RESEARCH ARTICLE

Development and Evaluation of Voriconazole Loaded Invasomes Gel for Enhanced Antifungal Activity

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ABSTRACT

Although it has limited solubility and poor permeability to the skin, voriconazole (VRC) is a viable choice for the topical treatment of fungal infections. Owing to these constraints, treating the inflection requires several injections over an extended period. The goal of this work was to produce a VRC invasome gel with improved topical antifungal activity. The Box-Behnken design (BBD) program was utilized to optimize the IVS after it was created using the thin-film hydration process. The optimized invasome formulation through BBD has 159.9 nm of vesicle size, 68.58% of entrapment efficiency, and 23.5 mV of zeta potential. The spherical shape of the vesicle was revealed using scanning microscopy. According to FTIR spectra, the medication and polymers do not interact. The optimized invasomal VRC gel exhibited an optimum of 345 to 589 cp. Ex-vivo permeation studies of IG4 exhibited a higher flux of 0.168 as compared to pure voriconazole. An antimicrobial study was accepted to check the antimicrobial efficiency of voriconazole gel (IG4). The study confirmed a good zone of inhibition of the fungus infection (*C. albicans*). The voriconazole invasome's potential diffusion and antibacterial efficacy are demonstrated here. Invasomal gel was found to be an effective carrier and a desirable strategy for improving the topical distribution of VRC to treat fungal infections.

Keywords: Fungal infection, Voriconazole, Invasomes, Gel, Box-Behnken design, Antifungal activity.

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INTRODUCTION

For the public, as well as those with weakened immune systems in developing nations, fungus and leishmania infections pose a serious risk to life. The emergence of germ resistance, inadequate diagnosis, and therapy restrictions are the reasons why the death rate remains high.[1,2] The two most prevalent fungi that cause infections on human mucous membranes, skin, and organs are candidiasis and aspergillosis. Aspergillus affects the skin, hair, eyes, and lungs, while Candida affects the mouth, nails, skin, foot, and bloodstream. The liver, spleen, and mucocutaneous tissue become severely infected with leishmania.[3] Voriconazole (VRC) has proven to be quite effective against Candida, Aspergillus, and Leishmania species in recent research on fungal and leishmaniasis disorders.[4]

Only oral and IV preparations of VRC are currently available for systemic administration. Low soluble and very permeable, VRC belongs to BCS class-II drugs. VRC is administered systemically, which results in renal damage. There have been several attempts to increase its solubility; nevertheless, notable advancements have been hindered by the lack of stability, control, and immediate release, which has increased the formulations' complexity. The permeability of the skin is reduced and skin irritation results from conventional topical application.[5]

As formulations are developed to improve the bioavailability of numerous medications, transdermal delivery is growing at a rapid pace. Medicines used topically have a deleterious effect on the skin barrier.[6,7] Various investigators have presented novel kinds of lipid vesicles throughout the past 20 years. Invasomes are a novel class of vesicular systems that have been studied more lately. To put it briefly, ethanol and terpenes, in addition to phospholipids, are found in invasomes and act as penetration enhancers by allowing the vesicles to be bent. Dermatological absorption of lipophilic and hydrophilic medications has been demonstrated to be enhanced by this approach.[8,9] The percutaneous absorption of ethanol and terpenes has been shown to benefit greatly from their synergistic interaction. With improved efficacy and patient compliance, the creation of invasomal formulation can function as an effective topical drug delivery method.[10] To assess the antifungal activity,

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Table 1: Factors considered and their quantity

Factor	*Name*	*Units*	*Minimum*	*Maximum*
A	Phospholipid	mg	50.00	300.00
B	D-Limonene	ml	0.1000	2.00
C	Span 60	mg	10.00	100.00

the current study was planned to create invasomes and then incorporate them into a gel form.

MATERIALS AND METHODS

Materials

Voriconazole was purchased from Manus Aktteva Biopharma. Phosphatidylcholine and span-60, D-limonene, Carbopol 934P, and PEG 400 were purchased from SD Fine Chem (Mumbai, India). All other chemicals used for the study and obtained from the laboratory are analytical grade.

Method of Preparation of Invasome

Utilizing the film hydration approach, an invasome filled with voriconazole was created. Seventeen formulations in total were produced, as the table indicates. The ethanol-dissolved phosphatidylcholine and span-60.[11] Following the transfer of the solution to a round bottom flask (RBF) in a rotary flask evaporator (RE-2010, Biobase, Mumbai, India). Next, an RBF film was obtained by fully drying the mixture at 40°C and 400 rpm. In another beaker drug and terpene was dissolved in ethanol. Phosphate buffer 7.4 was heated at 50°C then added to the above mixture. Subsequently, voriconazole, D-limonene, and ethanol are added to the phosphate buffer solution (pH: 7.4) and the deposited film is hydrated for 30 minutes. After cooling to room temperature, the obtained vesicles are vortexed and ultrasonicated (UAI-PS20khz-900W, India) at 2% amplitude for 10 minutes to obtain different pore sizes of the invasome.

Formulations suggested by Box-behnken design (BBD)

In the current investigation, A total of 17 experimental runs, a two-level, 3-factor BBD was employed to measure the impact of independent factors on the responses.[12] Three independent factors such as Phospholipid, D-Limonene, and Span 60, were considered. The responses noted in the experiment are particle size, zeta potential, and entrapment.

The polynomial equation was utilized to do mathematical fitting and analysis. A numerical technique and a graphical optimization methodology were used to solve the optimized solution, with a confidence interval value of $\alpha<0.05$.

Characterization of Invasome

A precise 100 mg equivalent of Invasome was weighed out and added to a 100 mL volumetric flask along with the bare minimum of ethanol, which was mixed well. The Ultrasonicator, CPX3800-E, Branson, was used to sonicate the dispersion for around ten minutes. The mixture that was left over was mixed with phosphate buffer that had a pH of 7.4. The volume was then adjusted to the appropriate value. After an extra ten minutes under a wash with sonication, the dispersion turned translucent. A Whatman membrane with a 0.45 µm pore

Table 2: Formulation table

	Factor I	*Factor II*	*Factor III*	*Response I*	*Response II*	*Response III*
Run	*A: Phospholipid*	*B: D-Limonene*	*C: Span 60*	*Particle size*	*Zeta potential*	*Entrapment*
	mg	*ml*	*mg*	*nm*	*mV*	*%*
1	300	0.1	55	159.9	23.5	67.37
2	175	2	10	153.4	15.1	64.58
3	300	1.05	10	181.2	32.7	70.11
4	175	0.1	10	148.6	11.8	60.58
5	175	1.05	55	149.7	13.8	63.87
6	300	2	55	177.3	27.7	73.29
7	50	2	55	140.1	12.2	59.66
8	50	0.1	55	138.7	11.9	54.18
9	175	1.05	55	143.4	13.5	62.39
10	175	1.05	55	143.8	12.9	61.64
11	50	1.05	10	145.1	11.6	57.82
12	175	1.05	55	142.9	13.3	62.75
13	175	2	100	80.9	17.8	66.56
14	175	1.05	55	143.5	13.7	62.11
15	50	1.05	100	71.9	10.5	56.04
16	175	0.1	100	72.9	14	58.79
17	300	1.05	100	85.3	22.9	68.53

size was used to filter the resultant combination. The amount of drug was measured using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan) set to 256 nm.

Attenuated Total Reflection (ATR) Study

Online monitoring of polymer composition is a particularly valuable application of ATR spectroscopy. IR can identify the components of a chemical process because of its capacity to fingerprint chemical components. The German company ATR Bruker Opus 7.0 carried out the investigation.[13]

Surface Morphology, Particle Size, and Zeta Potential

A scanning electron microscope (SEM) (S3700N-Hitachi, Japan) was used to examine the morphology of the optimized voriconaozle-loaded invasome. After spreading the material out across the slab surface, a glass slide was placed on it, and photos under a variety of magnifications were taken. In a similar way, the Malvern Zeta Sizer from ATA Scientific in the USA was used to assess the particle size and polydispersity index value of invasomes.

Differential Scanning Calorimetry (DSC) study

DSC (Shimadzu, USA, DSC-60) is a thermal analysis that tracks temperature and heat changes in a sample over time. It logs temperature and heat flow related to material transitions as a function of temperature and time.

X-ray Diffraction (XRD) study

By examining the crystal structure of a material, XRD analysis may determine which crystalline phases are present and, therefore provide details about the chemical composition of the material. Thermo Scientific, India's ARL EQUINOX 100 was used to conduct the XRD research.

Preparation of Gel loaded with Invasome

In a 100 mL beaker, Carbopol 934P was placed and lukewarm water was poured. Mixing was done in a magnetic stirrer at 50 RPM (Remi stirrer, Mumbai, India) to avoid the formation of a lump. The required quantity of triethanolamine was poured into the resulting mixture to adjust the pH to obtain gel. In another beaker, PEG 400, glycerol, and benzalkonium chloride were taken and mixed properly. To that mixture, Invasome is poured and mixed properly. Finally, the drug dispersion was poured into Carbopol 934P gel and stirred at 100 RPM by a magnetic stirrer. A total of seven formulations were developed and stored in a refrigerator (10°C) and evaluated for characterization.

Characterization of Gels

Drug content

One hundred milliliters of pH 7.4 ethanolic phosphate buffer was used to dissolve a precisely determined portion of the prepared gel. The gel solution was constantly shaken using a mechanical shaker and analyzed at 256 nm using a UV-visible spectrophotometer.

pH determination

A digital pH meter (Systronic Digital, Model: 335, Mumbai) was used to measure the gel's pH.

Viscosity

The produced gels' viscosity was measured by a Brookfield viscometer (model DV-II+Pro, USA) . Spindle No. 64 was used and recorded at 50 rpm for this purpose.

Spreadability (S)

A wooden block and a glass slide device were used to gauge the gels' spreadability. In the pan, there was around 500 mg of formed gel. There were seconds that the upper slide took to disengage from the fixed slides. The gel spreadability was assessed using the accompanying formula:

$$S=ML/T \quad \text{....(Equation 1)}$$

Where M = mass tied to the upper slide, L = length of a glass slide, and T = time taken by the slide to separate.

Homogeneity

To assess homogeneity, each generated gel formulation was characterized by visual inspection . The look and presence of any clogs in the gels were examined.

In-vitro diffusion study

Voriconazole was extracted from the gel using a Franz-diffusion cell method. Before being employed, the cellophane membrane (6 by 2.5 cm) was divided into equal portions and soaked in distilled water for an entire day. The drug release tests of the drug solution are conducted using 10 ml of phosphate buffer pH 7.4 saline, which is continuously heated using an IKA Auto Temp Regulator (Germany) and a magnetic stirrer to maintain a temperature of 37 ± 0.5°C. A single gram of gel was taken and added to the donor section. One milliliter aliquot sample were taken out and substituted with an equal volume of fresh buffer at regular intervals. To dilute the aliquots, fresh medium was added as needed. Measuring the quantity of drug that diffused across the membrane included using a UV spectrophotometer set to operate at 256 nm.

Ex-vivo permeation study

The study was performed using goat skin collected from a slaughterhouse. Phosphate buffer 7.4 (100 mL) was used for the knot of skin at both ends. Temperature of 37 ± 0.5°C for the investigation, which was carried out on a magnetic stirrer running at 50 rpm. An analysis of drug penetration was conducted using UV visible spectrophotometer at 256 nm on a 2 mL sample that was taken up to 6 hours beforehand. Voriconazole gel and optimised invasome underwent the same process again. A plot was created to show the drug's penetration rate against time.

RESULTS AND DISCUSSION

Entrapment Efficiency

It was noted that formulations with high phospholipid contents had significant EE. T3 and T17 are entrapped at 70.11 and 68.53%, respectively, whereas the formulation "T6" has a maximum of 73.29%. In a similar way, the percentage of D-Limonene indicated the EE. As can be observed, formulation

Table 3: Drug loading of formulations T1-T17

Run	*Drug loading*
T1	69.38 ± 2.5
T2	66.61 ± 1.8
T3	72.57 ± 3.3
T4	63.77 ± 2.9
T5	66.89 ± 1.5
T6	76.54 ± 2.2
T7	61.46 ± 3.7
T8	57.21 ± 1.9
T9	65.09 ± 2.5
T10	63.52 ± 1.9
T11	59.88 ± 1.7
T12	64.78 ± 2.8
T13	69.51 ± 3.5
T14	65.37 ± 2.2
T15	58.18 ± 1.8
T16	61.65 ± 3.4
T17	71.37 ± 2.5

(T16) with less D-Limonene, or 0.1 mL, had a relatively lower EE of 58.79%, while formulation (T13) with a higher amount of D-Limonene (2.0 mL) held higher EE (66.56%). This could be due to the lipophilic property of D-limonene which entrapped a higher amount of voriconazole. Ultimately, it can be said that an invasome with acceptable EE can be developed by combining D-limonene and phospholipid in a reasonable amount, preferably in less. Whereas, span 60 also contributed significantly; a higher amount of span 60 produced the invasome of lesser EE and a lesser amount of span 60 developed the invasome of higher EE as seen in T15 with 56.04% of the drug. Whereas, T4 with a lesser span of 60 exhibited a good EE of 60.58%.

Model Validation

ANOVA analysis of the independent variables about Responses and the appropriateness of the model selection must both be validated in this study.[14]

It can be seen in Table 4 that; the model is validated as the p-value is less than 0.05. Similarly, A, B, C, A^2, C^2, AB, and AC are significantly affecting Response 1.

In Response 1: Term A, Term B, Term C, A^2, and C^2 are significant. Along with that, interaction terms such as AB and AC are significant.

Response 1 [Particle size] =144.66+13.48A-3.95B-39.66C+4 AB 5.67AC +0.8BC+ $8.13A^2$+$1.207B^2$-$31.91C^2$ …….. (2)The response was shown in Figure 1.

Term A and quadratic term A2 are important in response 2. Response 2 [Zeta potential] = 13.44+7.57A+1.45B-0.75C+0.972AB-2.17AC +0.125BC +$5.06A^2$+ $0.317B^2$+$0.91C^2$……..(3) which was depicted in Figure 2.

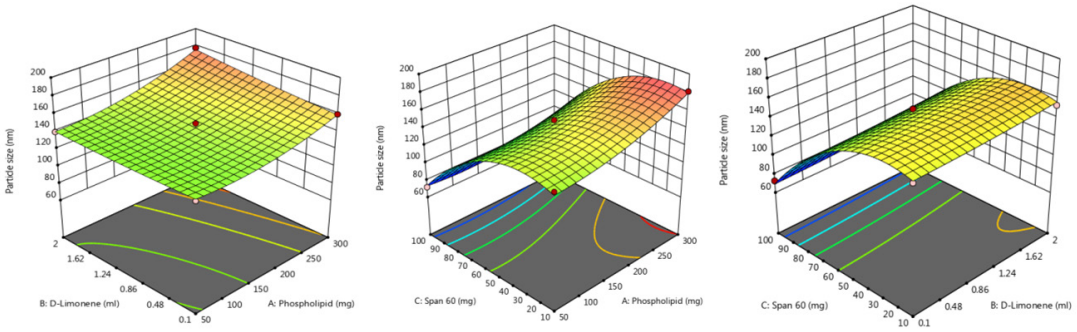

Figure 1: 3D simulation curve of Response 1(particle size)

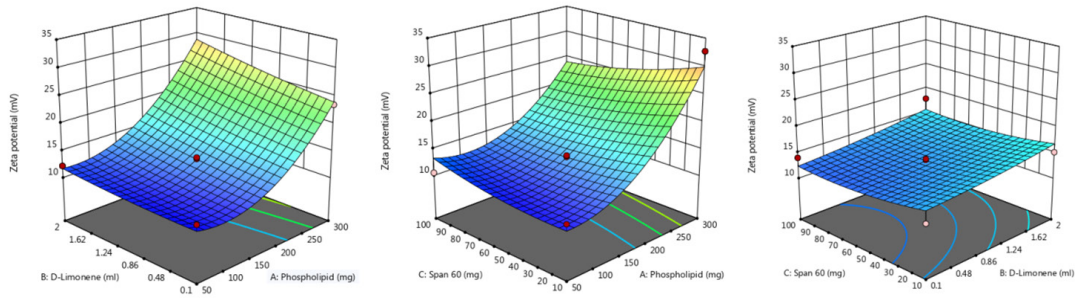

Figure 2: 3D simulation curve of Response 2(Zeta potential)

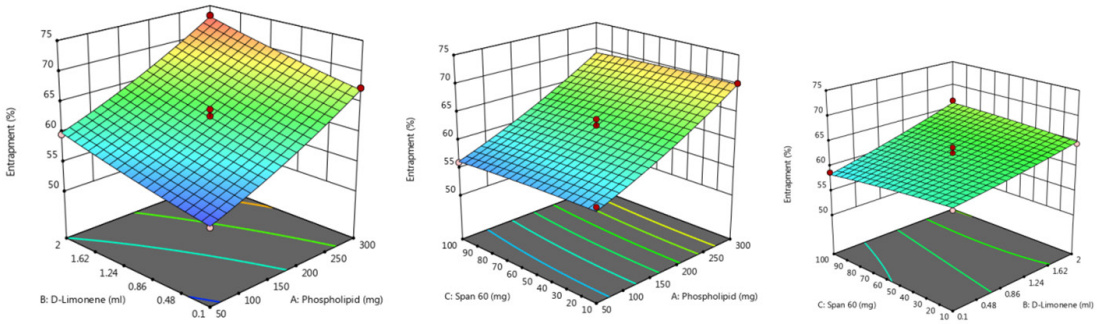

Figure 3: 3D simulation curve of Response 3(Entrapment Efficiency)

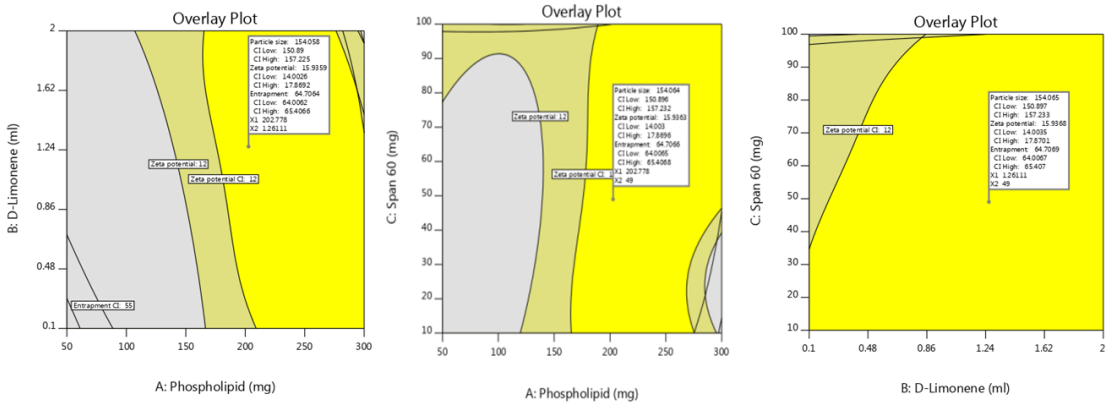

Figure 4: Region overlay graphic showing the idealized space and values

Term A and B are important in Response 3.

Response 3 Entrapment]= 62.55+6.45A+2.89B-0.39C-0.11AB+0.05AC +0.94BC+ $0.78A^2$+$0.28B^2$-$0.21C^2$…….. (4) was shown in Figure 3.

Optimization of Study

Polynomial equation showed that terms such as A, B, and C are significant; similarly, quadratic terms such as A^2 and C^2 are significant. However, it found interaction terms such as AB, and AC are significant. The optimal formulation that was developed from the mathematical and graphical approach of optimization is shown in Figure 4, along with the design space (yellow color region).

The observed value and predicted mean (obtained from response surface simulation) were compared.

ATR Study

The ATR spectrum of voriconazole in Figure 5. shows typical characteristic peaks at 3198.73 cm^{-1} (indicative of O-H stretching), 1498.45 to 1589.77 cm^{-1} (C-C stretching), 1276.13 cm^{-1} (aryl C-N stretching), and at 1128.93 to 1050.43 cm^{-1} (C-F stretching).

The ATR spectrum of optimized formulation Figure 6. shows typical characteristic peaks at 2923.88 cm^{-1} (indicative of O-H stretching), 1497.63 to 1589.25 cm^{-1} (C-C stretching), 1275.52 cm^{-1} (aryl C-N stretching), and at 1128.11 to 1048.60 cm^{-1} (C-F stretching).

Table 4: Optimization formulation table

Factor	*Name*	*Level*	*Low level*	*High level*
A	Phospholipid	202.78	50.00	300.00
B	D-Limonene	1.26	0.1000	2.00
C	Span 60	49.00	10.00	100.00

Table 5: Point of Prediction data of optimized formulation

Response	*Predicted mean*	*Predicted median*	*Observed*	*Std Dev*
Particle size	154.059	154.059	159.9	3.80178
Zeta potential	15.9367	15.9367	23.5	2.32016
Entrapment	64.707	64.707	68.58	0.840264

It was observed that no significant alterations occurred and that the peaks were situated in typical locations.

SEM Study

During the SEM study, it observed scattered dispersion of Invasomes. The study also showed that optimized Invasome surfaces and appearances are symmetrical. Figure 7 illustrates how evenly distributed the invasomes are throughout the sample and Figure 8 shows that the optimized formulation's particle size is uniform.

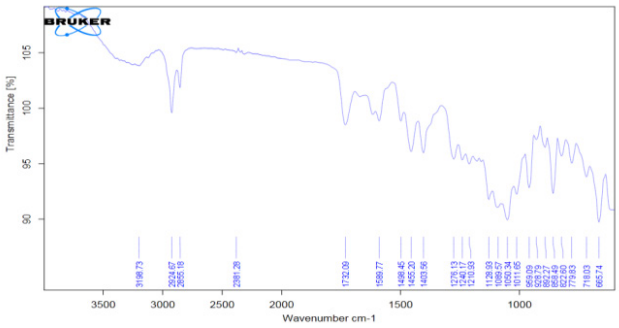

Figure 5: ATR spectra of voriconazole

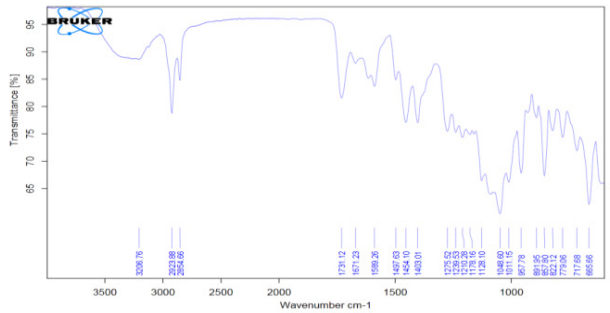

Figure 6: ATR spectra of optimized formulation

According to the particle size analysis, the optimized formulation's mean size was 159.9 nm. According to this, a value of 0.397 was found for the polydispersity index (PI). This indicates homogenous size dispersion of invasome in the formulation.

In our study, it showed a zeta value of -23.5 mV; indicating stability, was shown in Figure 9. The literature review already stated that the value above ± 15mV is stable and can be dispersed without raising the stability issue.

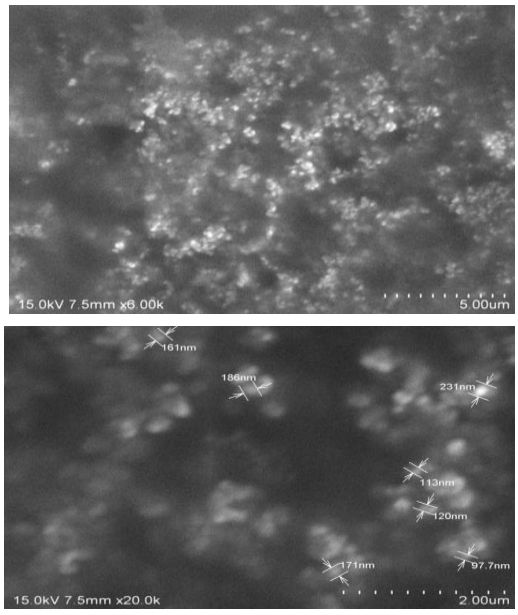

Figure 7: SEM study of optimized formulation at (5.0 & 2.0 µm)

DSC Study

The literature analysis indicates that a sharp endothermal peak was observed at 132.33°C with a heat of energy of -361.96 mJ. as shown in Figure 10.

A significant endothermal peak was seen in the DSC investigation (Figure 10) at 103.61°C, which is extremely close to the previously reported value of 132.33°C for pure voriconazole. The endothermal peak was found to have changed non-significantly. It also meant that throughout time, the pure drug in the formulation would not change. It was mentioned that phospholipid does not appear to have a discernible peak. This may be because, as Figure 14 illustrates,

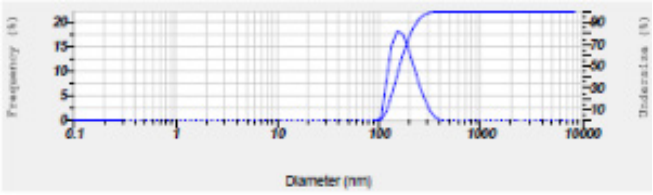

Figure 8: Particle size and distribution study of optimized formulation

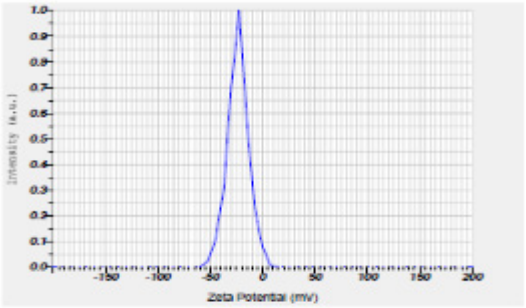

Figure 9: Zeta potential estimation of optimized formulation

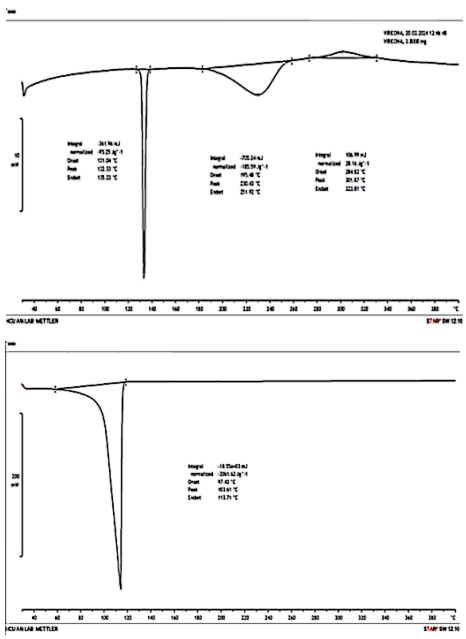

Figure 10: DSC of voriconazole and optimized formulation invasome

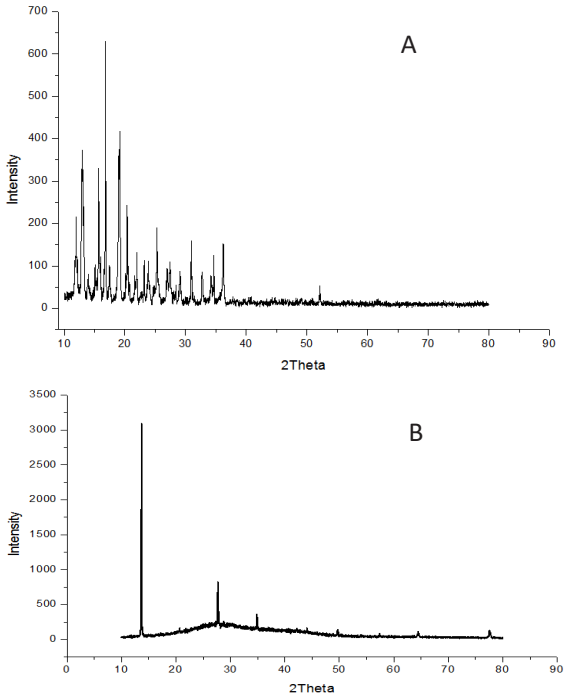

Figure 11: XRD of A) Voriconazole B) Optimized formulation invasome

Table 5: Formulation composition of gel loaded with optimized Invasome

Ingredients	*IG1*	*IG2*	*IG3*	*IG4*	*IG5*	*IG6*	*IG7*
Invasome (Equivalent quantity of 100 mg Voriconazole)	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
Carbopol 934P (g)	0.2	0.4	0.6	1.0	0.2	0.4	0.6
PEG 600 (mL)	30	20	15	5	-	-	-
Glycerol (mL)	-	-	-	-	30	25	15
Benzalkonium chloride (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine (mL)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

quaternary ammonium ions in phospholipid and D-limonene were the sources of an intermediate complex that formed throughout our investigation.

XRD Study

It was ascertained in the XRD study; that highlighted significant characteristic peaks at position 13.28, 27.11, and 34.75 (2Theta); those highlighted peaks were identical with pure voriconazole. This indicated the presence of voriconazole in the crystalline arrangement. Nevertheless, a few more distinctive peaks vanished; this might be because, as was indicated in the formulation development process, there was a solvent present and the crystallinity was decreased as shown in Figure 11 (B).

Invasomes Formulation

Drug content in all formulations as seen in Table 6; ranged from 83.1 to 92.4%. This shows satisfactory results. There were no such differences in pH values observed; which drop in the normal pH range of the skin. It ascertained viscosity ranged from 345 to 589cp. Formulations loaded with the highest amount of carbopol 934P (1.0 g) showed a maximum viscosity value of 589 cp; however, with the least amount of carbopol 934P (0.2 g) the viscosity was least recorded (345 cp). A similar pattern can be observed in the spreadability study; a high amount of carbopol 934P exhibited the least value and less amount of gelling agent exhibited higher spreadability. In the "Homogeneity" study all formulations showed no aggregated mass formed and clog mass appeared.

In-vitro Dissolution Study

It observed a significant contribution of Carbopol 934P as a gelling agent in drug release. It ascertained an increase in Carbopol 934P the drug release retarded drastically. The formulation "IG1" contributed to faster drug release of 99.8% at 10 hours. The formulation "IG4" exhibited a longer drug release of 76.9% at 14 hours. However, it can be seen that "IG7" with 0.6g of Carbopol 934P released 80.6% at 14 hours. Similarly, a comparison was also made between PEG 600 and glycerol-based formulation. The gels loaded with glycerol showed delayed drug release as compared to gels consisting of an equal amount of PEG 600. As it can be seen in IG7 and IG3. IG3 released 98.3% of drugs, whereas IG7 released 80.6% of drugs.

Table 6: Characterization of gels

Formulation	*Drug content (%)*	*pH*	*Viscosity (cp)*	*Spreadability (mm)*	*Homogeneity*
IG1	88.2 ± 6.1	6.5 ± 0.2	345 ± 6	85 ± 3	+++
IG2	83.1 ± 4.8	6.5 ± 0.4	413 ± 8	59 ± 5	+++
IG3	87.6 ± 3.9	6.7 ± 0.5	434 ± 7	67 ± 4	++
IG4	90.2 ± 5.8	6.4 ± 0.3	589 ± 4	48 ± 8	++
IG5	84.4 ± 3.7	6.7 ± 0.2	423 ± 8	78 ± 7	+++
IG6	87.1 ± 2.8	6.8 ± 0.1	466 ± 2	71 ± 1	+++
IG7	92.4 ± 4.7	7.1 ± 0.6	535 ± 4	62 ± 3	++

Every single set of data is shown as Mean ± Standard error of the mean (n = 3).

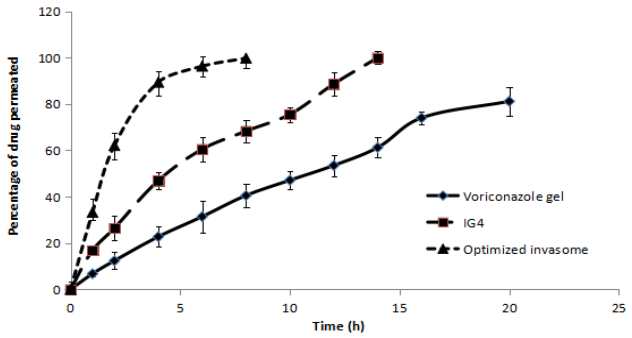

Figure 12: Voriconazole permeation study

Ex-vivo Permeation Study

Voriconazole released from the optimized invasome was considerably larger than that of pure voriconazole depicted in Figure 12. The IG4 exhibited a higher flux of 0.168 as compared to pure voriconazole. The Optimized invasome formulation flux (J) was determined to be 0.299 µg/cm^2/h, which is larger than the drug flux (0.104 µg/cm^2/h). Permeability coefficients (PC) were determined to be 0.00299 cm/s for the Optimised invasome formulation and 0.00168 cm/s for IG4. In contrast, voriconazole permeability flux was found to be comparatively less (0.00104 µg/cm^2/h) than developed IG4. These results were made possible by the presence of ethanol and D Limonene in the formulation system, which is responsible for enhancing Voriconazole permeability and facilitating the tight connection between the dermal membrane to open.

Antifungal Study

An antimicrobial study was carried out to check the antimicrobial efficiency of voriconazole gel (IG4). The test organisms used were (*Candida albicans, C. glabrata, Aspergillus niger,* and *A. flavus*; the growth medium used was nutrient agar. The disk diffusion method was used to carry out the antimicrobial study. The zone of inhibition (ZOI) was compared with that of the standard. The study confirmed a good zone of inhibition from Invasome loaded with the drug in the gel as compared to the drug in the gel. This shows the potential diffusion and antimicrobial efficiency of voriconazole invasome.

CONCLUSION

The BCS class II antifungal drug voriconazole has a broad spectrum of action. Its solubility is still a major problem for formulation scientists despite numerous attempts to improve it. One potentially novel nanocarrier that could be used to increase the solubility and permeation of poorly soluble and permeable drugs is an invasome. Invasome loaded with voriconazole prepared by film hydration technique. The impact of particular independent factors on the responses was assessed in the current study using BBD. The responses recorded in the experiment are particle size, zeta potential, and entrapment efficiency. A good zone of inhibition from Invasome loaded with the drug in the gel was validated by the antifungal investigation when compared to the drug in the gel. This demonstrates how well the voriconazole invasome diffuses and works as an antifungal medication.

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