RESEARCH ARTICLE

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

Amareshwar S[1*], Abbaraju Krishna Sailaja[2]

[1]Department of Pharmaceutics, University College of Technology, Osmania University, Hyderabad, Telangana, India.
[2]Department of Pharmaceutics, RBVRR Women's College of Pharmacy, Osmania University, Hyderabad, Telangana, India.

Received: 20th January, 2024; Revised: 27th February, 2024; Accepted: 11th July, 2024; Available Online: 25th September, 2024

ABSTRACT

The BCS class II antifungal drug voriconazole (VRC) has a broad spectrum of action. Its solubility is still a major problem for formulation scientists despite numerous attempts to improve it. The thin-film hydration technique produced the voriconazole-loaded Transfersomes, optimized using the Central Composite Design (CCD) and then included as a gel into Poloxamer 407P. Several parameters were assessed for the prepared VRCT, including particle size, zeta potential, surface shape, potential chemical interaction, and polydispersity index (PI). The SEM study also revealed the appearance and surface of optimized transferosomes are symmetrical. The optimized formulation's average size was found to be 238.8 nm by the particle size analysis. Similarly, a value of 0.449 was found by the polydispersity index (PI). To evaluate voriconazole Transfersomal gel's (TG4) antibacterial efficacy, an antimicrobial investigation was conducted. The creation of Transfersomal gel formulation offers a topical drug administration method that is more effective and improves patient compliance.

Keywords: Fungal infection, Voriconazole, Transfersomes, gel, Central composite design.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.32

How to cite this article: Amareshwar S, Sailaja AK. Development and Evaluation of Voriconazole Loaded Transfersomal Gel for Enhanced Antifungal Activity. International Journal of Drug Delivery Technology. 2024;14(3):1487-1494.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Among all the several drug delivery channels, the conventional drug administration system is the most common. When taken orally, certain drugs may have limited bioavailability because of partial absorption or first-pass metabolism.[1,2] The instantaneous drug release of traditional dose forms results in varying blood drug levels based on the dosage type. Consequently, vesicular drug delivery systems are required to keep the drug concentration within a therapeutically appropriate range. Colloidal particles are concentric bilayer molecules that are amphiphilic.[3] Vesicular delivery systems serve as prolonged-release methods and postpone the removal of drugs that are quickly metabolized.[4] It offers a competent method of delivery to the infection site, resulting in a reduction in drug toxicity without side effects. By increasing the bioavailability of drugs, vesicular drug delivery lowers therapy costs, particularly for poorly soluble drugs.[5]

Drugs can be delivered to the systemic circulation through the skin by transdermal drug delivery systems at a regulated and predefined rate. Compared to traditional dose forms, transdermal drug delivery systems have numerous advantages.[6] It reduces adverse pharmacological side effects brought on by transient overdose and improves patient compliance. The majority of chemicals have low skin penetration, which is the primary drawback of transdermal drug delivery. To compromise this skin barrier, several strategies have been devised. Utilizing vesicular systems is one method for improving drug penetration of the skin.[7]

Transferosomes are highly engineered, ultra-deformable lipid supramolecular aggregates that improve intact vesicle penetration. Because of their adaptability, transferosomes make excellent choices for the non-invasive administration of small, medium, and large-sized drugs.[8] The primary component of transferosomes is phospholipid, such as phosphatidylcholine, dipalmitoyl phosphatidylcholine, and soy lecithin. Phospholipids and edge activators make up the majority of the components of transferosomes. The single-chain surfactant that makes up the edge activator destabilizes the lipid bilayer while also making the bilayers more elastic. Transferosomes have a higher degree of flexibility than conventional liposomes, which makes them ideal for skin penetration.[9] To make use of phospholipid vesicles as transdermal drug transporters, transferosomes were created. Transferosomes squeeze themselves along the

**Author for Correspondence: amar.pharma99@gmail.com*

stratum corneum's intracellular sealing lipid to get around the barrier of skin penetration.[10] The drug voriconazole works as an antifungal. Both local and systemic fungal infections are treated with it. The commercially available forms of voriconazole include intravenous injections, oral pills, and oral solutions.

MATERIALS AND METHODS

Materials

Voriconazole was purchased from Chemo Pharma Laboratories Pvt. Ltd Hyderabad Telangana. Phosphatidylcholine, Span-60, and Poloxamer 407P were acquired from SD Fine Chem (Mumbai, India). Analytical-grade compounds were utilized for the other substances in the study.

Method of Preparation of Transferosome by thin-film hydration technique

In 10 mL of ethanol, span-60 and phosphatidylcholine were dissolved. After being moved to a round-bottom flask (RBF), the mixture was run through a rotary flask evaporator (Rotary evaporator, RE-2010, Biobase, Mumbai). To create a dried RBF film, the blend was then thoroughly dried at 40°C and 50 rpm. After that, the deposited film is hydrated for 30 minutes using an phosphate buffer pH 7.4 and voriconazole mixture. The transferosomal dispersions underwent a 10-minute sonication (UAI-PS20khz-900W, Ultra Autosonic, India) to generate smaller vesicles.[11] Using a cooling ultracentrifuge, the transfersomes were separated from the entrapped medication by high-speed centrifugation at 20,000 rpm for 30 minutes at −5°C. The voriconazole that was entrapped in the transfersomes continued as a precipitate. The precipitate was reconstituted in 10 mL of phosphate buffer pH 7.4 to assess it.

Formulations recommended by Central composite design (CCD)

The current study employed a two-level, two-factor CCD (Table 1) to assess the impact of particular independent factors on replies.[12,13] Two separate parameters were taken into consideration: Span-60 (B) and phospholipid (A). Vesicle size, entrapment, and permeability coefficient are the reactions that were noted during the experiment. The polynomial equation was used to perform mathematical analysis and fitting. Combining a numerical approach with a graphical optimization technique, the improved formula was solved with an alpha<0.05 confidence interval value.

Evaluation of Transferosome

In a 100-milliliter volumetric flask, a precisely weighed amount of Transferosome (equal to 100 mg of drug) was combined with the slightest amount of ethanol, and carefully mixed.[14] We sonicated the dispersion for about ten minutes (Ultrasonicator, CPX3800-E, Branson). After adding phosphate buffer with a pH of 7.4, the mixture's volume was adjusted to the required level. For five more minutes, the dispersion was bath-sonicated till it turned translucent. A UV-visible spectrophotometer (Shimadzu UV-1800, Japan) was used to measure the amount of medication present in the filtrate at 256 nm.

$$\text{Drug Loading (\% DL)} = \frac{\text{Drug content in Invasomes}}{\text{Total weight of Invasomes}} \times 100 \quad \text{.......(Equation 1)}$$

Entrapment Efficiency

$$EE = (\text{Total drug} - \text{Drug in supernatant})/(\text{Total drug}) \times 100 \quad \text{...(Equation 2)}$$

Attenuated Total Reflection (ATR) Study

Online polymer composition monitoring is a particularly valuable application of ATR (Bruker Opus 7.0) spectroscopy.[15]

Table 1: Formulation Table

Run	*Factor-I*	*Factor-II*	*Response-I*	*Response-II*	*Response -III*
	A: Phospholipid	*B: Span 60*	*Vesicle size*	*Entrapment efficiency*	*Permeability coefficient*
	mg	mg	nm	%	
1	175	55	215.4	65.41	0.00169
2	175	55	217.1	65.29	0.00167
3	175	55	216.7	66.04	0.0017
4	-1.7767	55	208.3	64.13	0.00196
5	175	118.64	239.1	64.09	0.0022
6	175	-8.63961	238.6	68.53	0.00151
7	300	100	261.5	70.83	0.00227
8	351.777	55	265.7	74.16	0.00151
9	50	10	217.4	62.34	0.00172
10	300	10	259.5	72.65	0.00151
11	50	100	211.8	60.71	0.0023
12	175	55	218.5	66.72	0.00172
13	175	55	218.3	66.38	0.0017

Surface Morphology, Particle Size, and Zeta Potential

The optimized Voriconazole-loaded transfersome morphology was examined by a scanning electron microscope (SEM) (S3700N-Hitachi, Japan). A glass slide was placed over the sample once it had been spread out over the slab surface, and photomicrographs were taken at various magnifications.[16] Similar to this, the scattering light intensity technique was used to measure the particle size and polydispersity index value of transfersomes (Malvern zeta sizer, ATA scientific, USA).

Differential Scanning Calorimetry (DSC) Study

The DSC (DSC-60, Shimadzu) is a thermal investigation that tracks the temperature and heat flow related to material changes computed as a function of temperature and time.[16]

X-ray Diffraction (XRD) Study

XRD analysis uses the study of crystal structure to determine which crystalline phases are present in a material and therefore, provides information about its chemical composition. ARL EQUINOX 100, Thermo Scientific, India, was used to conduct the XRD research.

Procedure for Preparation of Gel loaded with Transferosome

In a 100 mL beaker, Poloxamer 407P 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. In another beaker, PEG 400, glycerol, and Benzalkonium chloride were taken and mixed properly. To that mixture, the transferosome is poured and mixed properly. Finally, the drug dispersion was poured into Poloxamer 407P gel and stirred at 50 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

A precise quantity of the prepared gel was taken and added to 100 mL of pH 7.4 ethanolic phosphate buffer. For one hour, a mechanical shaker was utilized to continually agitate the gel solution. The resulting solution was subjected to spectrophotometric analysis at 256 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan).

pH determination

A digital pH meter (Beckman, Germany) was used to record the gel's pH. Every experiment was run in triplicate.

Viscosity

It was measured with a Brookfield viscometer (model DV-II+Pro, USA). Spindle number 64 was utilized for this purpose. The spindle was rotated at 50 rpm to record the viscosity.

Spreadability (S)

A wooden block and a glass slide device were used to assess the gels' spreadability. In the pan, there was around 500 mg of formed gel. The higher slide's separation time from the fixed slides was noted.

$$S = ML/T \quad \text{......(Equation 3)}$$

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 evaluate homogeneity, each generated gel composition was characterized. After the gel had settled in appropriate containers, this was accomplished by visual inspection of the gel. The look and presence of any clogs in the gels were examined.

In-vitro diffusion study

Voriconazole was released from the gel using a Franz-diffusion cell. The cellophane membrane was cut into equal pieces, each measuring 2.5 by 6 cm, and let to soak in distilled water for a full day before use. A magnetic stirrer equipped with a continuous heating mechanism was used to maintain the temperature of 10 milliliters of pH 7.4 saline phosphate buffer at 37 ± 0.5°C during the drug release investigations (IKA Auto Temp Regulator, Germany). Within the donor compartment, a single gram of gel was sampled. Every time a sample of one milliliter was taken out, it was replaced with an identical amount of brand-new buffer. In case fresh medium was required, the aliquots were diluted.[17]

Ex-vivo permeation study

Fresh abdominal goat skin was collected from the slaughterhouse and was used for the study. To make a pocket that is dipped in phosphate buffer 7.4 (100 mL), the skin was knotted at both ends. The temperature was kept at 37 ± 0.5°C using a magnetic stirrer running at 50 rpm. Drug penetration was assessed using UV-visible spectrophotometer at 256 nm on a 2 mL sample that was taken up to 6 hours before the scheduled time. As recommended by CCD, an identical process was carried out with every formulation. The amount of medication that penetrates vs time was shown on a graph. The coefficients of flux and permeability were computed.

RESULTS AND DISCUSSION

Entrapment Efficiency (Table 2)

It was noted that formulations containing large concentrations of phospholipid had significant EE. The formulation "T8" has a maximum of 74.16%, T10 and T7 entrapped 72.65% and 70.83% respectively. It also observed that a high amount of span 60 made lesser EE and a lesser amount of span 60 contributed higher EE. The %amount of span 60 indicated the EE as it can be seen that a lower amount of span 60 (10.0 mg) in T10 possessed higher EE (72.65%) whereas formulation (T7) with a high amount of phospholipid, i.e., 100.0 mg displayed relatively lesser EE of 70.83%. This may be because span 60 reduced the EE through emulsification. Ultimately, a transferosome with good EE can be created by combining the right amount of span-60 and phospholipid.

Table 2: Drug loading of formulations T1-T13

Run	*Drug loading*
T1	68.37 ± 2.7
T2	68.86 ± 5.3
T3	69.11 ± 1.9
T4	68.25 ± 6.4
T5	69.74 ± 5.5
T6	71.24 ± 4.6
T7	74.97 ± 5.2
T8	76.13 ± 3.8
T9	68.54 ± 4.4
T10	78.06 ± 5.8
T11	64.43 ± 3.6
T12	61.52 ± 1.8
T13	70.85 ± 2.9

Table 3: Optimization data

Factor	*Name*	*Level*		
		Level	*Low*	*High*
A	Phospholipid	243.81	50.00	300.00
B	Span 60	79.42	10.00	100.00

Model Validation

Both the model selection and the ANOVA analysis of the independent variables of Responses need to be validated in this study.[18] The model is validated as the *p-value* <0.05.

3D simulation curve & 2D contour plot of response 1(vesicle size) was shown in Figure 1.

3D simulation curve & 2D Contour plot of response 2 (Entrapment efficiency) was depicted in Figure 2.

3D simulation curve & 2D Contour plot of Response 2(permeability coefficient).was depicted in Figure 3.

The optimal formulation that was developed from the optimization is shown in Figure 4, along with the design space (yellow color region).[19] Before the optimal formulation was discovered, the target response ranges were established, as displayed in Figure 4.

As per the information provided in Table 3, the optimized transferosome was prepared and responses were recorded in Table 4 as observed values. The observed value and predicted mean (obtained from response surface simulation) were compared.

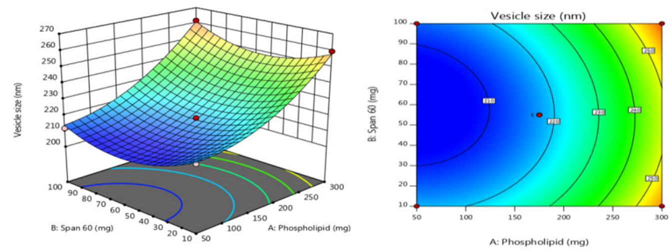

Figure 1: 3D simulation curve & 2D Contour plot of Response 1 (vesicle size)

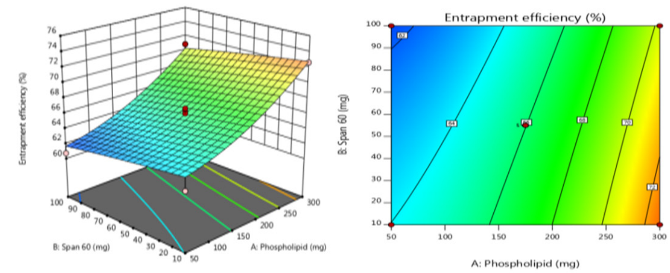

Figure 2: 3D simulation curve & 2D contour plot of response 2 (Entrapment efficiency)

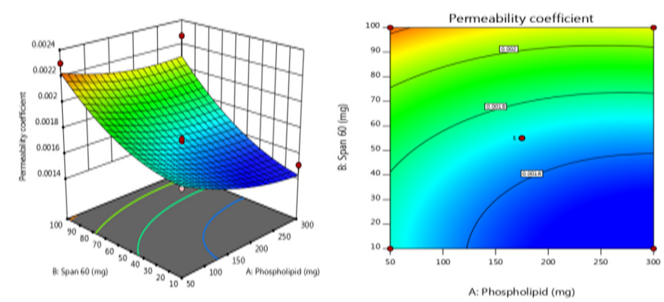

Figure 3: 3D simulation curve & 2D contour plot of response 3 (permeability coefficient)

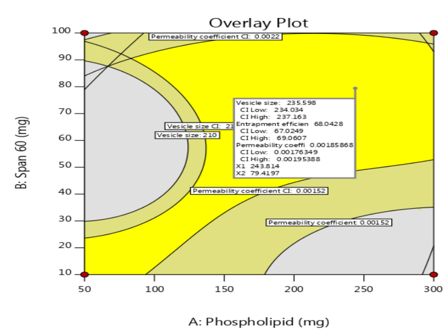

Figure 4: Overlay plot

ATR study

The ATR spectrum of voriconazole (Figure 5) shows typical distinguishing 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 optimized formulation underwent ATR analysis for group position and identifications. It was mentioned that peaks were at typical locations and that no such significant alterations had occurred. The ATR spectrum of optimized formulation (Figure 5) shows typical characteristic peaks at 2930.70 cm^{-1}

Table 4: Point of prediction data

Response	*Projected mean*	*Observed*	*Std dev*
Vesicle size	235.607	238.8	1.81695
Entrapment efficiency	68.044	73.16	1.18235
Permeability coefficient	0.0018587	0.00191	0.000110577

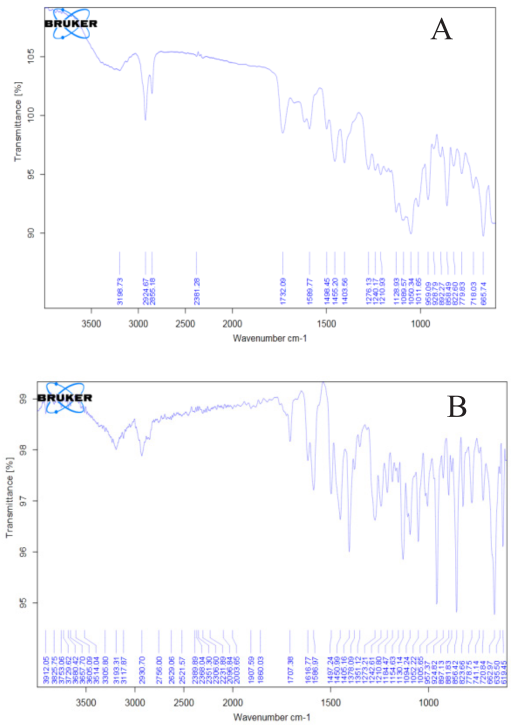

Figure 5: ATR spectra of A) Voriconazole and B) optimized formulation

(indicative of O-H stretching), 1497.24 to 1586.97 cm^{-1} (C-C stretching), 1273.21 cm^{-1} (aryl C-N stretching), and at 1130.14 to 1052.22 cm^{-1} (C-F stretching) which was shown in Figure 5.

Surface Morphology, Particle Size, and Zeta Potential

During SEM study it observed scattered dispersion of transferosomes. The study also revealed the appearances and surface of optimized transferosomes are symmetrical. It observed in Figure 6, transferosome are dispersed uniformly throughout the sample. Figure 6. Depicts the findings of (2.0 and 5 µm resolution) the uniform particle size of the optimized formulation.

The improved formulation's average size, according to the particle size analysis, was 238.8 nm. A score of 0.449 was also disclosed by the polydispersity index (PI). A PI value of less than 0.5, according to the literature, denotes homogeneous dispersion. This suggests that the Transferosome has a uniform size dispersion inside the formulation, as illustrated in Figure 7.

The literature review already stated that the value above ± 15 mV is stable and can be dispersed without raising the stability issue. In our study, it showed a zeta value of -20.5 mV as shown in Figure 8.

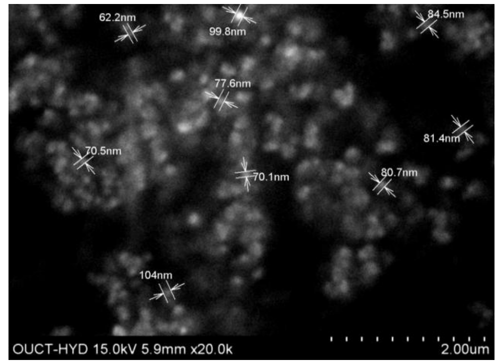

Figure 6: SEM study of optimized formulation

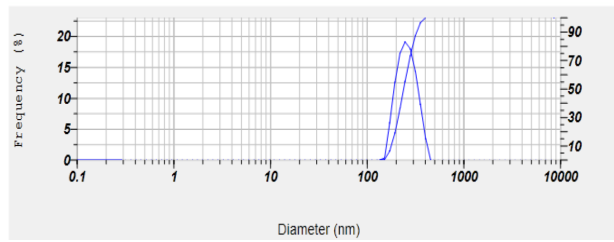

Figure 7: Particle size and distribution of improved formulation

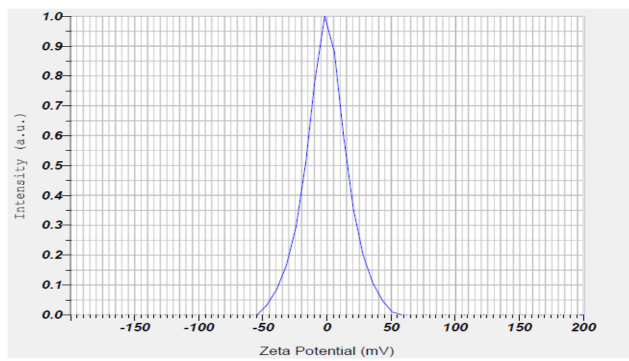

Figure 8: Zeta potential estimation of optimized formulation

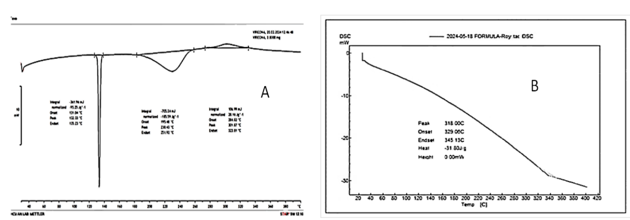

Figure 9: DSC of A) Voriconazole and B) Optimized formulation transfersome

DSC study

It observed a sharp endothermal peak with heat of energy of -361.96 mJ at 132.33°C which is depicted in Figure 9.

The DSC investigation (Figure 9) revealed a strong endothermal peak at 318.0°C, which is much higher than the previously reported value of 132.33°C for pure voriconazole. This determined that the endothermal peak had changed

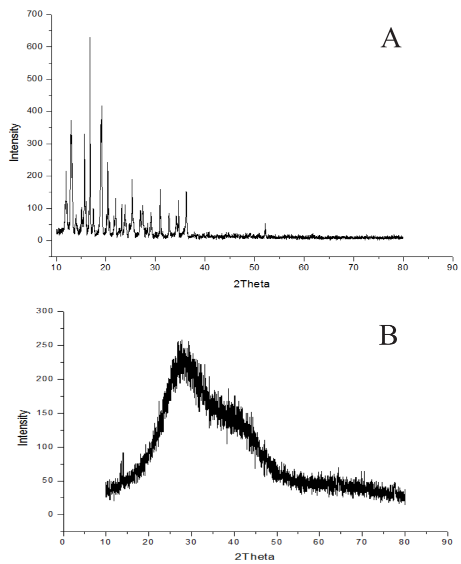

Figure 10: XRD spectra of A) Voriconazole and B) Optimized formulation transfersome

significantly. The formulation's pure drug's thermal stability was also deduced. It noted there is no such prominent peak appearing for phospholipid. This could be due to the formation of an intermediate complex developed by quaternary ammonium ion in phospholipid and cholesterol considered in our study.

XRD Study

Figure 10 displays the XRD study that highlighted the broadening of peaks as well a lesser number of significant characteristic peaks at positions 12.29 and 30.18 (2Theta); those highlighted peaks were identical to pure voriconazole. This indicated the presence of voriconazole in the crystalline arrangement. Nevertheless, a few more distinctive peaks vanished; this might be because of the solvent present and the formulation's decreased crystallinity.

Preparation of Gel loaded with Transferosome (Table 5)

Drug content in all formulations, as seen in Table 6, ranged from 80.5 to 90.1%. This shows satisfactory results. There were no such differences in pH values observed. It ascertained viscosity ranged from 229 to 473 cp. Formulations loaded with the highest amount of Poloxamer 407P (3.0 g) showed a maximum viscosity value of 473 cp; however, with the least amount of Poloxamer 407P (1.5 g), the viscosity was the least recorded. A similar pattern can be observed in the spreadability study; a high amount of Poloxamer 407P 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 Poloxamer 407P as a gelling agent in drug release. It ascertained an increase in Poloxamer 407P the drug release retarded drastically. The formulation "TG1" contributed to faster drug release by 99.1% at 10 hours. The formulation "TG4" exhibited longer drug release of 84.8% at 14 h. However, "TG7" with 2.5 g of Poloxamer 407P released 85.9% at 14 hours. Similarly, a comparison was also made between PEG 400 and glycerol-based formulations. The gels loaded with glycerol showed

Table 5: Formulation composition of gel loaded with optimized transferosome

Ingredients	*TG1*	*TG2*	*TG3*	*TG4*	*TG5*	*TG6*	*TG7*
Transferosome (Equivalent quantity of 100 mg Voriconazole) (mL)	5	5	5	5	5	5	5
Poloxamer 407P (g)	1.5	2.0	2.5	3.0	1.5	2.0	2.5
PEG 400 (mL)	40	25	20	10	-	-	-
Glycerol (mL)	-	-	-	-	40	25	20
Benzalkonium chloride (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 6: Characterization of gels

Formulation	*Drug content (%)*	*pH*	*Viscosity(cp)*	*Spreadability (mm)*	*Homogeneity*
TG1	85.8 ± 4.1	6.4 ± 0.4	234 ± 8	90 ± 6	+++
TG2	80.5 ± 2.6	6.2 ± 0.2	307 ± 9	64 ± 3	+++
IG3	84.9 ± 4.4	6.6 ± 0.6	229 ± 10	71 ± 5	+++
TG4	87.6 ± 3.3	6.7 ± 0.1	473 ± 5	53 ± 6	++
TG5	81.7 ± 4.1	6.6 ± 0.3	316 ± 7	84 ± 8	+++
TG6	84.5 ± 2.5	6.7 ± 0.5	351 ± 9	77 ± 9	+++
TG7	90.1 ± 3.8	7.3 ± 0.4	428 ± 5	66 ± 6	++

Data articulated as Mean ± Standard error (n = 3)

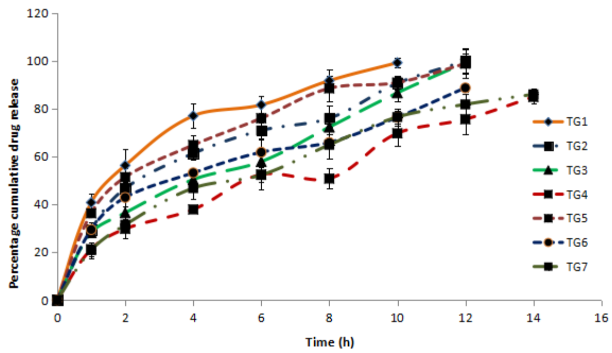

Figure 11: *In-vitro* dissolution study of gels loaded with optimized transferosome *ex-vivo* permeation study

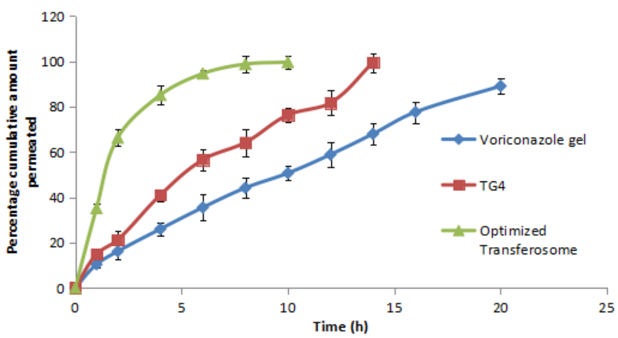

Figure 12: Voriconazole permeation study of TG4, voriconazole gel and optimized transferosome

delayed drug release as compared to gels consisting of an equal amount of PEG 400 as it can be seen in TG7 and TG3. TG3 released 99.1% of drugs, whereas TG7 released 81.7% of drugs. This information indicates a delay in drug release from formulations loaded with glycerol, as shown in Figure 11.

The amount of voriconazole released from the optimized transferosome was considerably larger than that of pure voriconazole. The TG4 exhibited a higher flux of 0.169 as compared to pure voriconazole in the gel. The optimized transferosome formulation flux (J) was determined to be 0.224 μg/cm^2/h, which is larger than the drug flux (0.1108 μg/cm^2/h). For the optimized transferosome formulation and TG4, the permeability coefficients (PC) were found to be 0.00224 and 0.00169 cm/s, respectively. In contrast, voriconazole permeability flux was found to be comparatively less (0.0011 μg/cm^2/h) than developed TG4. These results were made possible by the presence of span 60 in the formulation system, which is responsible for improving the permeability of voriconazole as well as the opening of the dermal membrane's tight junction as shown in Figure 12.

Antifungal Study

An antimicrobial study was done to check the antimicrobial efficiency of voriconazole gel (TG4). The test organisms used were (*Candida albicans, C. glabrata, Aspergillus niger, and A. flavus*; the growth medium used was nutrient agar. The disk diffusion technique was used to carry out the antimicrobial study. The study confirmed a good zone of inhibition from the Transferosome loaded with the drug in the gel as associated to the drug in the gel. This shows the potential diffusion and antimicrobial efficiency of voriconazole transferosome.

CONCLUSION

In this work, the process of creating VRC transfersomes and incorporating them into a gel to produce antifungal activity is explicated. The properties of transfersomes were assessed using drug release tests, polydispersity index (PI), zeta potential, entrapment efficiency, and particle size. Moreover, the antifungal efficacy and several physicochemical properties of the produced transfersomal VRC gel were analyzed. Transferosome gel dramatically increased the antifungal activity against susceptible organisms, as the results showed. It is ultimately possible to conclude that the produced transfersomal VRC gel is likely to be helpful in the treatment of a variety of fungal infections, including topical infections.

REFERENCES

1. Padhiar J, SV S DS. Formulation and Evaluation of Microemulsion Based Topical Gel of Carbamazepine. *Int. J. Drug Deliv. Technol*. 2018;8(2):44-52.
2. Aher VD, Rajesh V, Chitti R, Reddy BBK. Lornoxicam Solid Lipid Nanoparticle-Enriched Transdermal Gel: Development and Characterization. *Int. J. Drug Deliv. Technol*. 2024;14(2):792-796.
3. Bandara H.M.H.N., Samaranayake L.P. Viral, bacterial, and fungal infections of the oral mucosa: Types, incidence, predisposing factors, diagnostic algorithms, and management. *Periodontol. 2000.* 2019;80:148–176.
4. Pappas P.G., Lionakis M.S., Arendrup M.C., Ostrosky-Zeichner L., Kullberg B.J. Invasive candidiasis. *Nat. Rev. Dis. Primers.* 2018;4:18026.
5. Ostrosky-Zeichner L., Andes D. The role of in vitro susceptibility testing in the management of Candida and Aspergillus. *J. Infect. Dis.* 2017;216((Suppl. 3)):S452–S457.
6. Torres-Guerrero E., Quintanilla-Cedillo M.R., Ruiz-Esmenjaud J., Arenas R. Leishmaniasis: A review. *F1000Research.* 2017;6:750.
7. Oryan A., Bahrami S., Bemani E. Efficacy of voriconazole on leishmaniasis by *Leishmania major*: An in vitro and in vivo study. *Asian Pac. J. Trop. Med.* 2018;11:562–569.
8. Faisal W., Soliman G.M., Hamdan A.M. Enhanced skin deposition and delivery of voriconazole using ethosomal preparations. *J. Liposome Res.* 2018;28:14–21.
9. Garg V., Harmanpreet S. Systematic development of transethosomal gel system of piroxicam: Formulation optimization, in vitro evaluation, and ex vivo assessment. *AAPS Pharm SciTech.* 2017;18:58–71.
10. Mohammed B.S., Al Gawhari F.J. Transethosomes a Novel Transdermal Drug Delivery System for Antifungal Drugs. *Int. J. Drug Deliv. Technol.* 2021;11:238–243.
11. Khan D.H., Bashir S., Figueiredo P., Santos H.A., Khan M.I., Peltonen L. Process optimization of ecological probe sonication technique for production of rifampicin loaded niosomes. *J. Drug Deliv. Sci. Technol.* 2019;50:27–33.
12. Khan M.I., Madni A., Peltonen L. Development and in-vitro characterization of sorbitan monolaurate and poloxamer 184 based niosomes for oral delivery of diacerein. *Eur. J. Pharm. Sci.* 2016;95:88–95.

13. Pereira-Lachataignerais J., Pons R., Panizza P., Courbin L., Rouch J., López O. Study and formation of vesicle systems with low polydispersity index by ultrasound method. *Chem. Phys. Lipids*. 2006;140:88–97.
14. Roy H., Maddela S., Munagala A., Rahaman S.A., Nandi S. A quality by design approach of metronidazole bigel and assessment of antimicrobial study utilizing box-behnken design. *Comb. Chem. High Throughput Screen*. 2021;24(10):1628-43.
15. Teaima M.H., El Mohammady A.M., El-Nabarawi M.A., Mohamed A.I. Formulation and evaluation of niosomal vesicles containing ondansetron HCL for trans-mucosal nasal drug delivery. *Drug Dev. Ind. Pharm.* 2020;46:751–761.
16. Maddiboyina B., Roy H., Nakkala R.K., Gandhi S., Kavisri M., Moovendhan M. Formulation, optimization and characterization of raloxifene hydrochloride loaded PLGA nanoparticles by using Taguchi design for breast cancer application. *Chem. Biol. Drug. Des*. 2023;102(3):457-470.
17. Honary S., Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems—A review (Part 1) *Trop. J. Pharm. Res.* 2013;12:255–264.
18. Roy H. Box-Behnken design for optimization of formulation variables for fast dissolving tablet of Urapidil. *Asian J. Pharm.* 2018;12(03).
19. Singh C, Yashwant, Gupta AK, Garg V. Formulation Development and Evaluation of Divalproex Sodium Extended-release Tablets. *Int. J. Drug Deliv. Technol.* 2022;12(4):1769-1773.