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PREPARATION AND *IN VITRO* EVALUATION OF VORICONAZOLE LOADED UFASOMAL SUSPENSION

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ABSTRACT

Objective: Aim of this study was to develop and evaluate the Ufasomal suspension of Voriconazole. Voriconazole is a broad spectrum, triazole antifungal agent. Mostly Voriconazole is used topically for Cutaneous Fusarium Solani infection. But for topical administration there are no available dosage form. So, for better topical administration, Voriconazole is loaded into Ufasomes in which drug will be in nano size vesicles.

Methods: The present study aims to load Voriconazole into ufasomes by using thin film hydration method. Voriconazole loaded ufasomes were prepared using cholesterol and sodium oleate in different ratios. Prepared vesicles were characterized for size, polydispersity index , entrapment efficiency ,thermal behavior (Differential scanning calorimetry) ,*invitro* release studies and *invitro* skin permeation studies.

Results: Phospholipid and surfactant ratios played a remarkable role to determine the zeta potential and vesicle size of the vesicles. The vesicle size was found to be 201.30nm and the zeta potential was found to be -38.6mV. The entrapment efficiency of vesicles is in the range of 76.48% to 85.38%. The *invitro* drug release studies of all the five formulations shows drug release more than 62% by the end of 24hrs.

Conclusion: On the basis of findings, the study revealed that entrapment of hydrophobic drugs into lipid based vesicles can improve topical bioavailability. However, the results from this study fulfilled that the Voriconazole is loaded into ufasomes for better topical administration.

Keywords: Ufasomes, Voriconazole , fungal infection, sodium oleate , cholesterol, triazole

INTRODUCTION

The recent research works have urged that more than one billion people are affected by the skin infections. The majority of fungal infections are observed in those patients who are immune compromised with sever diseases such as AIDS, cancer, viral hepatitis and tuberculosis **[1]**. Most commonly recorded fungal infections are superficial in nature which effects skin, mucosa, nail, hair etc. **[2, 3]**. Topical fungal infections are mainly found in the subcutaneous tissue which may be invasive in nature and can penetrate deep into the epidermis **[4]**. Superficial fungal infections caused by dermatophytes effect around 40 million people in developed and underdeveloped countries **[5]**. Some of the common fungal pathogens that are responsible for fungal infections include *Candida, Aspergillus, Cryptococcus, Scedosporium* and other species. Candidiasis also called as yeast infection which is the most common fungal superficial infection caused by candida species **[6, 7, 8]**. Topical formulations are used to treat local infections on the superficial layer of the skin by effectively penetrating the drug into stratum corneum and results in destroying the fungi. Some of the advantages associated with topical formulations are limited systemic bioavailability of drug, reduced systemic adverse effects, patient compliance etc. However, there are some disadvantages including poor penetration into stratum corneum, poor dermal bioavailability, skin irritation, allergic reactions etc. **[9, 10]**. To overcome these above mentioned drawbacks there is a need of novel topical formulation. The novel topical formulations are obtained by using the Vesicular drug delivery systems (VDDS).

In recent years Vesicular delivery has become an important approach to drug delivery. Vesicles have been used in biological layer modeling and targeted transport of API **[11, 12]**. Vesicular drug delivery system can improve the bioavailability of drug and reduces the toxicity by drug targeting to the specific site **[13]**. Advantages associated with vesicular systems includes capable of entrapping both hydrophilic and hydrophobic drugs, reduce the expense of treatment, overcomes complications regarding stability and solubility, reduce the drug toxicity **[14]**. Lipid vesicles have gained a number of applications in various fields like immunology, membrane biology , theragnostic and genetic engineering research **[15]**.

Voriconazole, a broad spectrum , triazole antifungal agent. Its primary mode of action of action is inhibition of fungal cytochrome P450 mediated 14α-sterol

demethylation, which is an essential step in ergosterol biosysthesis. Voriconazole has high antifungal potency against *Candida* species and fungicidal activity against all *Aspergillus* species. It shows *invitro* activity against existing fungal pathogens such as *Scedosporium* or *Fusarium*. Voriconazole is more selective than other azole drugs for fungal infections. Voriconazole is designated chemically as (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol. Voriconazole is available in tablets, IV powder for injection and powder for oral suspension **[16]**. It is reported in the past literature that Voriconazole has been loaded into various novel drug delivery systems such as solid lipid nanoparticles, microemulgel, micro-emulsions, niosomes and liposomes. Ufasomes are unsaturated fatty acid vesicles that are closed lipid bilayered suspension. They are colloidal suspensions of fatty acids and ionized soaps which form lipid bilayers and have capability of entrapping the active lipophilic drugs. Most important feature of ufasomes is the fatty acids are readily available and they are composed of single chain amphipiles. Advantages associated with ufasomes are they are stable than liposomes, have therapeutic viability, have better drug retention, potential carrier for anti inflammatory drug delivery **[17, 18]**. Ufasomes being fatty acid vesicles, could interact with the stratum corneum and improve the topical bioavailability of Voriconazole. The present study aims to prepare and *invitro* evaluation of Voriconazole loaded ufasomes using cholesterol and sodium oleate by thin film hydration technique.

MATERIALS AND METHODS

Materials

Voriconazole was purchased from Yarrow Chem. Products (Mumbai). Sodium oleate was purchased from LOBA CHEMIE Pvt Ltd. Cholesterol was procured from Qualigens. Chloroform, Methanol and HPMC was obtained from SD Fine Chem Pvt (Mumbai, India). Dialysis membrane-50(LA387-5MT) was purchased from Hi Media. Ltd. Mumbai, India.

Methods

Thin film hydration method

Voriconazole loaded ufasomes were prepared by using Thin film Hydration method. Briefly all components of vesicles (Voriconazole, sodium oleate and cholesterol) at different proprotions were dissolved in 10 ml of chloroform-methanol solution(1:1 ratio) (Table1). Clear solution obtained was transferred to a beaker and kept on a Rotary Vacuum Evaporator for complete evaporation of solvent until a thin film was formed. The thin film was then hydrated with 5ml of PBS (pH 7.4). The formed vesicular dispersion was sonicated

for 15min to obtain ufasomes with uniform sizes. For permeation studies ufasomal gel was prepared by using 1% w/v(50mg) of HPMC to the optimized vesicular dispersion [19,20].

Vesicular size, Zeta potential and polydispersity index

The vesicle size and polydispersity index was determined by photon correlation spectroscopy with a zeta sizer Nano ZS-90. Zeta potential was determined by measuring the electrophoretic mobility. Ufasomal dispersion samples were diluted in double distilled de-ionozed water and the measurements were obtained by using Malvern Zetasizer.

Determination of percentage drug content and pH

1ml of vesicular suspension was pipette out and was lysed with 9ml of methanol. It was further diluted with 10ml of pH 7.4 phosphate buffer and samples were analysed spectrophotometrically at 256nm. pH of the ufasomal suspension was checked using pH meter. The electrode was submerged into the formulation at room temperature and the readings were noted.

Entrapment efficiency

Entrapment efficiency of formulations was determined using ultra-centrifugation method **[21]**. In brief, the vesicular dispersions were transferred to tubes and centrifuged at 15,000 rpm for 4 hrs. The supernatant was discarded to remove the unentrapped drug in the formulation. The lipid precipitate obtained was then mixed with methanol, bath sonicated for 30 min and kept overnight in shaking water bath for complete extraction of entrapped Voriconazole. The resultant solution was centrifuged at 15,000 rpm for 30 min to separate methanol and lipid layer. After centrifugation, supernatant was diluted appropriately. Entrapment efficiency was calculated using the following formula,

Entrapment effciency (%EE) = (Amount of voriconazole remained in vesicles)/ (Initial amount of voriconazole) * 100

DSC

Differential scanning calorimetry (DSC) is a widely used technique to understand the melting and recrystallization behaviour of drug molecule. DSC analysis was performed for lyophilized ufasomes, Voriconazole, sodium oleate and cholesterol using DSC (STA7300) (combined thermal analysis system) instrument. It is a thermo-analytical technique that determines the thermodynamics properties of materials by providing information about the polymorphic changes when subjected to a controlled heat flux. Sample were held 30˚C and 350˚C for 10 min, then heated 10˚C/min. DSC thermograms also confirm the internal arrangement of drug in the vesicles. Voriconazole is entrapped in cholesterol

bilayer and carboxylic groups of sodium oleate are on the surface of vesicles.

Fourier Transform Infrared (FTIR) spectroscopy

To investigate any possible interaction between the drug and the excipients, the Brucker IR- Affinity-1 FTIR spectrophotometer was used. The interaction between drug and excipient, if any was indicated either by presence of additional peaks or by absence of characteristic peaks in the IR spectrum of physical mixture that corresponding to the drug.

Optical Microscopic observation

The ufasomes were mounted on glass slides and viewed under a microscope with a magnification of 40X for morphological observation after suitable dilution. The photomicrograph of the preparation also obtained from the microscope by using a digital SL camera.

***Invitro* Drug Release studies**

This study is conducted to evaluate there leaserate/kinetics of the drug from ufasomes. This can be done by using Dialysis bag method. For this study known quantity (1 ml) of vesicular dispersion was added to dialysis membrane-50 (LA387-5MT). Dialysis membrane was then taken into 100 ml of phosphate buffer saline (pH 7.4) in a conical flask. The flask was kept in a orbital shaker and the speed of shaker maintained was 60 rpm at 37˚C. Samples were collected at different time intervals. Aliquots of samples are withdrawn at specified time intervals and replaced with equal volumes of fresh PBS (pH 7.4). The release studies have been done in duplicate pattern. The drug content in the sample was determined spectro-photometrically at 256nm. The percentage drug release of the drug has been calculated and the graph against percentage drug release versus time was plotted.

Release kinetic studies

The kinetic release study of Voriconazole from ufasomes was conducted using dissolution profile. The kinetic study was evaluated by using the *invitro* dissolution data in the below equations:

- Zero order $M_t = M_o + K_o t$
- First order : $\ln M_t = \ln M_o + K_1 t$
- Higuchi model: $M_t = K_H \sqrt{t}$
- Korsemeyer peppas model : $M_t / M_o = K_k t^n$

Where M_t is the amount of drug dissolved at time t, M_o is the initial amount of drug, K_1 is the first order release constant, K_H is the Higuchi constant, K_k is the Korsemeyer peppas model release constant and n is the diffusional release exponent indicative of the operating release mechanism. The correlation coefficient R^2 value was used as an indicator of best fitting for each of

models considered.

***Invitro* permeation studies**

Invitro permeation studies are conducted to predict skin (egg membrane) permeation of topical and transdermal formulations. For continous flow of receptor fluid to maintain skin conditions flow through cell was used. Moreover this type of system is more suitable to stimulate *invivo* conditions and preferred for several drug molecules. 24 hrs permeation studies were conducted on human skin for ufasome suspension and ufasome in hydroxyle propyl methyl cellulose (HPMC) gels with blanks as control.

Using Franz Diffusion cell the *invitro* skin permeation studies of Voriconazole from ufasomal formulation was studied. The *invitro* diffusion of drug was performed through one end of the hollow gas tube which act as a donor compartment. 10ml of PBS (pH 7.4) was taken in beaker and it is used as receptor compartment. A known quantity was spread uniformly on the egg membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.50˚C. The solutions to the receptor side were stirred by a small magnetic bead and were rotated at a constant speed. At predetermined time intervals, samples were withdrawn and replaced by 2ml of PBS. The drug concentrations in the aliquot were analyzed for drug content using UV spectrophotometer at 256nm against an appropriate blank. The optimized ufasomal formulation was taken and incorporated into the gel.

Table 1: Formulation of Voriconazole Ufasomal Suspension

Formulation code	Drug : cholesterol : Sodium oleate	Chloroform (ml)	Methanol (ml)
F1	1 (200mg) : 1 (200mg) : 1 (200mg)	5	5
F2	1 (200mg) : 1 (200mg) : 0.5 (100mg)	5	5
F3	1 (200mg) : 0.5 (100mg) : 1 (200mg)	5	5
F4	1 (200mg) : 2 (400mg) : 1 (200mg)	5	5
F5	1 (200mg) : 1 (200mg) : 2 (400mg)	5	5

RESULTS AND DISCUSSION

Determination of Size, Polydispersity Index and Zeta Potential

The size of vesicle was analyzed by using Zeta Sizer. Ufasomes with particle size <250nm was obtained with formulation F4 (1:2:1 ratio) (Voriconazole (200mg), cholesterol (400mg), sodium oleate (200mg)). However, the formulation F4 shows 201.30d.nm vesicle size **(Figure 1)**.

Increase in cholesterol with decrease in vesicle size which leads to high packing density. The polydispersity index of ufasomal formulation F4 was found to be 0.204 **(Figure 1)**. The Zeta potential value of ufasomal formulation F4 was found to be -38.6mV **(Figure 2)**.

Determination of Entrapment Efficiency, Drug content and pH

Entrapment efficiency of ufasomal formulation was measured to check the ability of ufasomal vesicle to entrap the drug. Entrapment efficiency is a vital parameter to characterize drug delivery from the vesicles. The high EE% will be useful in incorporating required dose in the minimum volume, facilitating local administration. Finally all formulations exhibited Voriconazole entrapment with entrapment efficiency ranging from 76.48% to 85.38% **(Figure 3)**. This indicates that ufasomes are the ideal carriers for entrapment of hydrophobic drugs. Formulation F4 had high entrapment efficiency of 85.38%. The greater entrapment was observed with the increased drug lipid ratio (1:2). The % drug content for formulations F1, F2, F3, F4, F5 are in the range of 75.54% to 84.83% **(Figure 4)**. The formulation F3 shows lowest drug content 75.54% and formulation F4 shows highest drug content 84.83%. pH of formulated ufasomal suspension were found to be in the range of pH 7 to 7.4. The pH of all formulations was compatible with skin pH.

Differential Scanning Calorimetry (DSC) study

DSC analysis was performed for lyophilized Ufasomes, Voriconazole, Sodium oleate and Cholesterol using DSC(STA7300) (combined thermal analysis system) instrument. The physical state of the drug inside the oleic acid vesicles is investigated by differential scanning calorimetry. The vesicles are placed in the conventional aluminium pan and as can speed of 2˚C/min was employed. Sample 4.9 mg of Voriconazole and 18.9mg of prepared ufasomal suspension was weighed in aluminium pans and hermetically sealed using a crimping device. An empty aluminium pan was used as a reference standard on the other side. Generally nitrogen was used as a purge gas during the analysis at a flow rate of 100ml/min. Sample were held 30˚C and 350˚C for 10 min then heated 10˚C/min. DSC thermogram of Voriconazole showed a characteristic endothermic peak at 132.7˚C **(Figure 5)**. DSC thermogram of prepared ufasomal suspension showed a characteristic endothermic peak at 131.6˚C **(Figure 6)**.

Fourier Transform Infrared (FTIR) spectroscopy

The interaction between drug and excipients were confirmed by FTIR

studies by using FTIR spectrophotometer. From the spectral study it was observed that the FTIR spectrum of pure Voriconazole there was no significant change in the peaks of pure drug and drug excipients mixture. Therefore it can be inferred that there was no specific interaction observed between the drug and excipients **(Figure 7, 8)**.

Optical Microscopic observation

All the formulations F1, F2, F3, F4, F5 are observed under the microscope and results in the formation of vesicles. In the optimized formulation F4 the vesicles shape was more spherical when compared to other formulations.

***Invitro* Drug Release Studies**

This study was performed by using Dialysis bag method. For this study known quantity (1 ml) of vesicular dispersion was added to dialysis membrane-50 (LA387-5MT). Dialysis membrane was then taken into 100 ml of phosphate buffer saline (pH 7.4) in a conical flask. The flask was kept in orbital shaker and the speed of shaker maintained was 60 rpm at 37˚C. Aliquots of samples are withdrawn at specified time intervals and replaced with equal volumes of fresh PBS (pH 7.4). The release studies have been done in duplicate pattern. The drug content in the sample was determined spectrophotometrically at 256nm. At the end of 8 hrs drug release is more than 30% for F1, F2, F3 and F4 shows 18.88%. At the end of 16hrs the drug release is more than 50% for F1, F2, F3 and F4 shows 38.956%. All the formulations released more than 62% by the end of 24 hrs **(Table 3)**. High proportions of cholesterol may leads to retardation of the drug release from the vesicles. This was observed in Formulation F4. The percentage drug release of the drug has been calculated and the graph against percentage drug release versus time was plotted **(Figure 9)**.

Release kinetics

As all the formulations got R^2 values higher for first order, so the drug release followed first order and the R^2 values for both higuchi and korsemeyer plots got higher values which indicates all the formulation followed diffusion and erosion release pattern **(Table 4)**.

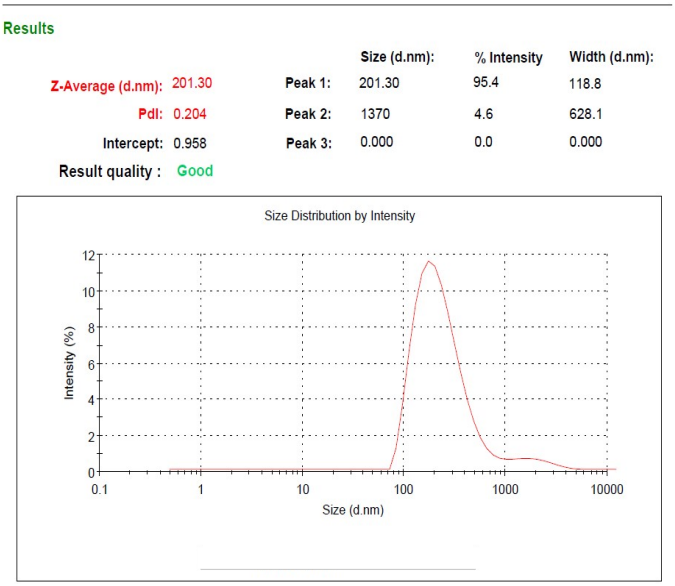

Figure 1: Size distribution curve of F4

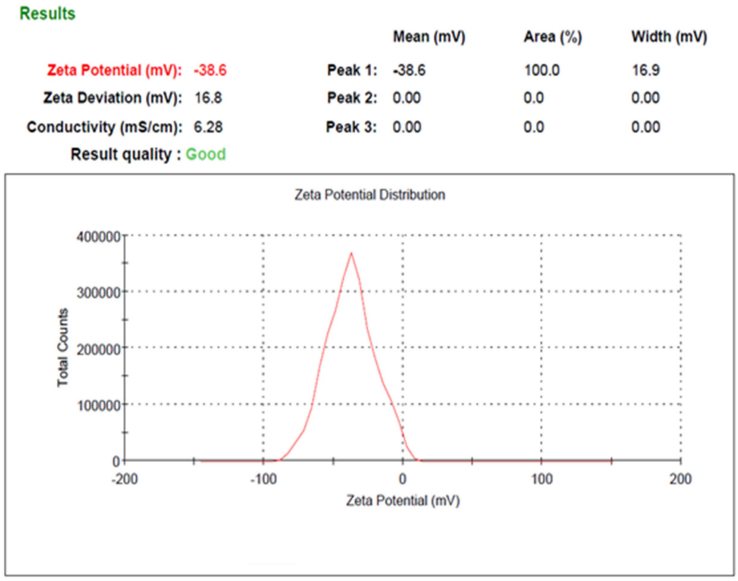

Figure 2: Zeta potential curve of F4

Table 2: Summary of Entrapment Efficiency, Drug content and pH results of ufasomes

Formulation code	Entrapment Efficiency (%)	Drug content	pH
F1	80.45	83.36	7.4
F2	76.48	79.23	7.1
F3	78.52	75.54	7.1
F4	85.38	84.83	7.2
F5	83.35	81.73	7.1

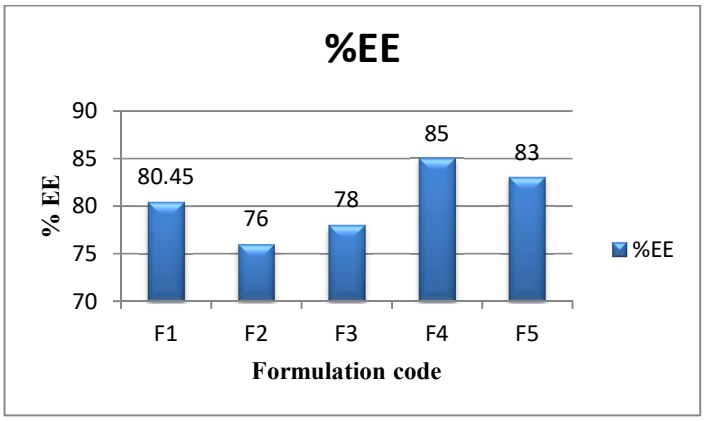

Figure 3: Entrapment Efficiency results of ufasomes

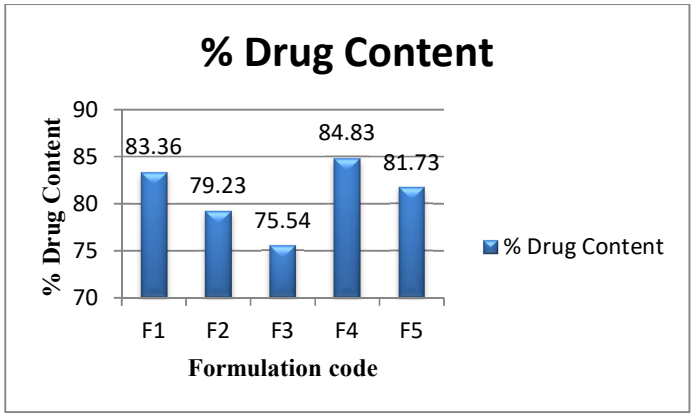

Figure 4: Drug content results of ufasomes

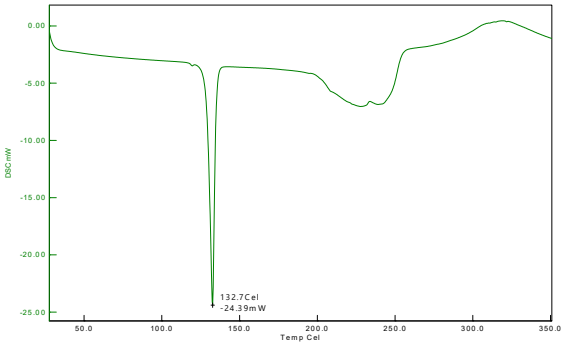

Figure 5: DSC thermogram of Voriconazole

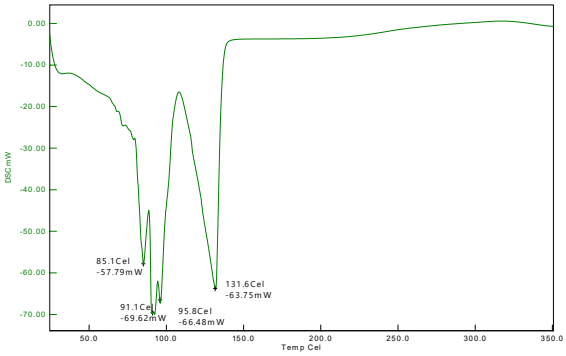

Figure 6: DSC thermogram of prepared ufasomal suspension

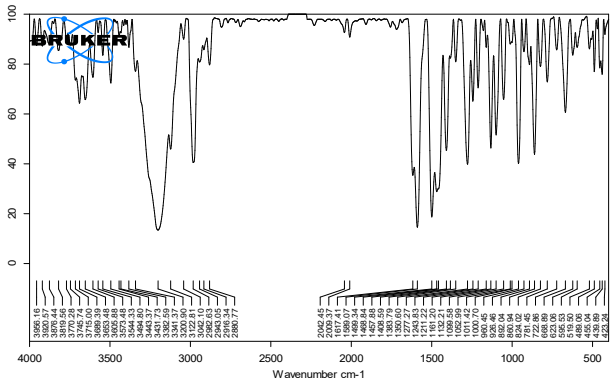

Figure 7: FTIR spectrum of Voriconazole

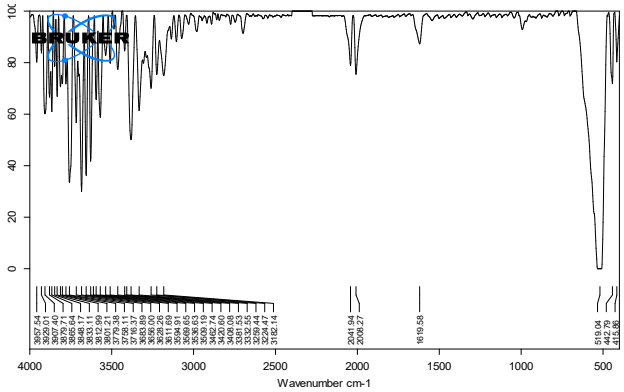

Figure 8: FTIR spectrum of Formulation

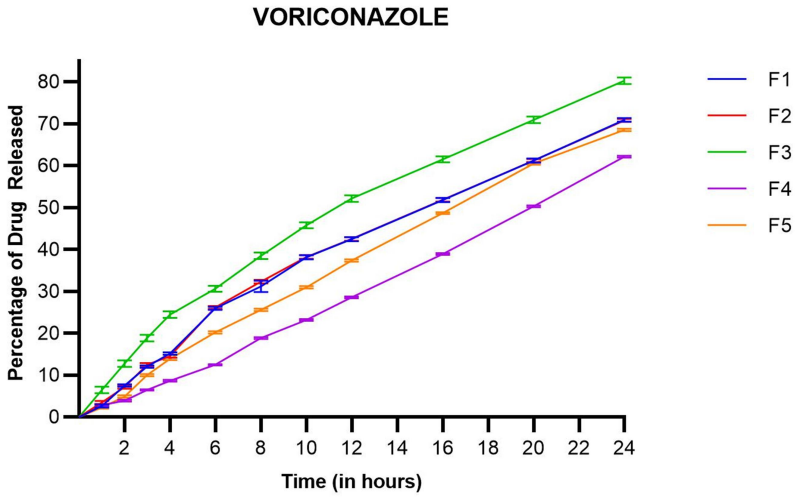

Figure 9: Graphical representation of *Invitro* drug release studies

Table 4: Kinetic profiling of all ufasomal formulations

Formulation code	Zero order	First order	Higuchi	Korsemeyer peppas
F1	0.980	0.995	0.962	0.968
F2	0.977	0.994	0.965	0.989
F3	0.968	0.993	0.982	0.991
F4	0.997	0.967	0.895	0.989
F5	0.995	0.988	0.939	0.985

CONCLUSION

Entrapment of hydrophobic drugs into lipid-based vesicles can improve the topical bioavailability. In the present study we successfully prepared Voriconazole loaded ufasomes using cholesterol and sodium oleate. Ufasomes were prepared using various excipients like methanol and chloroform as permeation enhancers, sodium oleate for vesicles formation and cholesterol for maintaining rigidity. By taking different ratios of cholesterol and sodium oleate, Voriconazole was incorporated into the ufasomes successfully.

Drug excipient compatibility studies were done between the drug, cholesterol, sodium oleate and formulation F1. FTIR studies confirmed that there was no specific interaction between the drug and excipients. DSC was performed for the drug and formulation. DSC thermogram of Voriconazole showed a characteristic endothermic peak at 132.7˚C. DSC thermogram of prepared ufasomal suspension showed a characteristic endothermic peak at 131.6˚C which indicates that there was no significant change in the pure drug in formulation. The prepared ufasomal suspension was characterized for vesicle size, entrapment efficiency, drug content, pH, zeta potential. The drug content is in the range of 75.54% to 84.83% and pH were within the limits. The % EE of all the prepared ufasomal formulations was found to be in the range of 76.48% to 85.38%. Among all formulations, F4 shows higher entrapment value.

The *invitro* drug release studies were also conducted for all the formulations. As there is increased proportion of cholesterol in the formulations the amount of drug release from vesicles is declined. This is due to the membrane rigidity property of cholesterol. Based on this study the amount of drug release in the formulations is F3>F2>F1>F5>F4. Sodium oleate doesn't have significant effect on the drug release from the vesicles.

Zeta potential of the optimized formulation F4 indicated good stability of the formulations. Evaluation of prepared ufasomes was done and among all the formulations, F4 has small vesicle size, high drug entrapment efficiency and drug release is more than 62% by the end of 24 hrs. Based on these results F4 is selected as optimized formulation. The *invitro* permeation studies were conducted for the optimized formulation F4. At the end of 24hrs amount of drug release in receptor compartment is very less. However the results from this study fulfilled that the Voriconazole is loaded into ufasomes for better topical administration.

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