DESIGN, DEVELOPMENT AND EVALUATION OF CUBOSOMAL GEL USING ANTI-FUNGAL AGENT

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ABSTRACT:

The objective of the present study is to developed voriconazole loaded cubosomal gel for localized drug delivery through the skin, to improve drug bioavailability, improved therapeutic efficacy, while minimizing side effects and reducing dosing frequency. Voriconazole pre-formulation studies was carried out such as melting point, FTIR, DSC analysis, determination of wavelength, determination of calibration curve study. Cubosomal formulation were formulated by using top-down technique, were developed by using glyceryl monooleate as a lipid and poloxamer 407 as a stabilizer, Then it is formulated as a Carbopol gel base, Optimization was carried out by using 3^2 factorial design The optimized batch was subjected to in vitro anti-fungal activity, morphological analysis using a high resolution scanning electron microscopy, zeta potential, polydispersity index, drug content and entrapment efficiency. From F1-F9 formulations, Studies showed that F5 batch was optimized batch, a various parameters such as organoleptic properties, pH, spredability, viscosity and drug release were evaluated. The optimized cubosomal formulation exhibits a Vesicle size 10.63 nm, polydispersity index 0.254, zeta potential 0.0211 mV, % EE 92.67%. % Drug content 96.42%. The pH of gel 5.7, spreadability of 46.46 g·cm/sec, viscosity of 6674± 3.22 cps, the optimized formulation exhibits better In-vitro anti-fungal activity against Candida albicans showed a 12 mm zone of inhibition, In-vitro drug release rate 62.58% at 12 hours, Long-term stability analysis confirmed the formulation stability under cold condition. In conclusion, VRC loaded cubosomal gel presents a promising avenue to treating the skin fungal infections, offering sustained drug release and improved patient compliance.

Keywords: Voriconazole, Cubosome, Fungal Infection, Topical drug delivery, Optimization, Top-down technique, Lipid-based nanocarrier.

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INTRODUCTION:

Fungal infections is a substantial and underappreciated global health challenge, affecting billion individuals worldwide and responsible for over 1.5 million fatalities every year. Burden of mycotic disease is disproportionately higher among immunocompromised populations, including patients with human immunodeficiency virus infection, malignancies, organ transplants, chronic respiratory disorders, and those receiving long-term immunosuppressive or corticosteroid therapy. Invasive fungal infections, particularly candidemia and aspergillosis, are associated with prolonged hospitalization, increased healthcare costs, and high mortality rates. [1] Notably, candidemia has been reported to exhibit mortality rates approaching 60% in critically ill patients, while untreated invasive aspergillosis is almost universally fatal. In parallel, superficial fungal infections caused by dermatophytes continue to affect nearly 25% of the global population, leading to chronic discomfort, recurrent infections, and reduced quality of life.[2] keratinophilic fungi distinct group of fungi genera *Microsporum*, *Trichophyton*, and *Epidermophyton*, which colonize the stratum corneum, hair, and nails. Although dermatophytic infections are generally non-invasive, their chronic and recurrent nature, coupled with suboptimal treatment outcomes, underscores the need for more effective topical therapeutic strategies. The widespread use of antifungal agents has further contributed to the emergence of drug-resistant fungal strains, particularly among *Candida* species, posing a serious threat to current treatment regimens.[3] Antifungal pharmacotherapy primarily comprises polyenes, azoles, allylamines, and echinocandins. Among these, triazole antifungals are frequently employed due to their broad-spectrum activity and favourable efficacy profiles. However, the increasing incidence of azole resistance and dose-

limiting systemic toxicity has significantly constrained their long-term clinical utility. These limitations have driven growing interest in alternative drug delivery strategies that can maximize local drug concentration at the site of infection while minimizing systemic exposure. [4,5]

Voriconazole (VRC), a second-generation triazole and synthetic derivative of fluconazole, is a potent broad-spectrum antifungal agent with excellent activity against *Aspergillus* spp. and fluconazole-resistant *Candida* strains. Its antifungal mechanism involves inhibition of cytochrome P450-dependent 14-α-lanosterol demethylase, resulting in impaired ergosterol biosynthesis and disruption of fungal cell membrane integrity. Despite its superior antifungal potency, the clinical use of voriconazole is limited by poor aqueous solubility, extensive first-pass metabolism, nonlinear pharmacokinetics, and a high incidence of adverse effects, including hepatotoxicity, neurotoxicity, visual disturbances, and significant drug–drug interactions. Consequently, systemic administration of voriconazole is often unsuitable for the management of localized cutaneous fungal infections.[6] Currently, no topical Voriconazole formulation available in the market. most of the research work conducted on the ocular drug delivery system, one of the barriers in topical delivery is the impeded by the formidable barrier function of the stratum corneum, which restricts drug permeation into deeper skin layers. To overcome this barrier of stratum corneum researchers investigated nano-carrier based system to enhanced the efficacy of drug delivery to the topical region. [7-9]

Among lipid-based nanocarriers, cubosomes have gained increasing attention due to their unique internal architecture and exceptional drug-loading capabilities. Cubosomes are nanoscale dispersions of bicontinuous cubic liquid crystalline phases formed by the self-assembly of amphiphilic lipids and stabilized by suitable surfactants. Their characteristic three-dimensional honeycomb-like structure comprises two interpenetrating aqueous channels separated by a lipid bilayer, resulting in an extraordinarily large internal surface area. Cubosomes typically exhibit particle sizes ranging from 10 to 500 nm and are thermodynamically stable systems capable of encapsulating both hydrophilic and lipophilic drugs. Their lipidic composition and structural resemblance to the lamellar and cubic phases present in the stratum corneum a distinct advantage for and transdermal drug delivery. Previous studies have demonstrated that cubosome-based formulations can enhance skin penetration, prolong drug residence time within the skin, and provide controlled release profiles, thereby improving therapeutic outcomes while minimizing adverse effects.[10]

Physicochemical limitations of voriconazole and the challenges associated with conventional topical formulations; the development of a cubosome-based topical gel represents a promising strategy to enhance its dermal delivery. Incorporation of voriconazole into cubosomal nanocarriers may improve its solubility, protect it from degradation, facilitate penetration across the stratum corneum, and sustain drug release at the site of infection. Therefore, the present study aims to develop and characterize a voriconazole-loaded cubosomal gel and to evaluate its potential as an advanced topical delivery system for effective management of cutaneous fungal infections.

MATERIALS AND METHODS:

Materials:

Glyceryl monooleate (GMO) was obtained as a gift sample from Mohini Organics Pvt. Ltd., Mumbai. Poloxamer 407 was purchased from S.M. Chemical, Mumbai. Voriconazole were purchased from MSN laboratories, Hyderabad, Carbopol 940 and triethanolamine were of commercial grade. All other chemicals and reagents used in the study were of analytical grade.

Stastical Design:

Response Surface Methodology (RSM) was employed to optimize the formulation variables and evaluate their influence on the characteristics of the voriconazole-loaded cubosomal formulation. A 3^2 factorial design was selected, comprising two independent variables, Glyceryl Monooleate (GMO) (X_1) and Poloxamer 407 (X_2), each studied at three levels (-1, 0, and +1). The selected dependent responses included % entrapment efficiency (Y_1), cumulative drug release (Y_2), and viscosity (Y_3). The experimental data were analyzed using Minitab® statistical software, and quadratic polynomial equations were generated to describe the relationship between the formulation variables and the responses. Analysis of variance (ANOVA), regression analysis, contour plots, and three-dimensional response surface plots were used to evaluate the significance of the model and optimize the formulation.

METHODS:

PREPARATION OF CUBOSOME:

Cubosomal dispersions were prepared by the top-down technique. Accurately weighed Glyceryl Monooleate (GMO) was taken in a beaker and heated to 70°C until a clear and homogeneous molten mass was obtained. The required quantity of drug was then added to the molten GMO and stirred until completely dissolved. In a separate beaker, Poloxamer 407 was heated to 70°C until completely melted. The molten GMO-drug mixture was then added to the molten Poloxamer 407 and mixed thoroughly to obtain a uniform lipid phase. Preheated distilled water (60°C) was added dropwise to the lipid phase under continuous stirring to form a coarse cubosomal dispersion. The

resulting dispersion was then subjected to bath sonication for period of 15-30 min with intermittent shaking and stirring. The end result will be milky white dispersion without presence of any aggregates. The prepared cubosomal dispersion was allowed to cool to room temperature and stored in a well-closed container protected from direct sunlight until further evaluation.[11]

Preparation of voriconazole loaded cubosomal Gel:

The Carbopol 940 (1%w/w) was dispersed in sufficient quantity of distilled water and allowed to soak for 12 hours for complete hydration. Triethanolamine 1%v/v was added dropwise with continuous stirring to neutralized the gel and adjust the pH until clear gel base was formed. The resulting gel was diluted with an appropriate amount of cubosomal dispersion at 1:1 ratio of dispersion to gel.[12]

Table 1: Formulation Design of Cubosome

Formulation Batch	Glyceryl monooleate (gm)	Poloxamer 407 (gm)	Voriconazole (mg)	Distilled water (ml)
F1	2.0	0.30	300	30
F2	2.0	0.35	300	30
F3	2.0	0.40	300	30
F4	2.5	0.30	300	30
F5	2.5	0.35	300	30
F6	2.5	0.40	300	30
F7	3.0	0.30	300	30
F8	3.0	0.35	300	30
F9	3.0	0.40	300	30

Analytical Method Used in the determination of Voriconazole:

a) Determination of λmax of Voriconazole:

The maximum absorbing wavelength (λmax) of Voriconazole was determined using a UV-Visible spectrophotometer (Shimadzu UV-1800) with Phosphate Buffer pH 7.4 containing 5% methanol as a solvent system. Accurately weighed 10 mg of Voriconazole was transferred into a 100 mL volumetric flask and dissolved in a small quantity of Phosphate Buffer pH 7.4 containing 5% methanol. The volume was then made up to 100 mL with the same solvent to obtain a stock solution of 100 µg/mL. From this stock solution, 1 mL was withdrawn using a pipette and transferred into a 10 mL volumetric flask. The volume was adjusted up to 10 mL with the same solvent system to obtain a working solution of 10 µg/mL. The prepared solution was scanned in the UV range of 200– 400 nm against the solvent blank using a UV-Visible spectrophotometer. The wavelength at which maximum absorbance was observed was recorded as the λmax of Voriconazole.

b) Standard calibration curve of voriconazole in pH 7.4 containing 5% Methanol:

1. Preparation of standard solution:

100 mg of Voriconazole was accurately weighed; it was transferred in to 100ml volumetric flask and drug dissolved in small quantity (15- 20 ml) of Phosphate Buffer pH 7.4 containing 5% methanol respectively. The volume was made up to the mark with same solvent system to get the concentration of 1000µg/ml. This is treated as primary stock solution.

2. Preparation of working standard solutions:

From the primary stock solution (1000 µg/mL), 10 mL was pipetted out and transferred into a 100 mL volumetric flask. The volume was made up to the mark with Phosphate Buffer pH 7.4 containing 5% methanol to obtain a secondary stock solution of concentration 100 µg/mL. From the secondary stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mL were withdrawn separately into a series of 10 mL volumetric flasks and the volume was adjusted up to the mark with Phosphate Buffer pH 7.4 containing 5% methanol to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL, respectively. The absorbance of each prepared solution was measured at the determined λmax using a UV-Visible spectrophotometer against the reagent blank. A graph of absorbance versus concentration was plotted to obtain the standard calibration curve of Voriconazole.[13]

c) Drug Identification & Drug- Excipient Compatibility Study:

1. FTIR Study

FTIR spectroscopy was performed to identify the functional groups and confirm the chemical structure of the individual components used in the study. FTIR spectroscopy was further performed to evaluate possible interaction between the drug and individual excipient used in the formulation. The spectra analysis was carried out by using FTIR Affinity-1 instrument in the range of 4000-400 cm^{-1} at a scanning speed of 20 cycles. For sample preparation, 5 mg of sample was mixed with approximately 200 mg of dry KBr powder and triturated uniformly to obtain a fine mixture. The mixture was compressed using a hydraulic press to form a transparent pellet. Each pellet was scanned individually, and the characteristic absorption peak were recorded. The spectra of drug excipient combination (1:1) ratio were compared with that of the pure drug to detect any significant shift, disappearance, or appearance of new peaks. [14]

2. DSC Study

Differential Scanning Calorimetry (DSC) study was carried out to evaluate the thermal behaviour and compatibility of Voriconazole with selected excipients. The thermograms of pure drug, and physical mixtures (1:1) ratio were recorded using a Mettler Toledo DSC instrument equipped with

STARe SW 12.10 software. Accurately weighed sample 3 mg were placed in aluminium pans and sealed properly. An empty aluminium pan was used as reference. The samples were heated over a temperature range of 30°C to 300°C at a heating rate of 10°C/min under a nitrogen atmosphere to maintain inert conditions. The thermograms obtained were analysed for characteristic endothermic or exothermic peaks, melting behaviour, and possible changes in thermal characteristics of the drug in the presence of excipients. The obtained data were used to confirm thermal behaviour and crystalline nature of the pure drug.[15]

CHARACTERIZATION OF THE PREPARED CUBOSOME:

Determination of Vesicle size and size distribution and zeta potential analysis:

The vesicles size was determined by dynamic light scattering (DLS), using a computerized inspection system (Malvern Zeta sizer Nano-ZS, Malvern, U.K.) For vesicles size measurement, vesicular dispersion about 1 ml was diluted with distilled water and put into the cuvettes of zeta sizer. Then the measurements were conducted at dynamic scattering angle of 173 at 25°C. The DLS measurements were performed during increasing and decreasing temperature cycles at each temperature the sample allowed to equilibrate for at least 3 minutes before performing the measurement. The average hydrodynamic diameter of the cubosome under consideration corresponds to the Z-average diameter by DLS. Hence, the data was collected for vesicles size and size distribution. Polydispersity index was also calculated. Zeta potential of cubosomal formulation was determined using Zeta Sizer at 25°C. [16]

Determination of Percentage Drug Entrapment Efficiency:

The cubosomal dispersion was centrifuged at 10,000 rpm for 30 min by using ultra centrifugation. to separate the free drug. A clear solution of supernatant and pellets of cubosome was obtained. Here clear supernatant was taken and analysed under UV spectrometer. It gives unentrapped drug concentration. The absorbance of the drug was noted at 255nm. Entrapment efficiency was calculated using formula:[17]

$$\%\mathbf{EE} = \frac{\mathbf{Total\ Drug\ concentration - Free\ Drug\ Concentration}}{\mathbf{Total\ Drug\ Concentration}} \times \mathbf{100}$$

Determination of Percentage Drug Content:

The drug content of the cubosomal formulation was determined by dissolving 1 mL of formulation in methanol to disrupt the vesicular structure and release the entrapped drug. The resulting solution was suitably diluted with methanol and analysed spectrophotometrically using a UV–Visible spectrophotometer (Shimadzu UV-1800) at wavelength of 255 nm and the percentage drug content was calculated by using formula:[18]

$$\%\ \mathbf{Drug\ Content} = \frac{\mathbf{Actual\ amount\ of\ drug\ present}}{\mathbf{Theoretical\ amount\ of\ drug\ present}} \times \mathbf{100}$$

Scanning Electron Microscopy:

The morphology and surface characteristics of the prepared cubosomes were evaluated using Scanning Electron Microscopy (SEM) employing a JSM-6360OL scanning electron microscope (Tokyo, Japan). For SEM analysis, a small quantity 1 ml of the cubosomal dispersion was carefully mounted onto an aluminium sample stub using double-sided adhesive carbon tape to ensure proper adhesion of the sample. The mounted samples were then subjected to gold sputter coating to improve electrical conductivity and prevent charging during electron beam exposure. A thin gold layer of approximately 200 nm thickness was deposited under reduced pressure maintained at 0.001 mmHg. After coating, the samples were examined under the SEM at suitable accelerating voltage and different magnifications to study the surface morphology, shape, and structural characteristics of the cubosomes. The obtained micrographs revealed the presence of discrete cubosomal particles with nearly spherical morphology, confirming the successful formation of cubosomal nanoparticles.[19]

EVALUATION OF CUBOSOMAL GEL:

Organoleptic Characteristics:

The cubosomal gel preparation was determined by visual examination for appearance, homogeneity and consistency.[20]

Measurement of pH:

The pH of various gel formulations was determined using a calibrated digital pH meter. Accurately weighed 1 g of gel was dispersed in 100 mL of distilled water and allowed to stand for 1 hours to obtain complete equilibration. The pH of each formulation was then measured by immersing the electrode of the pH meter into the prepared gel. The measurements were carried out in triplicate, and calculate average pH values.[21]

Spreadability:

The spreadability of gel formulation was determined by using horizontal glass slide method. Accurately weighed 1 g of gel was placed between two glass slides (10×10 cm). A standardized weight of 50 g was placed over the upper slide for 5 minutes to obtain uniform thickness of the gel layer. After removal of excess gel, the upper slide was tied with a weight and allowed to move over the lower slide. The time taken by the upper slide to move a specified distance and separate completely from the lower slide was noted. Spreadability was calculated using following formula:

$$S = M \times \frac{L}{T}$$

Where,
S = Spreadability
M = Weight tied to upper glass slide (g)
L = Length moved by glass slide (cm)
T = Time taken to separate the slides (sec) [22]

Viscosity:

The rheological behaviour of the prepared voriconazole cubosomal gel was evaluated using a Brookfield viscometer fitted with spindle No. 4. adequate quantity of gel was placed in a suitable container and the viscosity of each formulation was measured at different rotational speeds of 10, 20, 50, and 100 RPM. The spindle was allowed to rotate for 60 seconds at each speed before recording the reading. The viscosity readings for each batch were taken in triplicate and the mean ± standard deviation (SD) values were calculated. The variation in viscosity with change in RPM was used to determine the flow behaviour of the gel formulations. The obtained data were further used for rheological analysis and graphical representation.[23]

***In-Vitro* Anti-Fungal Study:**

The *in-vitro* antifungal activity of the prepared Voriconazole cubosomal gel formulation was evaluated against Candida albicans using the disc diffusion method. Sterile Sabouraud Dextrose Agar (SDA) medium was prepared and sterilized by autoclaving at 121°C for 15 minutes. The sterilized molten medium was poured into sterile Petri plates and allowed to solidify under aseptic conditions. A standardized fungal suspension of Candida albicans was prepared and uniformly spread over the surface of the solidified SDA plates using a sterile cotton swab. Sterile Whatman filter paper discs of 6 mm diameter were impregnated separately with the optimized Voriconazole cubosomal gel formulation and pure Voriconazole drug solution used as the reference standard. The impregnated discs were carefully placed on the inoculated agar surface using sterile forceps. The Petri plates were incubated at 28 ± 2°C for 24–48 hours. After incubation, the antifungal activity was determined by measuring the diameter of the zone of inhibition surrounding each disc in millimetres using a transparent ruler. Larger zones of inhibition indicated greater antifungal activity of the cubosomal gel formulation.[24]

***In- vitro* drug release study:**

The in vitro drug release study of the cubosomal gel formulation was carried out using by the dialysis membrane diffusion technique. In this method, a predetermined quantity of cubosomal gel 1 g was accurately weighed and placed into a pre-soaked dialysis membrane bag, A membrane with a molecular weight cut-off (MWCO) of 12,000-14,000 Da. which was then securely tied at both ends to prevent leakage. The dialysis bag containing the formulation was immersed in a beaker containing suitable dissolution medium phosphate buffer 7.4 maintained at 37±0.5°C The dissolution medium was continuously stirred using a magnetic stirrer at a constant speed to maintain uniform conditions throughout the experiment. At predetermined time intervals, a definite volume of sample 2 ml was withdrawn from the receptor medium and immediately replaced with an equal volume of fresh dissolution medium maintained at the same temperature to maintain sink conditions. The withdrawn samples were suitably diluted, whenever necessary, and analysed spectrophotometrically at the wavelength 255nm. reported in the respective monograph of the drug. The cumulative percentage drug release was then calculated and plotted against time to evaluate the release pattern of the cubosomal gel formulation.[25]

Stability Studies:

According to, International Conference on Harmonization (ICH) specifies the length of study and storage conditions:

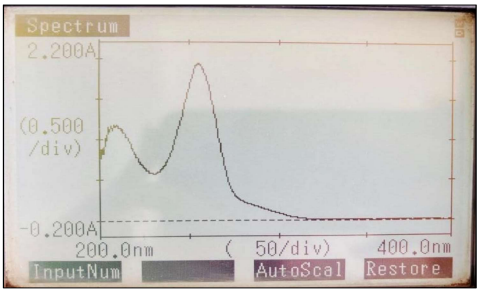
- Long term testing: 25 °C ±2°C / 60%RH±5% for 12 months.
- Accelerated testing: 40°C ±2°C / 75% RH ±5% for 6 months.

In the present study, stability studies were carried out at 2-8 °C, room temperature and at 40°C ±2°C / 75% RH ±5% for a specific time period up to 30 days for the optimized formulation.[26]

RESULTS AND DISCUSSION:

Determination of λmax:

UV-Visible spectrum of voriconazole was recorded using 10 µg/mL sample prepared with PBS 7.4 containing 5% methanol as a solvent system scanned between range of 200-400nm, the drug showed maximum absorption at 255nm. So, the λmax of voriconazole was found to be 255 nm.



Graph No:1 Absorbance maxima of Voriconazole

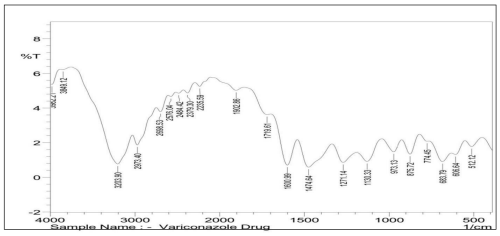
Standard calibration curve of voriconazole in pH 7.4 containing 5% Methanol:

Standard calibration curve data was given in table no 1. The UV–Visible spectrophotometric analysis of voriconazole in phosphate buffer 7.4 containing 5% methanol showed a maximum absorbance (λmax) at 255 nm, which was selected for all quantitative estimations due to its higher sensitivity and reproducibility. The calibration curve was

constructed over the concentration range of 2–20 µg/mL, and the absorbance values showed a proportional increase with concentration. y intercept and R^2 values was found to be 0.0271 and 0.9935 respectively.

Table no: 1 Standard calibration curve data for voriconazole

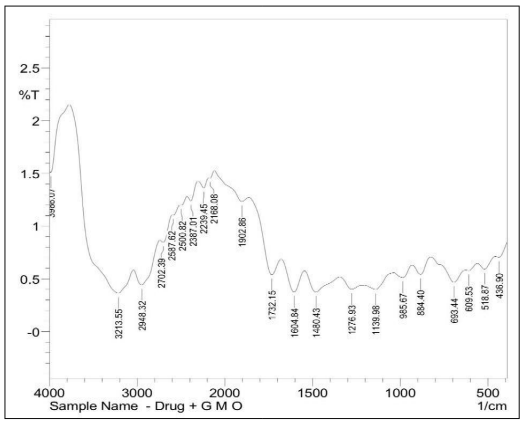
Sr no:	Concentration (ug/ml)	Absorbance
1	2	0.07
2	4	0.10
3	6	0.16
4	8	0.21
5	10	0.26
6	12	0.31
7	14	0.39
8	16	0.42
9	18	0.47
10	20	0.57



Graph No: 2 Calibration Curve of Voriconazole

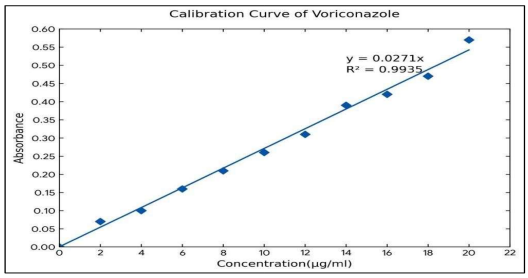
FTIR Spectroscopy:

The FTIR spectrum of Voriconazole and Drug with excipient was recorded to confirm the identity the characteristics functional group present in the molecule.



Graph No: 3 FTIR Spectra of Drug

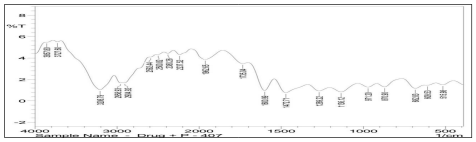
From the FTIR result of voriconazole, A broad peak observed at 3203.90 cm^{-1} was attributed to O–H stretching vibration, which falls within the standard range of 3200–3550 cm^{-1}. The peak at 2973.40 cm^{-1} corresponded to C–H stretching vibrations of aliphatic groups. A characteristic peak at 1600.99 cm^{-1} indicated the presence of C=N stretching, while the absorption peak at 1474.64 cm^{-1} was assigned to C=C stretching vibrations of the aromatic ring. The peaks observed at 1130.33 cm^{-1} and 1271.14 cm^{-1} confirmed the presence of C–F stretching vibrations, which are characteristic of fluorinated compounds. Additionally, a sharp peak at 1796.61 cm^{-1} corresponded to C=O stretching vibration. All the observed peaks were found within their respective principal ranges, confirming the characteristic functional groups of Voriconazole and indicating the purity and compatibility of the drug for further formulation development.



Graph No: 4 FTIR of Drug + Glyceryl monooleate

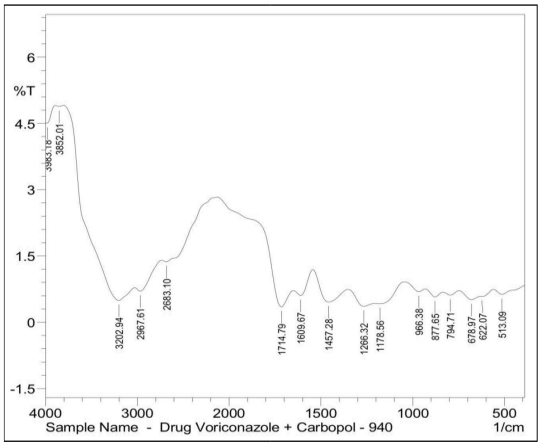
From the FTIR result of drug and excipient, The FTIR spectrum of the Voriconazole–GMO mixture (1:1) was recorded, spectrum showed a characteristic peak at 3213.55 cm^{-1} corresponding to O–H stretching vibration, while the peak at 2948.32 cm^{-1} was attributed to C–H stretching vibration. The absorption peak observed at 1604.84 cm^{-1} confirmed the presence of C=N stretching, and the peak at 1480.43 cm^{-1} corresponded to C=C stretching vibration. The characteristic peak at 1276.93 cm^{-1} indicated C–F stretching vibration, whereas the sharp peak at 1732.15 cm^{-1} represented

C=O stretching vibration. All the observed peaks were found within their respective principal ranges and retained their characteristic positions without any significant shifting or disappearance. This confirmed the absence of chemical interaction between Voriconazole and GMO and demonstrated good compatibility of the drug with the lipid excipient for cubosomal formulation development.



Graph No: 5 FTIR Spectra of Drug and Poloxamer-407

From the FTIR result of drug and excipient, The FTIR spectrum of the Voriconazole Poloxamer 407 mixture (1:1) was recorded, spectrum exhibited a characteristic peak at 3206.79 cm^{-1} corresponding to O–H stretching vibration, while the peak at 2958.93 cm^{-1} was attributed to C–H stretching vibration. The absorption peak observed at 1600.99 cm^{-1} confirmed the presence of C=N stretching, and the peak at 1472.71 cm^{-1} corresponded to C=C stretching vibration. The characteristic peak at 1269.22 cm^{-1} indicated C–F stretching vibration, whereas the peak at 1136.12 cm^{-1} represented C–O–C stretching vibration of the ether linkage present in Poloxamer 407. All the observed peaks were found within their respective principal ranges and retained their characteristic positions without any significant shifting or disappearance. This confirmed the absence of chemical interaction between Voriconazole and Poloxamer 407 and demonstrated good compatibility of the drug with the excipient for cubosomal formulation development.

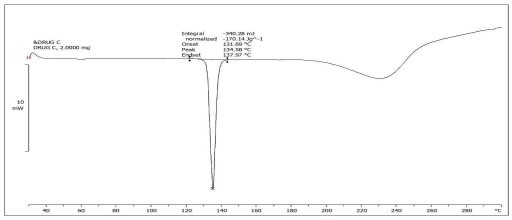


Graph No 6: FTIR Spectra of Drug and Carbopol-940

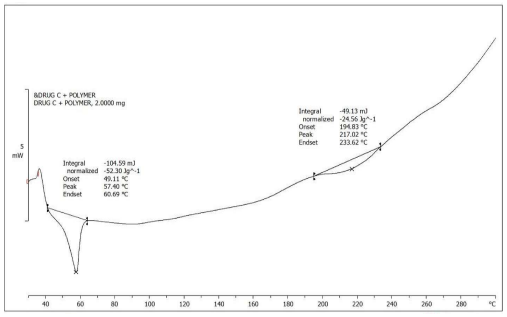
From the FTIR result of drug and excipient, The FTIR spectrum of the Voriconazole–Carbopol 940 mixture (1:1) was recorded, spectrum exhibited a characteristic peak at 3202.94 cm^{-1} corresponding to O–H stretching vibration. The peaks observed at 2967.61 cm^{-1} and 2683.10 cm^{-1} were attributed to C–H stretching vibrations. A sharp absorption peak at 1714.79 cm^{-1} confirmed the presence of C=O stretching vibration, while the peak at 1457.28 cm^{-1} corresponded to C=C stretching vibration. The characteristic peak at 1609.67 cm^{-1} indicated C=N stretching vibration, and the peak at 1266.32 cm^{-1} represented C–F stretching vibration. All the observed peaks were found within their respective principal ranges and retained their characteristic positions without any significant shifting or disappearance. This confirmed the absence of chemical interaction between Voriconazole and Carbopol 940 and demonstrated good compatibility of the drug with the excipient for cubosomal formulation development.

DSC Studies:

The DSC thermogram of voriconazole was measured to assess the thermal properties and melting characteristics of drug and confirm the crystalline nature.



Graph No 7: DSC Thermogram of Voriconazole

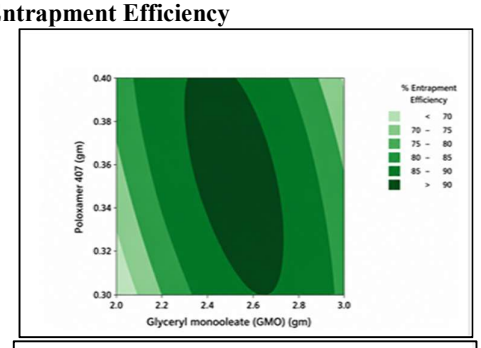


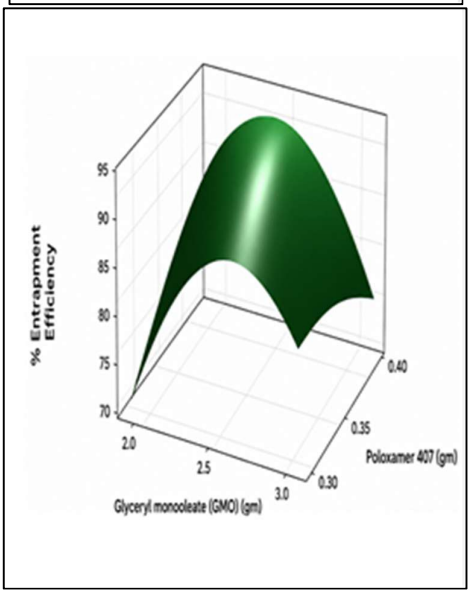
Graph No 8: DSC Thermogram of Voriconazole and Excipient Mixture

From the result The Differential Scanning Calorimetry thermogram of pure Voriconazole showed a distinct endothermic peak at 134.58°C with onset and end set temperatures at 131.69°C and 137.97°C, respectively. The sharp melting endotherm confirmed the characteristic corresponding to the melting point and crystalline state of the drug. The enthalpy value observed was −340.28 mJ with a normalized value of −170.14 J/g. No additional peaks or thermal degradation were observed before melting, indicating good thermal stability and purity of Voriconazole. The DSC profile obtained was consistent with the reported thermal characteristics of the drug. Hence,

the study confirmed that Voriconazole remained stable and suitable for formulation development. From the result (DSC) thermogram, thermogram of the physical mixture (1:1) containing Voriconazole, Poloxamer 407, GMO, and Carbopol 940 showed characteristic endothermic peaks at 57.40°C and 217.02°C. The peak observed at 57.40°C corresponds to the thermal transition of polymeric components, while the broad peak at 217.02°C indicates the thermal behaviour of the excipient matrix. The distinct melting peak of pure Voriconazole at 134.58°C was shifted/disappeared in physical mixture, indicating uniform dispersion of the drug within the polymer matrix and decline in crystallinity. No additional or unexpected peaks were observed in the thermogram, suggesting the absence of any significant drug–excipient interaction. The DSC analysis confirmed good compatibility of Voriconazole with Poloxamer 407, GMO, and Carbopol 940 incorporated into the formulation. Hence, the selected excipients were found to be appropriate for development of Voriconazole cubosomal gel formulation.

Effect of Formulation Variables on % Entrapment Efficiency





Graph No : 9 Contour Plot of

Graph No: 10 Surface Plot of % Entrapment Efficiency

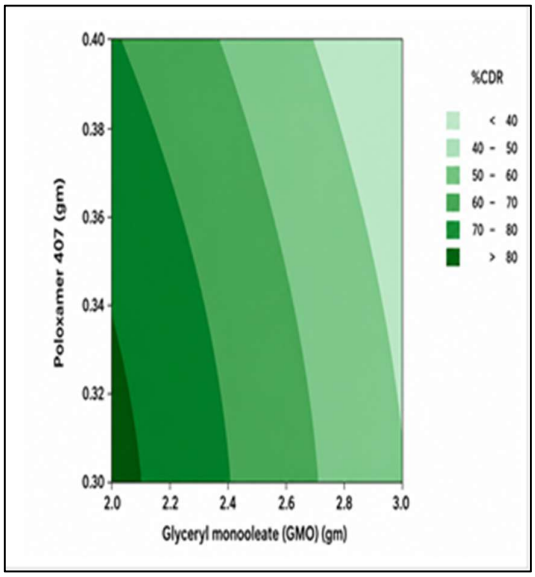
% Entrapment Efficiency

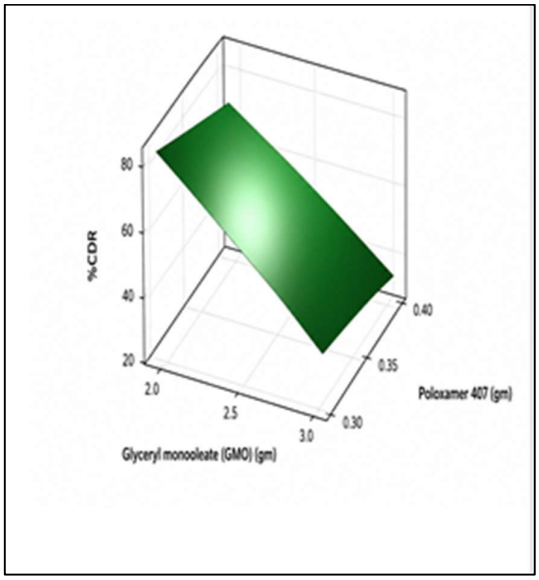
Regression Equation for %EE

EE (%) = $-503.4+320.2A+1078B-47.95A^2-733B^2-220.9AB$

The quadratic model was statistically significant (P = 0.010) with an R^2 value of 97.95%, indicating an excellent fit between the predicted and experimental responses. The quadratic effect of GMO and the interaction between GMO and Poloxamer 407 significantly influenced the entrapment efficiency. The contour and response surface plots demonstrate that both GMO and Poloxamer 407 affected the entrapment efficiency. Entrapment efficiency increased with increasing GMO concentration up to the middle level and then decreased at higher concentrations because of the negative quadratic effect. The highest entrapment efficiency was observed near 2.5 g GMO and 0.35 g Poloxamer 407, indicating the optimum formulation region for maximum drug entrapment.

Effect of Formulation Variables on % Response Surface Analysis of % Cumulative Drug Release (%CDR):





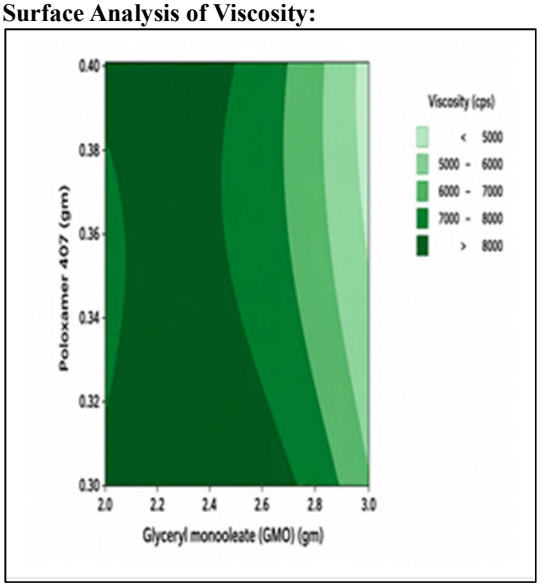
Graph No: 11 Contour Plot of % CDR
Graph No: 12 Surface Plot of % CDR

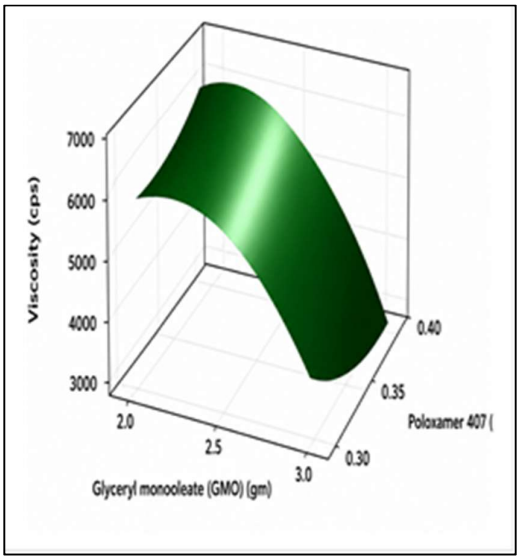
Regression Equation for %CDR

%CDR = 145.7−29.28A+58.7B −2.09A^2 −342B^2+28.41AB

The contour plot demonstrates that drug release decreased with increasing concentrations of both GMO and Poloxamer 407. Higher lipid content produced a denser cubosomal matrix that prolonged drug diffusion, resulting in sustained drug release. The optimized formulation exhibited controlled release characteristics. The response surface clearly indicates a gradual reduction in cumulative drug release as the concentration of GMO increased. The interaction between GMO and Poloxamer 407 also contributed to the release behaviour, producing a sustained-release profile desirable for topical delivery. The regression model for cumulative drug release was highly significant ($P < 0.001$) with an excellent fit ($R^2 = 99.96\%$). Both GMO and Poloxamer 407 significantly influenced drug release, confirming that formulation composition governs the sustained-release characteristics of the cubosomal system.

Effect of Formulation Variables on % Response Surface Analysis of Viscosity:





Graph No:13 Contour Plot of Viscosity
Graph No: 14 Surface Plot of Viscosity

Regression Equation for Viscosity

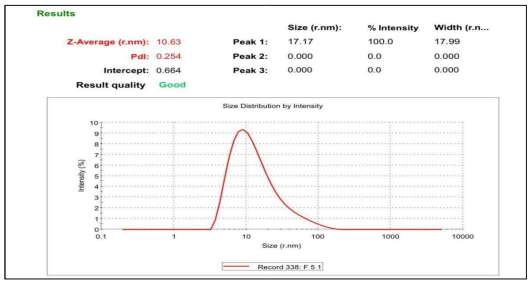
Viscosity = −3440+16080A− 45155 B− 2924A^2+ 88151B^2−8022AB

The three-dimensional response surface indicates that viscosity was primarily governed by GMO concentration. The curvature of the response surface suggests a quadratic relationship between formulation variables and viscosity. The viscosity model showed an R^2 value of 87.50%, indicating acceptable predictive capability. Among the formulation variables, GMO exerted the greatest

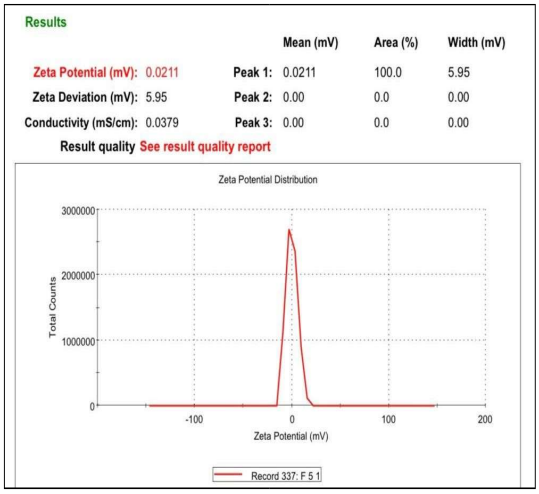
influence on viscosity (P = 0.035), while the effect of Poloxamer 407 was comparatively smaller. These findings suggest that optimization of GMO concentration is essential for obtaining the desired viscosity and ensuring suitable rheological properties for the cubosomal gel formulation.

Evaluation of Voriconazole Cubosome:

Particle Size and Polydispersity Index:



Graph No 15: Particle size measurement of Cubosome



Graph No 16: Particle size measurement of Cubosome

Among all prepared formulations (F1–F9), batch F5 containing 2.5 g GMO and 0.35 g Poloxamer 407 showed optimum characteristics. F5 Formulation had a particle size of a particle size of 10.63 nm with (PDI) of 0.254, indicating uniform particle size distribution and good homogeneity. The narrow peak observed in particle size distribution confirmed formation of stable cubosomal nanoparticles. A zeta potential of F5 was observed to be 0.0211 mV, indicating physical stability of the formulation due to steric stabilization provided by Poloxamer 407.

Entrapment Efficiency (%):

%EE range from 70.55% - 92.67%. The highest entrapment efficiency was found to be in the batch F5, consisted of 2.5 g of GMO, and 0.35 g of Poloxamer-407, The entrapment efficiency was dependent on the amount of lipid and stabilizers, The result showed that greater entrapment efficiency may be due to the increased the lipid concentration which can provide sufficient space for drug accommodation. Additionally, Poloxamer 407 stabilized cubosome structure and reduce drug leakage into external phase, resulting in improved drug entrapment.

Drug Content (%):

% drug content in various formulation varied from 73.15% - 96.42%. The high drug content observed in F5 may be attributed to the optimum amount of lipid and stabilizer, thereby facilitated efficient drug loading within the cubosomal matrix. These findings indicate that formulation composition Contributes significantly to determining drug-loading capacity of cubosomes.

Scanning Electron Microscopy:

Scanning electron microscopy was employed to determine structural characteristics and surface morphology of the prepared nanoparticles. SEM analysis revealed the external morphology, particle shape, surface texture of the nanoparticles. The SEM microphotographs of the optimized formulation batch (F5) revealed well-defined, distinct nanostructures distributed within the nanometre size range.

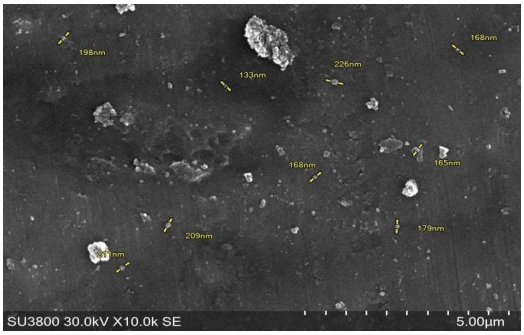


Figure No: 1 Scanning Electron Microscopy

Evaluation of Cubosomal Gel:

Appearance:

The Appearance of cubosomal gel was examined visually for its appearance, color, and homogeneity. All the formulation were found to be homogenous, smooth, white in colour. The result of cubosomal gel formulation (F1-F9) revealed that all formulation were white, smooth and homogenous in consistency.

Figure No: 2 Formulation of 9 batches of cubosome gel

pH of Cubosomal Gel:

The formulated gel exhibited a pH of 5.7, which is compatible to the physiological pH of the skin.

Spreadability of Cubosomal Gel:

F5 showed a spreadability of 46.46 g·cm/sec with a spreading time of 10.76 sec, facilitating convenient application, while ensuring uniform coverage of the skin. The optimized formulation possessed satisfactory spreading properties, making it acceptable for topical delivery.

Viscosity of Gel:

The viscosity of F5 batch was found to be 45034.67± 256.44 cps at 10 rpm, 26441.67± 98.19 cps at 20 rpm, 13147.33± 3.22 cps at 50rpm, and 6674± 3.22 cps at 100 rpm. As per the rpm increases the viscosity was decreases, which indicating a shear thinning a pseudoplastic behaviour of the gel formulation. The rheological characteristics was desired for the topical application.

***In- vitro* Anti- fungal study**

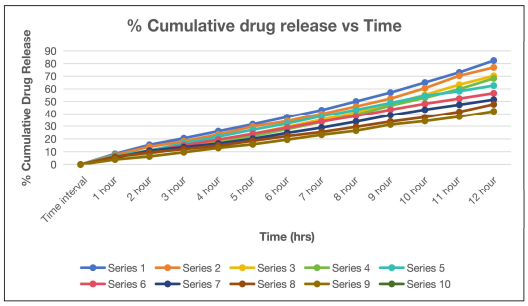
Figure No: 3 *In- vitro* Anti- fungal study

Table No: 6 Results of Anti-fungal study of cubosomal gel

Sr. No	Test Sample	Microorganism	Zone of Inhibition
1	e Anti-fungal drug	Candida albicans	12mm
2	al Cubosomal Gel	Candida albicans	14mm

The antifungal activity of the Voriconazole-loaded cubosomal gel carried out to check the antifungal effectiveness of the formulation against *Candida albicans* was assessed by employing the agar disc diffusion method. The formulation showed a (14 mm zone of inhibition, which was comparable to the reference drug (12 mm), indicating that Voriconazole retained its antifungal activity after incorporation into the cubosomal gel and was effectively released to inhibit fungal growth.

***In- Vitro* drug release study:**



Graph No:17 *In- Vitro* drug release study

Formulation batch	Cumulative Drug Release (%) (12hr)
F1	82.37%
F2	77.61%
F3	70.30%
F4	68.09%
F5	62.59%
F6	56.50%
F7	51.26%
F8	47.64%
F9	40.30%

Stability Studies:

Parameters	1st Month		2nd Month		3rd Month	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
Phase separation	–	–	–	–	+	+
Discoloration	–	–	–	–	–	–
Creaming	–	–	–	–	–	–
pH change	–	–	–	–	–	–
Drug precipitation	–	–	–	–	–	–
Homogeneity	Uniform	Uniform	Uniform	Uniform	Slightly changed	Slightly changed
Appearance	Smooth	Smooth	Smooth	Smooth	Acceptable	Acceptable

(-) = Absent / No Change observed, (+) = Present / Present / Change observed

CONCLUSION:

The current research emphasized the formulation and characterization of Voriconazole loaded cubosomal gel. A BCS Class II drug with low aqueous solubility and high permeability was selected, and drug loading was carried out to

enhance its solubility. to overcome this barrier and better results obtained in the form of cubosome, which significantly improved the drug solubility and permeability, as a result conclude that the voriconazole loaded cubosomal gel is effective for the treatment of fungal infection when administered as a cubosomal gel. The formulated gel containing optimized formulation (F5) when evaluated showed good rheological properties, stability testing, and sustained drug release. Hence it might be ideal formulation for the treatment of the fungal infection.

CONFLICT OF INTREST:

The author declares that there is the no conflict of interest.

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