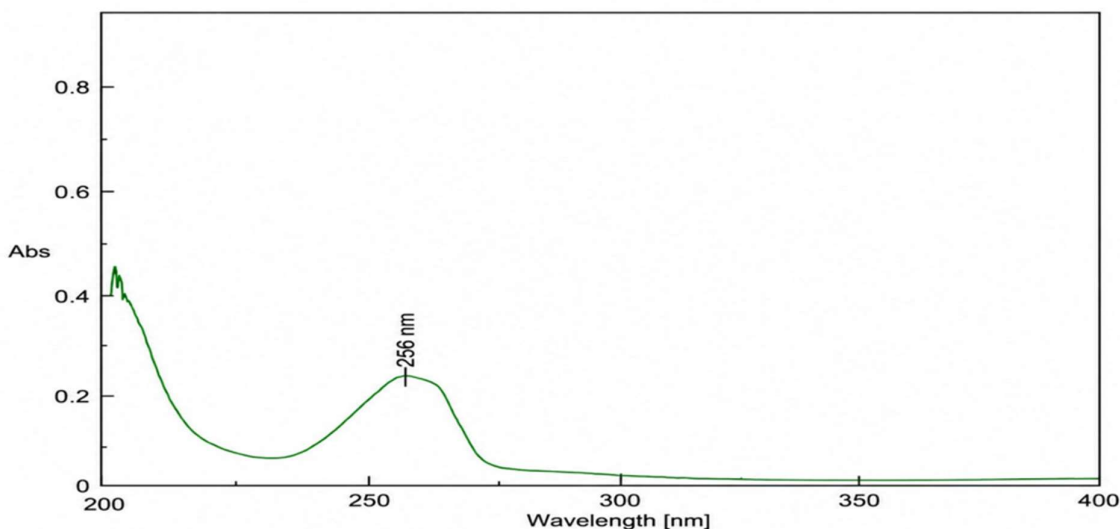


Figure 3: UV spectra of Voriconazole in Phosphate buffer pH 7.4

5. Calibration Curve of Voriconazole in Methanol, Water and Phosphate Buffer 7.4:

- The standard calibration curve of Voriconazole was prepared in Methanol. Absorbance at 256 nm and result was reported in Table No.7 and graphically this calibration curve was presented in Figure 4

Table 7: Calibration Curve of Voriconazole in Methanol

Sr. No.	Concentration	Absorbance
1	0 ppm	0
2	10 ppm	0.0918

3	20 ppm	0.1564
4	30 ppm	0.2476
5	40 ppm	0.3147
6	50 ppm	0.3876

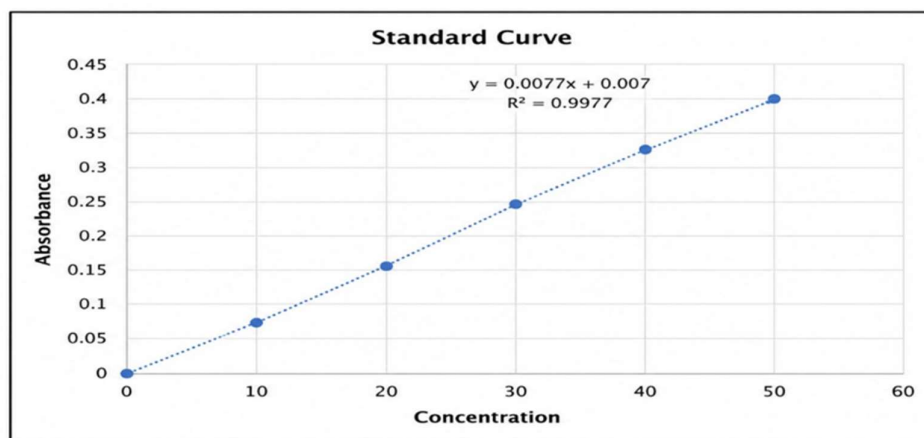


Figure 4. Calibration Curve of Voriconazole in Methanol

- The standard calibration curve of Voriconazole was prepared in Water Absorbance at 256 nm and result was reported in Table 8 and graphically this calibration curve was presented in Figure 5

Table 8: Calibration Curve of Voriconazole in Water

Sr. No.	Concentration	Absorbance
1	0 ppm	0
2	10 ppm	0.1113
3	20 ppm	0.2292
4	30 ppm	0.3639
5	40 ppm	0.4825
6	50 ppm	0.6001

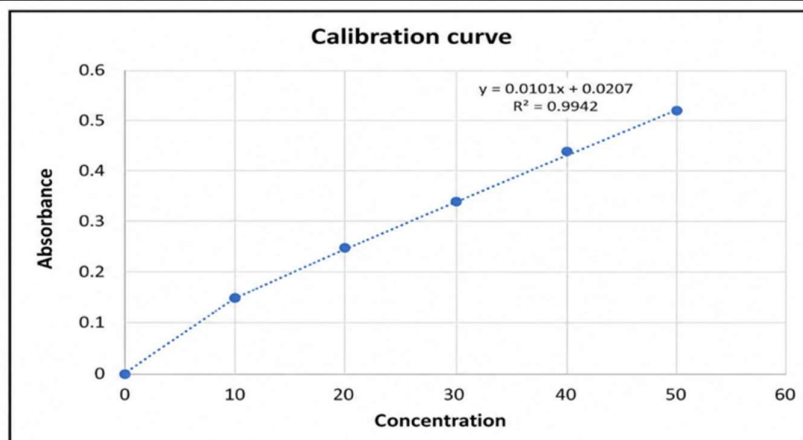


Figure 5.: Calibration Curve of Voriconazole in Water

- The standard calibration curve of Voriconazole was prepared in Phosphate buffer pH 7.4 Absorbance at 256 nm and result was reported in Table No.9 and graphically this calibration curve was presented in Figure 6

Table 9: Calibration Curve of Voriconazole in Phosphate buffer 7.4

Sr. No.	Concentration	Absorbance
1	0 ppm	0
2	10 ppm	0.1420
3	20 ppm	0.2237
4	30 ppm	0.3320
5	40 ppm	0.4190
6	50 ppm	0.5160

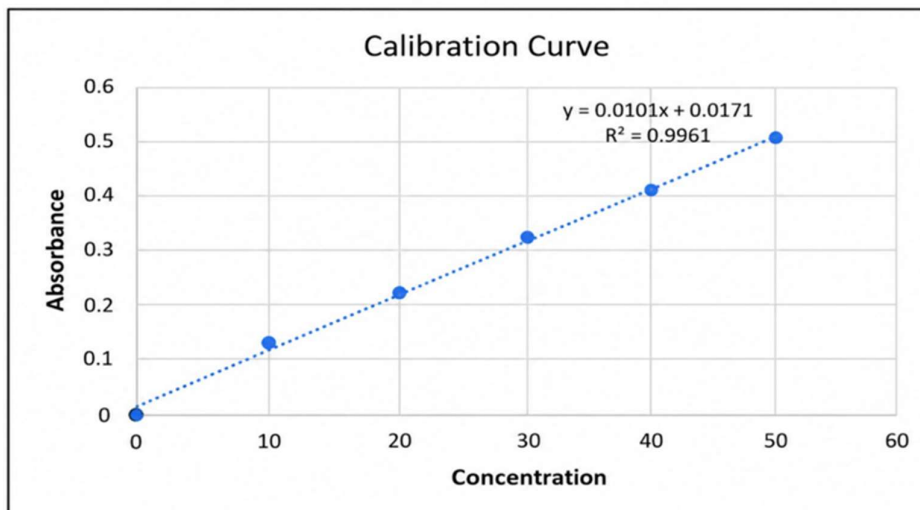


Figure 6: Calibration Curve of Voriconazole in Phosphate buffer 7.4

6. Fourier Transform Infrared (FTIR) Spectroscopy Study of Drug:

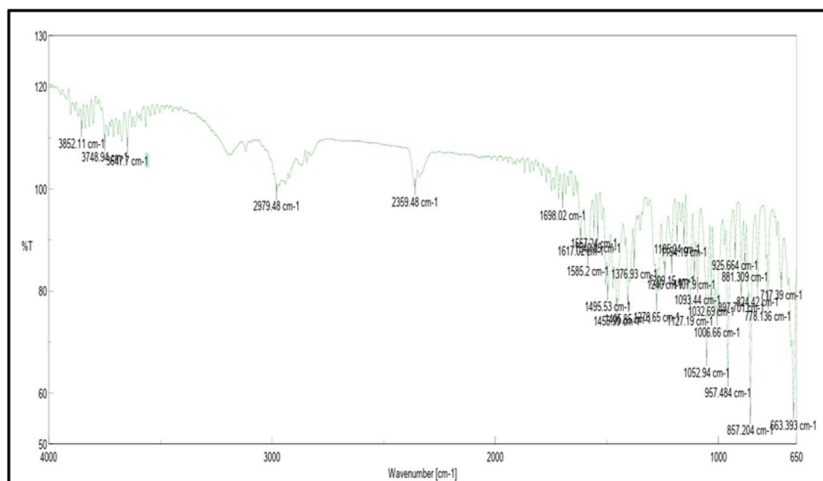


Figure 7: IR Spectra of Voriconazole

Table No.10. Interpretation of FTIR spectrum of Voriconazole

Functional Group	Standard Peak Range (cm ⁻¹)	Observed Peak (cm ⁻¹)
O-H Stretching	3200–3600	3456
Aliphatic C-H Stretching	2850–3000	2979
C=N Stretching (Triazole/Pyrimidine Ring)	1600–1700	1698

Aromatic C=C Stretching	1450–1500	1495
C–N Stretching	1020–1360	1376
C–F Stretching	1000–1300	1240
C–N Stretching	1000–1250	1093
Aromatic C–H Bending	850–1000	957
C–F / Aromatic Ring Deformation	650–850	788

The above FTIR shown the characteristics peak of shown in (Figure 7 & Table 10) from this result it was conclude that the sample of Voriconazole was pure.

7. COMPATIBILITY STUDY OF DRUG WITH EXCIPIENTS:

Fourier Transform Infrared (FTIR) Spectroscopy Study of Drug + Excipients

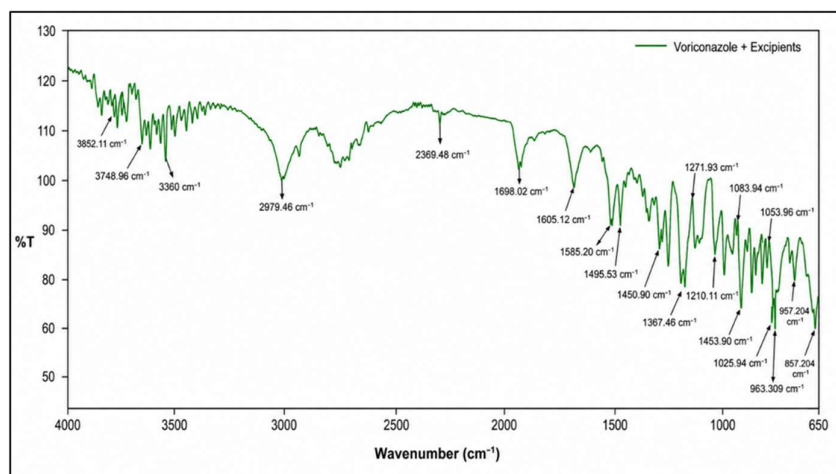


Figure 8: IR Spectra of Voriconazole+ Excipients

Table no 11 – Interpretation of FTIR spectrum of Voriconazole IP + Excipients

Sr. No.	Functional Group	Standard Value (cm ⁻¹)	Voriconazole (cm ⁻¹)	Voriconazole + Excipients (cm ⁻¹)	Compatible or Not
1	O–H Stretching	3200–3600	3366.14	3365.80	Compatible
2	C–H Stretching	2850–3000	2972.73	2971.95	Compatible
3	C=N Stretching	1600–1700	1698.02	1696.85	Compatible
4	Aromatic C=C Stretching	1450–1600	1455.99	1454.28	Compatible
5	C–N Stretching	1020–1360	1376.03	1375.22	Compatible
6	C–F Stretching	1000–1400	1032.69	1024.02	Compatible
7	C–O–C Stretching	1140–1250	1185.40	1184.75	Compatible
8	COO ⁻ Stretching	1600–1650	1617.02	1615.86	Compatible
9	Ester C=O Stretching	1720–1750	—	1732.48	Compatible
10	Carboxylic O–H	3000–3500	—	3358.64	Compatible

The FTIR spectrum of the physical mixture exhibited all the characteristic peaks of Voriconazole without any significant shift, disappearance, or appearance of new peaks. The major functional group peaks of the drug were retained in the spectrum of the physical mixture, indicating that the chemical structure of Voriconazole remained unchanged in the presence of the excipients. shown in (Figure 8 & Table 11)

8. DIFFERENTIAL SCANNING CALORIMETRY OF VORICONAZOLE

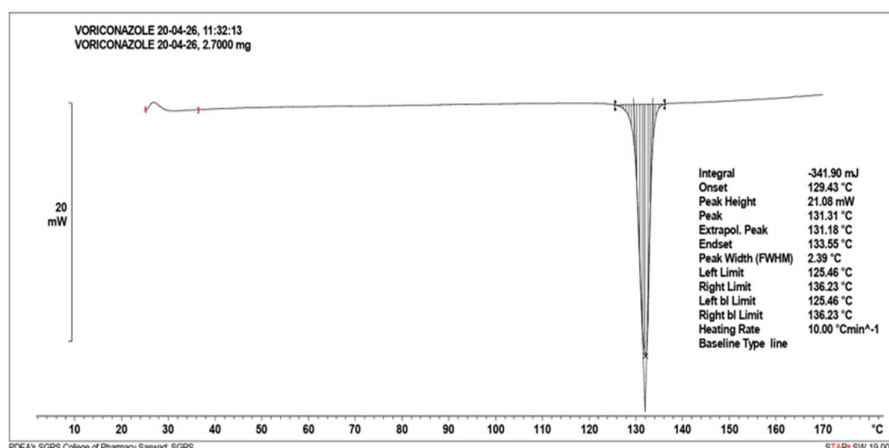


Figure 9 DSC of Voriconazole

The Differential Scanning Calorimetry (DSC) analysis was performed to determine the thermal behavior, melting point, and crystalline nature of Voriconazole. The DSC thermogram exhibited a sharp endothermic peak at 131.31°C, corresponding to its melting point. The onset and endset temperatures were observed at 129.43°C and 133.55°C, respectively. The presence of a single well-defined endothermic peak indicates that the drug is pure, crystalline, and free from significant impurities, confirming its thermal stability. shown in Figure No.9

Preparation of Voriconazole-Loaded Microspheres

Voriconazole-loaded microspheres were successfully prepared using the ionotropic gelation method. During formulation development, Batch F1 failed to produce microspheres, indicating insufficient polymer concentration for effective cross-linking and microsphere formation. In Batches F2 and F3, microspheres were formed; however, they exhibited poor stability and dispersed in the calcium chloride solution, suggesting inadequate structural integrity.

In contrast, Batches F4, F5, and F6 produced well-formed, discrete, and spherical microspheres with good physical stability. The increase in polymer concentration improved the cross-linking efficiency, resulting in stronger and more stable microspheres. Based on their satisfactory morphology and stability characteristics, formulations F4, F5, and F6 were selected for further evaluation studies.

EVALUATIONS OF PREPARED VORICONAZOLE-LOADED MICROSPHERES:

1. Determination of Particle Size and Zeta Potential

The particle size and zeta potential of Voriconazole-loaded microspheres (F4–F6) were evaluated using Dynamic Light Scattering (DLS). The particle size ranged from 429 to 525 µm, while the zeta potential values ranged from -23.0 to -29.0 mV. An increase in polymer concentration resulted in larger particle sizes due to increased solution viscosity. The negative zeta potential values indicated good physical stability and reduced particle aggregation. Among all formulations, F6 exhibited the largest particle size (525 µm) and highest negative zeta potential (-29.0 mV), demonstrating superior stability, structural integrity, and reproducibility. Therefore, F6 was selected as the optimized formulation for further studies.

Table No 12 Characterization of Microsphere Formulation

Characterization	F4	F5	F6
Particle Size (nm)	429	476	525
Zeta Potential (mV)	-23.0	-25.0	-29.0

2. Percentage Yield

The percentage yield of the prepared Voriconazole-loaded microspheres was determined by comparing the practical yield obtained after preparation with the theoretical yield. The percentage yield of formulations F4, F5, and F6 was found to be 81.42%, 87.65%, and 93.18%, respectively. The results demonstrated a progressive increase in percentage yield with increasing polymer concentration. This improvement may be attributed to enhanced cross-linking efficiency, better microsphere formation, and reduced loss of material during the preparation process. Among the evaluated formulations, F6 exhibited the highest percentage yield (93.18%), indicating efficient entrapment and recovery of the polymeric matrix during microsphere preparation. These findings suggest that higher polymer concentrations favor the production of stable microspheres with improved manufacturing efficiency.

Table No.13.: Percentage Yield of Voriconazole-Loaded Microspheres

Sr. No.	Formulation Batch	Percentage Yield (%)
1	F4	81.42 $\pm$ 0.85
2	F5	87.65 $\pm$ 0.72
3	F6	93.18 $\pm$ 0.64

3. Estimation of Entrapment Efficiency

The entrapment efficiency of Voriconazole-loaded microspheres (F4–F6) was evaluated to determine the drug-loading capacity of the formulations. The entrapment efficiency ranged from 75 $\pm$ 2.10% to 94 $\pm$ 1.35%. Among all batches, F6 showed the highest entrapment efficiency (94 $\pm$ 1.35%), indicating superior drug encapsulation due to the optimal concentration of sodium alginate and Eudragit RS100. The dense polymeric network formed in F6 effectively retained the drug and minimized drug loss during microsphere preparation. Therefore, F6 was considered the optimized formulation based on its maximum entrapment efficiency.

Table No. 14. Entrapment efficiency of Microsphere Formulation

Sr. No.	Batch	Entrapment Efficiency (% Mean $\pm$ SD)
1	F4	75 $\pm$ 2.10
2	F5	87 $\pm$ 1.75
3	F6	94 $\pm$ 1.35



Figure10. Entrapment efficiency of Microsphere Formulation

In Vitro Drug Release Study

The in vitro drug release study demonstrated a sustained release pattern of Voriconazole from all microsphere formulations. The cumulative drug release after 12 hours was 78.45%, 86.72%, and 94.38% for formulations F4, F5, and F6, respectively. Among all batches, F6 showed the highest drug release (94.38%), indicating its superior release performance. The enhanced release from F6 may be attributed to the optimum concentration of Sodium Alginate and Eudragit RS100, which enabled effective drug encapsulation and controlled drug diffusion. Therefore, F6 was selected as the optimized formulation for further studies.

Table No. 15. In Vitro Drug Release Study of Microsphere Formulation

Time (h)	F4 (% Release)	F5 (% Release)	F6 (% Release)
1	12.5	13.8	15.2
2	24.8	27.1	29.6
4	41.5	45.3	48.7
6	57.2	61.4	65.8
8	70.1	75.2	80.4
10	75.8	84.1	89.6
12	78.45	86.72	94.38

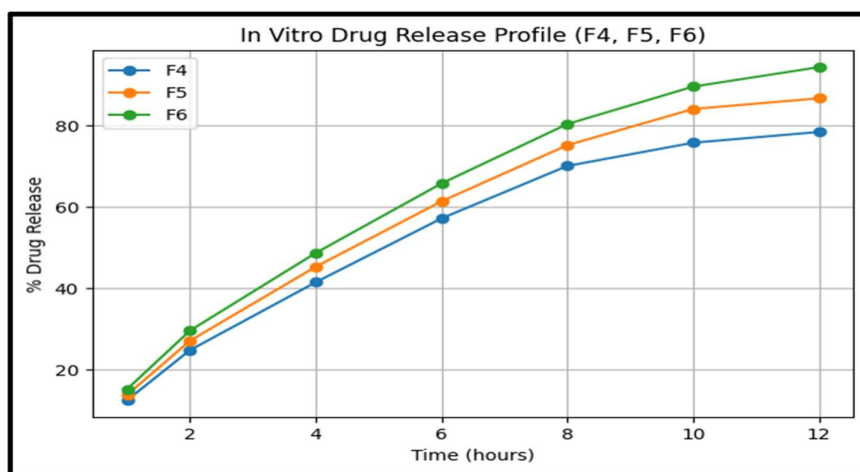


Figure11. In Vitro Drug Release Study of Microsphere Formulation

Swelling Index

The swelling index of Voriconazole-loaded microsphere formulations F4, F5, and F6 was found to be 68.25%, 76.84%, and 84.53%, respectively. The swelling index increased with increasing concentrations of Sodium Alginate and Eudragit RS100 due to their hydrophilic nature. Among all formulations, F6 exhibited the highest swelling index (84.53%), indicating greater water absorption capacity and enhanced swelling behavior. The improved swelling characteristics of F6 contributed to its controlled drug release and overall formulation performance. Therefore, F6 was selected as the optimized formulation.

Table No. 16. Swelling Index of Microspheres

Batch	Swelling Index (%)
F4	68.25
F5	76.84
F6	84.53

Drug Content of Voriconazole-Loaded Microspheres

The drug content of Voriconazole-loaded microsphere formulations F4, F5, and F6 was found to be 85.42%, 90.16%, and 96.84%, respectively. The results confirmed successful incorporation of Voriconazole into the

microspheres with minimal drug loss during preparation. Among all formulations, F6 showed the highest drug content (96.84%), indicating efficient drug entrapment and uniform drug distribution within the polymeric matrix. Therefore, F6 was considered the optimized formulation based on its superior drug content.

Table No.17.: Drug Content of Prepared Voriconazole-Loaded Microspheres

Batch	Drug Content (%)
F4	85.42
F5	90.16
F6	96.84

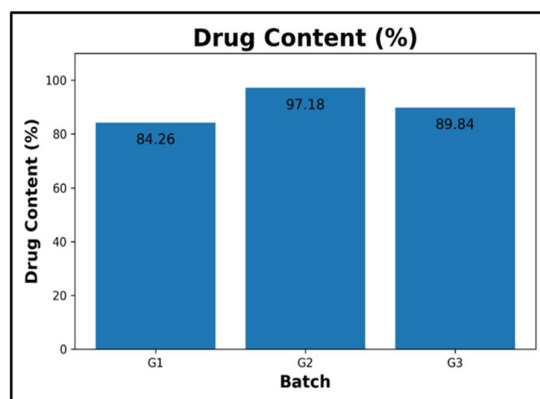


Figure12.Determination of Drug Content

Preparation of Voriconazole Microsphere-Loaded Gel

Voriconazole-loaded microsphere gel formulations were successfully prepared using Carbopol 934P as the gelling agent. All formulations were smooth, homogeneous, and showed uniform distribution of microspheres without any phase separation or aggregation. Among the prepared batches, G2 exhibited excellent homogeneity, smooth texture, suitable consistency, and good physical stability. The results confirmed the successful incorporation of Voriconazole-loaded microspheres into the gel base, and therefore G2 was selected as the optimized formulation for topical application.

EVALUATION PARAMETERS OF PREPARED VORICONAZOLE MICROSPHERE LOADED GEL

• Physical Appearance

The optimized Voriconazole-loaded microsphere gel (G2) was found to be smooth, homogeneous, and free from grittiness, lumps, and phase separation. The gel exhibited good consistency and uniform distribution of microspheres throughout the gel matrix without any aggregation or sedimentation, indicating good physical stability. The satisfactory appearance and texture of the formulation confirmed the suitability of the gel base for topical application.

pH Determination

Table No. 18. Determination of pH

Sr. No.	Batch	pH
1	G1	6.7 ± 0.10

2	G2	6.9 ± 0.12
3	G3	7.1 ± 0.09

The pH of all Voriconazole-loaded microsphere gel formulations was found to be in the range of 6.7 ± 0.10 to 7.1 ± 0.09 , which is within the normal physiological pH range of the skin. The optimized formulation G2 showed a pH of 6.9 ± 0.12 , indicating its suitability for topical application without causing skin irritation. Therefore, G2 was considered the optimized gel formulation based on its acceptable pH and skin compatibility.

Viscosity

The viscosity of Voriconazole-loaded microsphere gel formulations was evaluated at different shear rates and showed a decrease with increasing rpm, indicating pseudoplastic (shear-thinning) behavior, which is desirable for topical application. The viscosity values ranged from 2032.4 ± 1.1 cP to 5890.6 ± 1.4 cP. Among all formulations, G2 exhibited optimum viscosity (2105.7 ± 0.6 cP to 4925.5 ± 0.9 cP), providing an ideal balance between spreadability and retention at the application site. Therefore, G2 was selected as the optimized gel formulation due to its suitable rheological properties and ease of application.

Table No. 19. Determination of Viscosity

Sr. No.	RPM	G1 (cP)	G2 (cP)	G3 (cP)
1	10	3520.3 ± 1.2	4925.5 ± 0.9	5890.6 ± 1.4
2	20	3248.9 ± 1.3	4110.2 ± 1.1	5227.4 ± 1.0
3	50	2940.1 ± 1.0	3305.4 ± 1.1	4780.3 ± 0.7
4	100	2032.4 ± 1.1	2105.7 ± 0.6	4022.6 ± 0.3

• In Vitro Diffusion Study

The in vitro diffusion study of the Voriconazole-loaded microsphere gel formulations was carried out, and the percentage drug diffusion was found to be G1 ($82.45 \pm 0.52\%$), G2 ($95.68 \pm 1.12\%$), and G3 ($88.34 \pm 0.96\%$).

Table No. 20. Determination In Vitro Diffusion Study

Time (h)	G1 (% Drug Diffusion)	G2 (% Drug Diffusion)	G3 (% Drug Diffusion)
1	10.2 ± 0.41	12.5 ± 0.38	11.1 ± 0.35
2	18.6 ± 0.52	22.4 ± 0.44	20.1 ± 0.47
4	35.8 ± 0.60	41.7 ± 0.58	38.9 ± 0.55
6	52.4 ± 0.63	60.3 ± 0.61	56.2 ± 0.59
8	66.7 ± 0.55	75.9 ± 0.53	70.4 ± 0.50
10	76.8 ± 0.48	88.2 ± 0.46	81.5 ± 0.49
12	82.45 ± 0.52	95.68 ± 1.12	88.34 ± 0.96

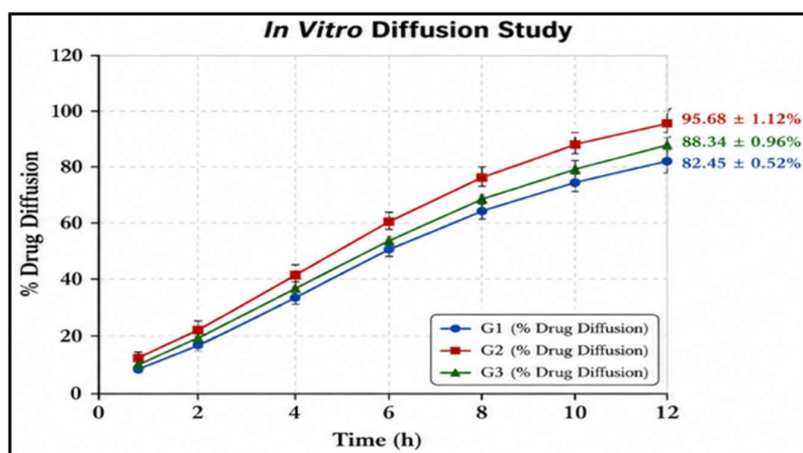


Figure13.In Vitro Diffusion Study

The in vitro drug diffusion study demonstrated a sustained and controlled release of Voriconazole from all microsphere-loaded gel formulations. Among all formulations, G2 showed the highest cumulative drug diffusion of $95.68 \pm 1.12\%$ at the end of 12 hours. The enhanced drug diffusion may be attributed to the optimum concentration of Carbopol 934P and the uniform distribution of Voriconazole-loaded microspheres within the gel matrix. The controlled release behavior ensured prolonged drug availability at the site of application, which may improve therapeutic efficacy and reduce the frequency of application. Therefore, G2 was selected as the optimized gel formulation.

Comparative In Vitro Diffusion Study

A comparative in vitro diffusion study was carried out between the optimized microsphere-loaded gel formulation (G2) and a conventional gel. The results showed that G2 exhibited significantly higher cumulative drug release ($93.10 \pm 1.08\%$) at the end of 12 hours compared to the conventional gel (37.13%). The enhanced drug release from G2 may be attributed to the sustained and controlled release properties of the microspheres, higher drug entrapment efficiency, and improved drug diffusion through the gel matrix. In contrast, the conventional gel showed limited drug diffusion due to the absence of a controlled drug delivery system. These findings indicate that G2 provides superior topical drug delivery with prolonged release and improved therapeutic efficacy compared to the conventional gel.

Table No. 21. Determination In Vitro Diffusion Study

Time (hrs)	G2 (Microsphere Loaded Gel) %	Controlled Gel %
1	8.20 ± 0.45	5.10
2	15.60 ± 0.62	9.40
3	23.80 ± 0.88	13.20
4	32.10 ± 0.95	17.60
5	40.25 ± 1.02	21.80
6	48.90 ± 1.10	25.40
7	58.30 ± 1.15	28.60
8	67.45 ± 1.20	30.10
9	75.80 ± 1.18	31.50
10	83.20 ± 1.12	33.00
11	89.10 ± 1.05	35.20
12	93.10 ± 1.08	37.13

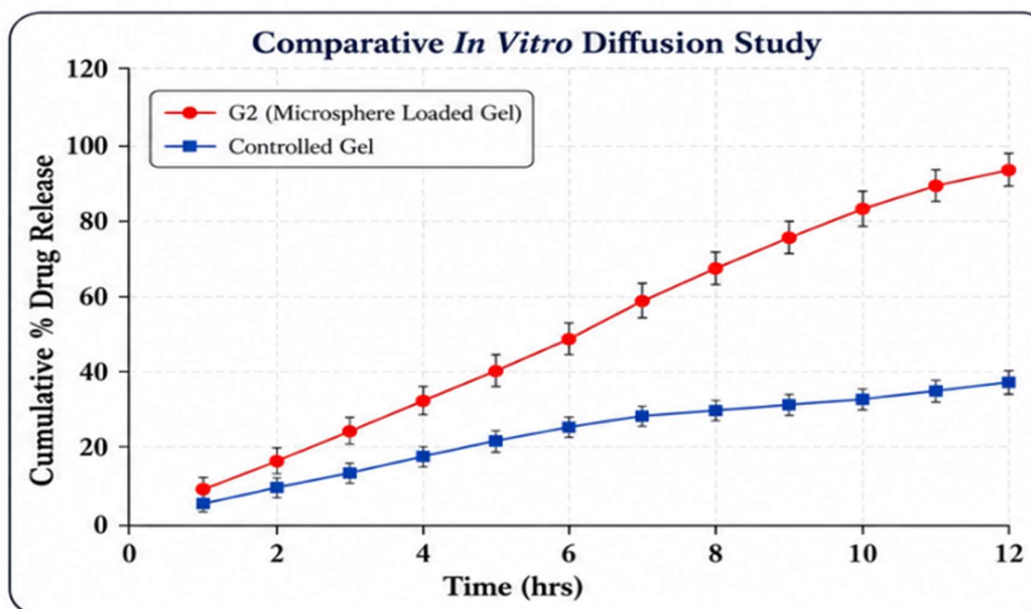


Figure No. 14. Comparative In Vitro Diffusion Study

- **Antifungal Activity**

The antifungal activity of the optimized Voriconazole microsphere-loaded gel formulation (Batch G2) was evaluated against *Candida albicans* using the agar well diffusion method.

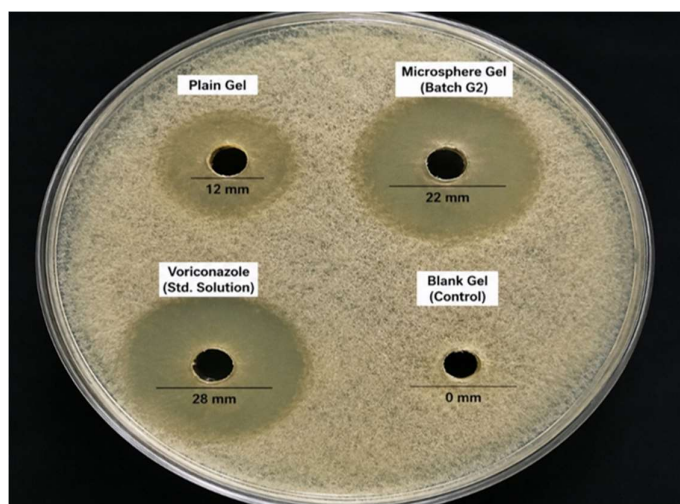


Figure No. 15. Antifungal Activity

Table No. 22. Antifungal Activity

Sr. No.	Fungal Strain	Sample Name	Zone of Inhibition (mm) $\pm$ SD
1	<i>Candida albicans</i>	Plain Voriconazole Gel	12.00 $\pm$ 0.58
2	<i>Candida albicans</i>	Voriconazole Microsphere Gel (Batch G2)	22.00 $\pm$ 0.70
3	<i>Candida albicans</i>	Voriconazole Standard Solution	28.00 $\pm$ 0.65
4	<i>Candida albicans</i>	Blank Gel (Control)	0.00 $\pm$ 0.00

The antifungal activity study demonstrated that the Voriconazole microsphere-loaded gel exhibited a

significant zone of inhibition of 22.00 ± 0.70 mm, indicating strong antifungal efficacy. In comparison, the plain Voriconazole gel showed a zone of inhibition of 12.00 ± 0.58 mm, while the standard Voriconazole solution produced the highest inhibition zone of 28.00 ± 0.65 mm. No zone of inhibition was observed with the blank gel (0.00 ± 0.00 mm), confirming that the gel base itself possessed no antifungal activity. The enhanced antifungal effect of the microsphere-loaded gel may be attributed to improved drug entrapment, sustained drug release, and better drug diffusion from the microsphere matrix. These results confirm that the optimized microsphere-loaded gel possesses effective antifungal activity and offers potential for prolonged therapeutic action in the treatment of fungal infections.

• Stability Study

The stability study of the optimized formulation (Batch G2) was conducted in accordance with ICH guidelines. The prepared microsphere-loaded gel was filled into suitable airtight containers and stored under accelerated stability conditions at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for a period of 3 months.

Table No. 23. Stability Study of Microsphere Loaded Gel Formulation

Batch No.	Evaluation of Gel	Initial Result of Evaluation of Gel	After 3 Months Result of Evaluation of Gel
Batch G2 (Optimized Batch)	pH	6.8 ± 0.12	6.7 ± 0.10
	Viscosity (cP)		
	Low (10 RPM)	4865.4 ± 0.82	4838.6 ± 0.56
	Low (20 RPM)	4028.7 ± 0.94	3995.3 ± 0.71
	Moderate (50 RPM)	3245.8 ± 0.65	3212.4 ± 0.48
	High (100 RPM)	2089.6 ± 0.52	2056.8 ± 0.35
	Drug Content (%)	97.18 ± 1.10	96.42 ± 0.95
	In Vitro Drug Release (% at 24 hrs)	92.84 ± 1.05	91.76 ± 0.88

The result showed no significant changes in these parameters, indicating that the formulation has good physical and chemical stability during the period of study.

CONCLUSION

The present study successfully developed and evaluated Voriconazole-loaded microspheres incorporated into a Carbopol gel for topical antifungal delivery. Among the prepared microsphere formulations, batch F6 showed the best performance with high entrapment efficiency (94%), drug content (96.84%), percentage yield (93.18%), and sustained drug release (94.38% in 12 h). The optimized gel formulation (G2) exhibited suitable pH, viscosity, excellent drug diffusion (95.68%), and enhanced antifungal activity against *Candida albicans* compared to the plain drug gel. Stability studies confirmed that the formulation remained stable under accelerated storage conditions. Overall, the developed Voriconazole microsphere-loaded gel demonstrated sustained drug release, improved antifungal efficacy, and potential as an effective topical treatment for fungal infections.

REFERENCES

1. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. *Mycoses*. 2008;51(Suppl 4):2–15.
2. Peres NT, Maranhão FC, Rossi A, Martinez-Rossi NM. Dermatophytes: host-pathogen interaction and antifungal resistance. *An Bras Dermatol*. 2010;85(5):657–67.
3. Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clin Infect Dis*. 2003;36(5):630–7.

4. Theuretzbacher U, Ihle F, Derendorf H. Pharmacokinetic and pharmacodynamic profile of voriconazole. *Clin Pharmacokinet*. 2006;45(7):649–63.
5. Varde NK, Pack DW. Microspheres for controlled release drug delivery. *Expert Opin Biol Ther*. 2004;4(1):35–51.
6. Jain NK. *Controlled and Novel Drug Delivery*. 1st ed. New Delhi: CBS Publishers & Distributors; 2002. p. 236–255.
7. Patel RP, Patel MM. Formulation and evaluation of microsphere-based topical drug delivery systems. *Int J Pharm Sci Res*. 2011;2(5):1120–1127.
8. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm*. 2010;67(3):217–223.
9. Indian Pharmacopoeia Commission. *Indian Pharmacopoeia*. Ghaziabad: IPC; 2022.
10. Beckett AH, Stenlake JB. *Practical Pharmaceutical Chemistry*. 4th ed. New Delhi: CBS Publishers; 2005.
11. *United States Pharmacopeia and National Formulary (USP-NF)*. Rockville, MD: United States Pharmacopeial Convention; 2023.
12. Sharma BK. *Instrumental Methods of Chemical Analysis*. 24th ed. New Delhi: Goel Publishing House; 2014.
13. Silverstein RM, Webster FX, Kiemle DJ. *Spectrometric Identification of Organic Compounds*. 8th ed. New York: Wiley; 2015.
14. Pavia DL, Lampman GM, Kriz GS, Vyvyan JA. *Introduction to Spectroscopy*. 5th ed. Boston: Cengage Learning; 2015.
15. Giron D. Applications of thermal analysis and coupled techniques in pharmaceutical industry. *J Therm Anal Calorim*. 2002;68(2):335–57.
16. Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F, Couvreur P. Development of a new drug carrier made from alginate. *J Pharm Sci*. 1993;82(9):912–7.
17. Anal AK, Stevens WF. Chitosan–alginate multilayer beads for controlled release. *Int J Pharm*. 2005;290(1–2):45–54.
18. Rani KR, Priya RM, Buella GB. Design and characterization of voriconazole microspheres. *Indo Am J Pharm Sci*. 2016;3(10):1172–1181. doi:10.5281/zenodo.164928.
19. Kumar G, Bhatt M, Badoni PP. Micro beads loaded bio adhesive vaginal gel of voriconazole: development and characterization. *Indian J Novel Drug Deliv*. 2021;13(3):125–135.