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Formulation and Evaluation of Cubosomal In-Situ Gel for Topical Aaplication

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ABSTRACT

Background: Nanotechnology has emerged as a transformative approach in drug delivery, particularly for enhancing the therapeutic efficacy and targeting of poorly soluble drugs.The present study focuses on the formulation and evaluation of a thermosensitive cubosomal *in-situ* gel for the vaginal delivery of voriconazole, aimed at enhancing local antifungal therapy. Methods: Cubosomes were prepared using the thin film hydration method incorporating voriconazole, poloxamer 188, GMO and a suitable solvent, followed by hydration with distilled water to form a stable cubosomal dispersion. The dispersion was further integrated into an in-situ gel system using thermosensitive polymers Poloxamer 407 and Carbopol 940P prepared under cold conditions (2–8°C) to prevent premature gelation.

Results: Among the formulations, VF5 exhibited superior cubosomal characteristics with a drug content of 78.88%, entrapment efficiency of 73.24% and *in-vitro*drug diffusion 77.72% at 12 hours. Particle size analysis showed a Z-average of 102.0nm, PDI at 0.381 and Zeta potential at -10.2 mV, indicating good stability. Upon incorporation into the *in-situ* gel, formulation V2 demonstrated optimal physicochemical properties, including a pH at 5.4, Viscosity at 1128.33±12.89 cps and excellent spreadability. Gelation temperature was found to be 36±0.30°C with strong gel strength (+++) and a gelation time of 11seconds. V2 also showed the highest Drug Content (81.84%), Entrapment Efficiency (73.93%) and Drug Diffusion (79.57% at 12hrs) highlighting its potential as an effective topical drug delivery system.

Conclusion: These findings suggest that the cubosomal*in-situ* gel system offers a promising platform for controlled and targeted delivery of antifungal agents.

Keywords: *Thin film hydration method, Cubosome, Thermo-sensititve, Cold method, Topical application.*

1. INTRODUCTION

Nanotechnology has emerged as a transformative force in the field of drug delivery, particularly over the past century. Various nanocarriers such as cubosomes, liposomes, polymeric nanoparticles, dendrimers, micelles and solid lipid nanoparticles have been developed each with specific advantages for drug encapsulation, stability and controlled release.Cubosome is bi-continuous cubic liquid crystalline structure formed as colloidal dispersion in water with the help of suitable surfactants can result into nanostructured systems.[1-2] Generally cubosomes have particle size 100 to 300nm.[3-4] The 'in-situ gel' system has become one of the most effective new drug delivery methods. Its unique 'Sol to Gel' transition helps to improve patient compliance and comfort while facilitating a regulated and prolonged release of the medications. *In situ* gelling system is a formulation that is in solution form before entering in to the body, but it will change to gel form under various physiological conditions.[5-7]Around 70% of women experience this vaginal candidiasis infection at least once in their lifetime and approximately 8% suffer from recurrent episodes. The primary pathogen is *Candida albicans* responsible for about 90% of cases followed by *Candida glabrata*. This infection is basically not a sexually transmitted one. Women have a very high chance of developing vaginal candidiasis and probably have at least one during their lifetime. Having a pregnancy or diabetes makes them more vulnerable to this vaginal infection.[8] It can cause itching and pain on the vagina, a thick discharge of cheese like appearance, scorching sensation surrounding the vagina and irritation while having intercourse.

2. MATERIALS AND METHODS

The materials used in the formulation of cubosomal*in-situ*gel are obtained from different source such as voriconazole from auirobindo, Aurangabad, GMO from Meher chemicals, Mumbai, Poloxamer 188, Poloxamer 407, Glycerin from COSMO Chem. Pune,Carbopol940 from HI Media Laboratories Pvt. Ltd.,etc.

3. METHODS

Pre-Formulation Study:

Physical appearance

The sample of voriconazole was checked for its color, odor and natureby visualization.

Melting Point

The melting point was determined by using capillary method. This method consist of capillary tube, thermometer etc. A small amount of drug was placed in a clean capillary tube. Then the drug loaded capillary tube was introduced into melting point apparatus with gradually heat the sample and observe. The reading was taken with change in appearance.

UV Calibration:

Determination of ƛ max:

Accurately weighed 50mg of voriconazole was dissolved in ethanol in 50ml. the wavelength max of voriconazole was detected by using wavelength ranges of 400-200nm by using UV-Spectrophotometer (Model: Shimadzu UV-1800, Japan).

UV Spectroscopy of Voriconazole

Accurately weight 50mg of pure Voriconazole drug was diluted in 50ml ethanol (1000µg/ml).From this solution remove 1mL and transferred to another 100ml volumetric flask and adjust volume upto 100mL by using PBS 7.4. (10µg/mL- Stock I).From Stock-I;2, 4, 6, 8 and 10ml was taken and transferred to 100ml volumetric flask and volume was made up to 100ml using PBS 7.4 to make 0.2, 0.4, 0.6, 0.8 and 1µg/ml solution respectively.

Fourier Transform Infrared Spectrum study (FTIR):

FT-IR was used to check compatibility of drug & excipientsover a range of 4000 - 400Cm^{-1}. On the surface of the FTIR, A dry sample was placed to obtain IR spectrum; then compared to voriconazole standard group frequencies.

Method of Formulation:

A. Preparation of Voriconazole Loaded Cubosomes [9-10]

Cubosome were prepared by thin film evaporation method. A weighed amount of voriconazole, poloxamer 188 and suitable solvent were combined in a rotary evaporator and subjected to evaporation at 40^0C until complete removal of the solvent. The resulting thin film was then hydrated with 20ml of distilled water to form a cubosomal dispersion form then evaluate.

Table No.1: Preparation of Cubosomes

INGREDIENTS	VF1	VF2	VF3	VF4	VF5	VF6
Voriconazole (mg)	20	20	20	20	20	20
GMO (mL)	20	25	30	35	40	45
Poloxamer 188(mg)	02	04	06	08	10	12
Water (mL)	20	20	20	20	20	20

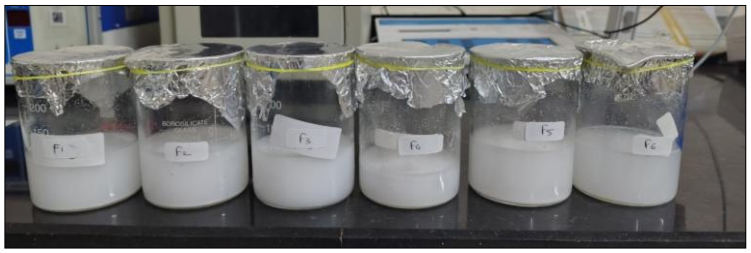

Fig. No.1: Preparedbatches of cubosome

B. Preparation of VZ-Cub-*in-situ* gel [9-10]

Separately, Thermosensitive polymer Poloxamer 407 and Carbopol 940P were dispersed in chilled distilled water, maintaining the temperature between 2-8^{0}C to ensure proper dissolution and prevent premature gelation. After complete dispersion of the polymers, this cold polymeric solution was added to the previously prepared & optimized cubosomal dispersion under continuous stirring to obtain the final *in-situ* gel formulation & evaluate.

Table No.2: Formulation of cub-*in-situ* gel

Ingredients	VF1	VF2	VF3
Cubosomal dispersion (mL)	20	20	20
Poloxamer 407 (mg)	18	20	22
Carbopol 940P (mg)	450	500	550
Glycerine (mL)	0.05	0.05	0.05
Benzoic Acid (mL)	0.01	0.01	0.01
Solvent (mL)	Q.S.	Q.S.	Q.S.

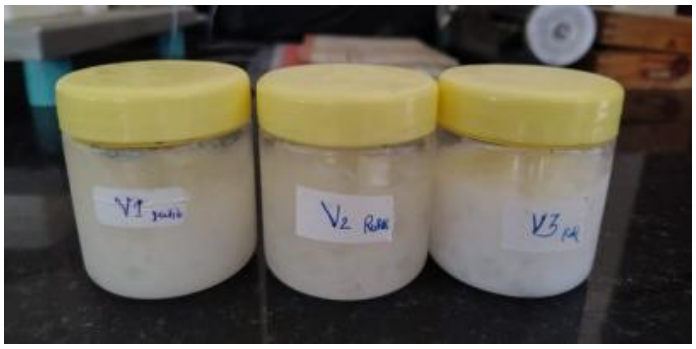

Fig. No. 2: Prepared cub-*in-situ* gel batches

Evaluation of Cubosome

Appearance

The appearance of cubosome was carried out by visual observation.

Drug content (%)

An appropriate conc. of drug was stirred in 10ml of PBS 7.4 for 1hr.Check the filtered sample at 256 next to the blank with an ultraviolet spectrophotometer. [11]

% Drug Content = (Actual concentration of drug in the formulation ÷ Theoretical concentration of drug) × 100

Entrapment Efficiency (%)

Drug % EE of drug can be calculated by weight amount of voriconazole added cubosomal*in-situ* gel were added in phosphate buffer 7.4 and diluted with 100ml phosphate buffer 7.4 solutionand sonicate for 10 minutes, then centrifuged at 1000rpm for 15min then the supernatant was withdrawn and further diluted by buffer 7.4 solution and analyzes at wavelength max at 256nm of voriconazole using UV spectroscopy.

***In-Vitro* Drug Diffusion**

In-vitro drug diffusion study was conducted using a cellophane membrane soaked in pH 7.4 PBS for 12 hrs. The drug-loaded *in-situ* gel was enclosed in the membrane sealed at both ends and placed in a receptor compartment containing PBS at 37±0.5°C. Samples were collected at intervals and analyzed by UV-spectrophotometry.

Microscopic analysis

The microscopic analysis of prepared cubosome was studied under the light microscope. A sample was placed on a slide and slide was studied under the microscope.

Particle size & Zeta potential

The particle size analysis and zeta potential of formulation was performed by photon correlation spectroscopy that analyzes the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a zetasizernano series &horiba. Before measurement the sample was diluted with double distilled water measured.

Scanning Electron Microscopy (SEM)

The morphology of sample was examined using a Scanning Electron Microscope with an image analysis system. Double-sided adhesive tape was used to secure the sample to a brass stub, and it was vacuum coated with platinum for 120 seconds at 15 kV. The temperature ranged from 25 to 3000 degrees Celsius. Nitrogen was purged at a rate of 100 milliliters per minute to maintain an inert environment.

1) Evaluation of Cubosomal*in-situ* gel

pH

A calibrated pH meter was used to measure the gel's pH. [12]

Viscosity

Viscosity of the gels was measured using a Brookfield DV-II Viscometer with spindle no.6 at 50rpm. Initial viscosity was recorded at room temperature, then after gel formation at 37±1°C. For Carbopol-based gels pH was also adjusted. Measurements were done in triplicate and averaged. [12]

Spreadability:

Using a two-slide device, 1g of gel was sandwiched between glass

plates and a 50g weight was applied for a minute to test spreadability. Tests were then conducted in duplicate, and M is equal to the the spread area's diameter was measured.(13)

S = ML/T

weight tide to the upper slide (g). L = length (in centimeters) traveled on the glass slide T = time spent (sec)

Gelation Time

Gelation time determined by at which time to sol to gel transition happens.

Gelling Temperature

The gelation temperature was determined by placing the solution in test tube dipped in water bath whose temp was raised steadyabove 35^0C. The temp at which solution was converted to gel was noted down by placing the thermometer in the test tube.[14]

Gel Strength:

An accurate weighed 30gm of formulation was placed in a 50ml graduated measuring cylinder and was allowed to form gel in a water bath at 37^0C. By applying to sink 5cm down through the gel, strength was measured.

Similar procedures were referred to conduct drug content (%), drug entrapment efficiency, and *in-vitro* drug diffusion studies of cubosomal*in-situ* gel.

Stability study:

Optimized *in-situ* gel was assessed for stability test for 3 months by exposing the sample at accelerated stability condition (40±5^0C, 75% RH), after stability of the *in-situ*gel is evaluated for Drug content, pH, Viscosity, Entrapment efficiency.

4. RESULTS AND DISCUSSION

Pre-formulation study:

Physical appearance:

It is a white to off-white, amorphous powder that has a bitter flavor and is odorless or almost odorless.

Melting point: It was discovered that voriconazole had a melting point of 1290C.

UV-Calibration: The absorbance rises linearly with concentration, suggesting that Beer-Lambert's law is being followed. This shows a direct association between concentration (2–10 µg/ml) and absorbance values (0.132–0.668).

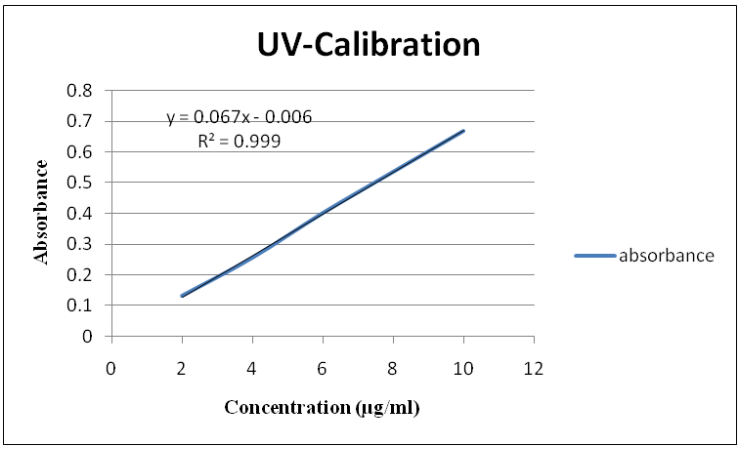

Fig. No.3:UV-Calibration of drug

Fourier Transform Infrared Spectrum (FTIR)

The FT-IR study of voriconazolewas performed to check the interaction with polymer. The Perkin Elmer FT-IR Spectrometer was used to perform the drug's FT-IR spectra.

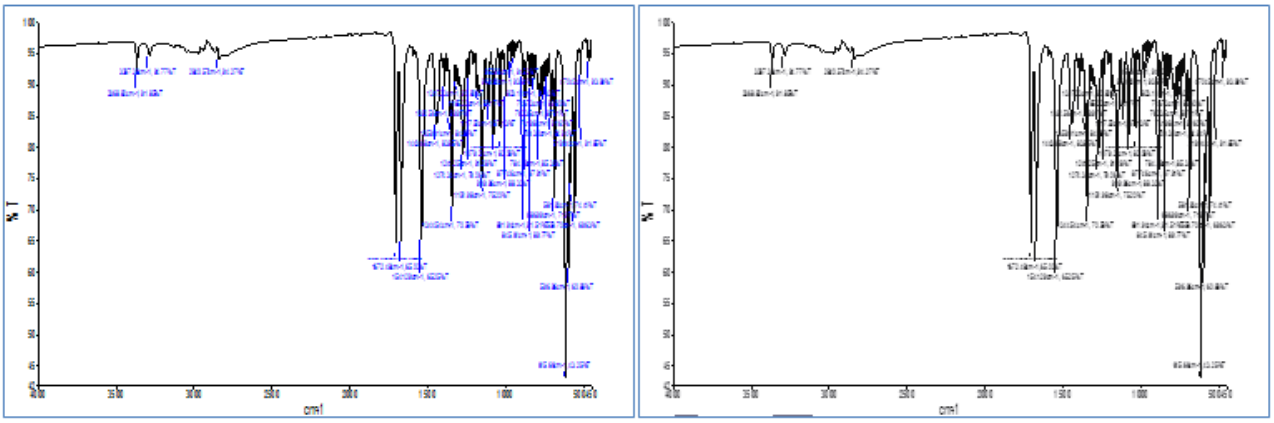

Fig. No.4: FTIR of API & physical mixture of API with excipients

Table No.3: FTIRInterpretations for physical mixture of API with excipients

Sr. No.	Functional Groups	Standard Frequency Cm^{-1}	Observed Peak Frequency Cm^{-1}
1	O-H	3200-3600	3368.82
2	C=C	1450-1600	1541.29
3	C-F	1000-1400	1359.91
4	C-O	1050-1300	1274.34

It was states that there are no any incompatibilities when API & excipients are mixed.

- **Evaluations of Cubososme**

- **Drug Content%&Entrapment Efficiency %**

The drug content of each batch was studies and the drug content formulation VF5 was found to be 78.88%. Each batch's entrapment efficiency was examined, and formulation V2's drug entrapment rate was determined to be 73.93%.

Table No.4: Drug Content % &Entrapment Efficiency %

Formulation Code	Drug content (%)	Entrapment efficiency (%)
VF1	72.52	70.23
VF2	73.01	69.90
VF3	69.90	59.99
VF4	77.78	54.01
VF5	78.88	73.24
VF6	67.72	54.22

- ***In-Vitro* Drug Diffusion**

The result of the optimized batch VF5shows that drug diffuse at 12hrs 84.92%. As compared to the other formulation VF5 has better drug diffusion.

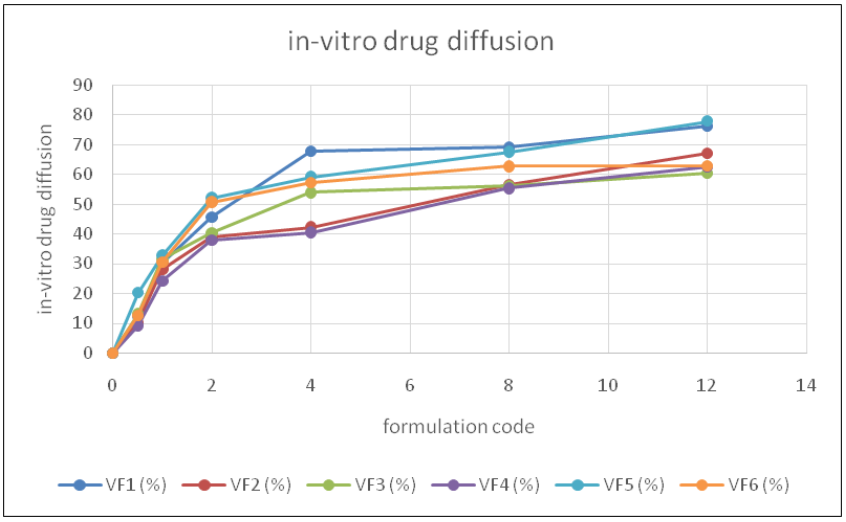

Fig. No.5: *In-vitro* Drug Diffusion

- **Microscopic analysis and SEM**

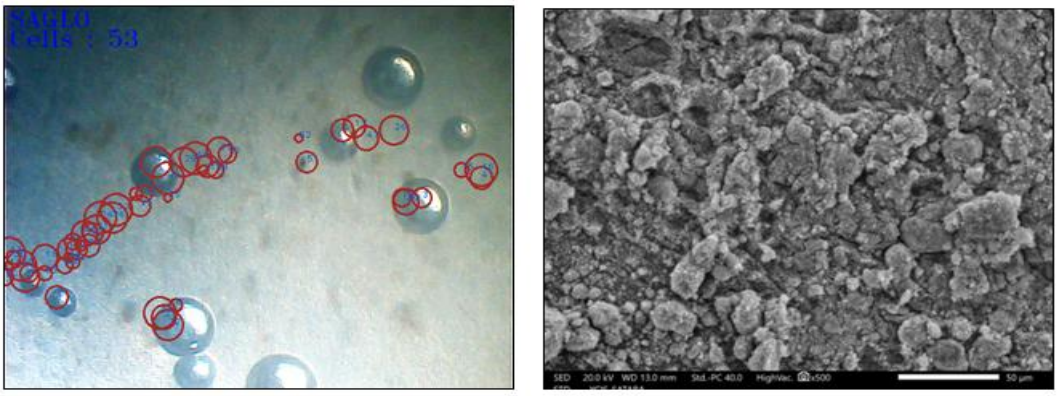

Fig. No.6: Microscopic image & SEM image

- **Particle Size &Zeta Potential**

The formulation showed a Z-Average is 102.0 nm and Zeta Potential is -10.2 mV.

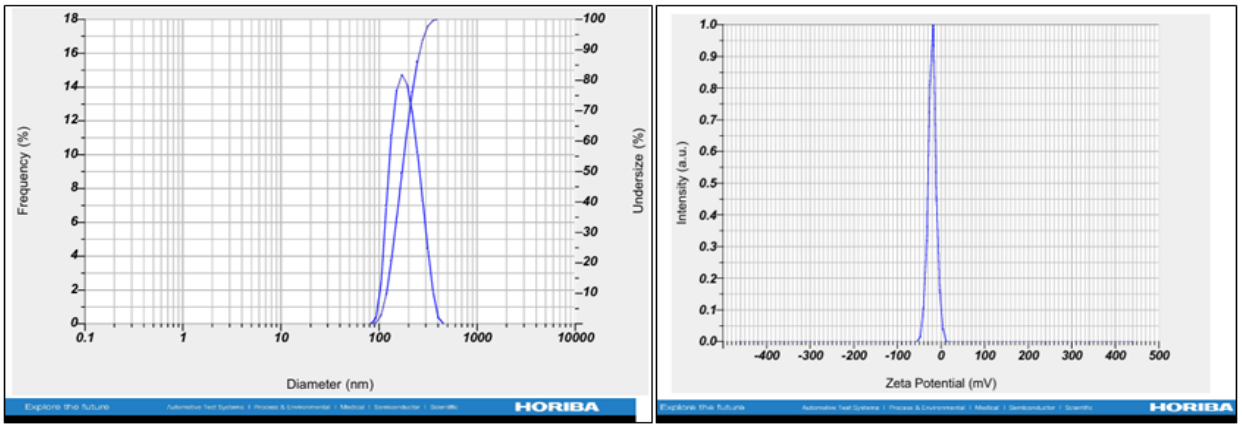

Fig. No.7:Particle size&Zeta Potential

➢ **Evaluations of cub-*in-situ* gel**

- **pH**

The pH values of V1(5.5), V2(5.4) and V3(5.6) fall within a slightly acidic range, making them suitable for mucosal applications. Shown in table no.5

- **Viscosity**

Formulations V1,V2 and V3 showed increasing viscosities (982.33±5.50, 1128.33±12.89 and 1254.33±3.21cps) suggesting higher polymer content or gel strength, with low standard deviations indicating good consistency.Shown in table no.5

- **Spreadability**

Formulation V2 shows the highest spreadability (13.41±1g.cm/sec) with the least variability, indicating better and more consistent performance compared to V1 and V3.Shown in table no.5

- **Gelation Strength**

Formulations V1 and V3 showed moderate gel strength (++) while V2 exhibited stronger gel consistency (+++) likely due to higher polymer content or better cross linking. This firmer gel supports prolonged drug retention and diffusion. Results are summarized in Table No.5

- **Gelation Time**

V2 showed the fastest gelation (11sec) compared to V1 (28sec) and V3 (31sec), enabling quicker *in-situ* gel formation and better retention. See Table No.5

- **Gelation Temperature**

Formulations V1, V2 and V3 showed gelation temperatures near body temperature (36±0.20°C, 36±0.30°C and 35±0.50°C) confirming suitability for *in-situ* gelation. Results are shown in Table No.5

Table No.5: Characterization results of *in-situ*gel

Formulation code	Gelation temperature (^{0}c)	Gel strength	Gelation time (sec)	Viscosity (cps)	Spredability (g.cm/sec)	pH
V1	36±0.20	++	28	982.33±5.50	12.41±0.2	5.5
V2	36±0.30	+++	11	1128.33±12.89	13.41±1	5.4
V3	35±0.50	++	31	1254.33±3.21	12.80±0.3	5.6

Fig. No.8: Sol-Gel Transition

- **Drug Content % & Entrapment Efficiency %**

The drug content of each batch was studies and the drug content formulation V2 was found to be 81.84%. The entrapment efficiency of each batch was studies and the drug entrapment of formulation V2 was found to be 73.93%.

Table No.6: Results of Drug content and EE%

Formulation Code	Drug content (%)	Entrapment Efficiency (%)
V1	78.96	54.73
V2	81.84	73.93
V3	77.77	53.12

- ***In-Vitro* Drug Diffusion**

The result of the optimized batch V2 shows that drug diffuse at 12 hrs 79.57%. All compared toother formulation. V2 has better drug diffusion.

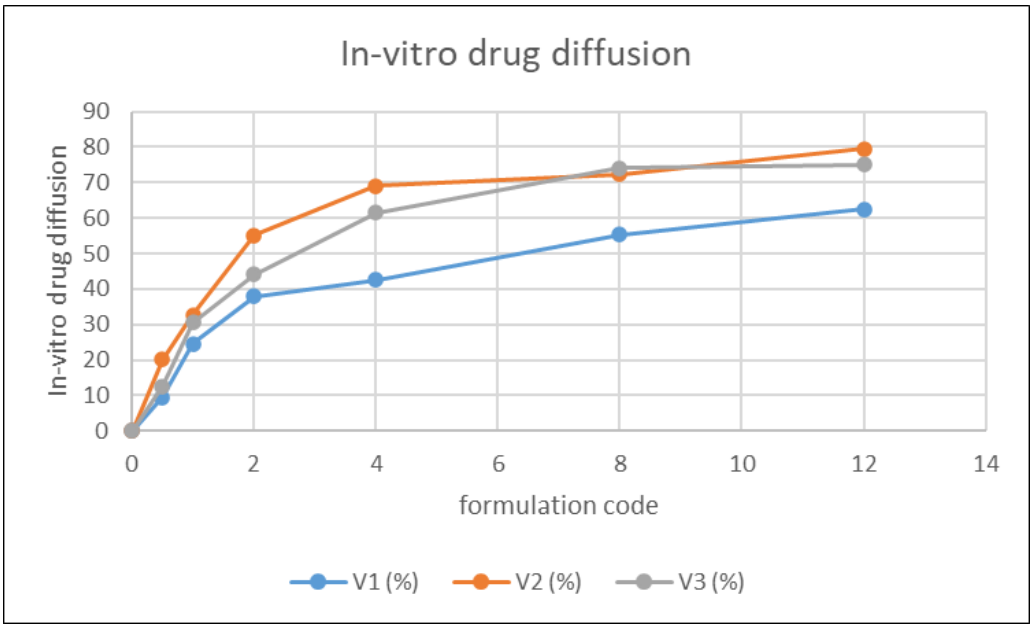

Fig. No.9: *In-Vitro* drug diffusion

- **Stability study**

The stability study was conducted as per the ICH Guidelines 3 months study at $40^0C \pm 2^0C$ and 75±5% RH.

Table No.7: Stability study

Stability Period	pH	Viscosity (cps)	(%) Drug Content	Entrapment Efficiency (%)
Initial	5.4	1128.33±12.89	81.84	73.93
1 month	5.4	1128.33±12.89	81.84	73.93
2 months	5.4	1118.33±0.29	81.27	73.11
3 months	5.4	1048.28±23.12	80.96	72.42

5. CONCLUSION

In this study, voriconazole-loaded cubosomalin-situ gel was effectively created for topical medication administration, with formulation V2 demonstrating the best results. The approach provides better local bioavailability, controlled release, and increased medication retention. Future research is required for in-vivo validation, but it shows promise in treating vaginal candidiasis.

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