UNSATURATED FATTY ACID VESICULAR TOPICAL FORMULATION FOR THE TREATMENT OF SUPERFICIAL FUNGAL INFECTION

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ABSTRACT

Enhancement of skin bioavailability through topical formulation is challenging due to the presence of the barrier stratum corneum. Elastic, deformable lipid vesicles are easily penetrated through the skin barrier. The present study involves unsaturated fatty acid for vesicular preparation, ufosomes of miconazole nitrate. Fatty acid acts as skin penetration enhancer and improves skin retention. The effect of formulation parameters on efficacy of formulation was studies using 3^2 full factorial design. The particle size and % entrapment efficiency was found to be in the range of 157 to 291.6 nm with irregular spherical shape and 54.14% to 85.84%, respectively. The *in vitro* drug release, *ex vivo* drug release and skin retention of optimized ufosomes were found to be 78.424 %, 20.793 % and 43.36 %, respectively. The optimized batch displayed Higuchi drug release kinetic model with desired physicochemical properties.

Keywords: Miconazole nitrate(MN), unsaturated fatty acids, ethanol injection, skin bioavailability, amorphization, ufosomes

INTRODUCTION

About more than a billion people are suffering from skin, hair and nail fungal infections, about 10 billion from mucosal candidiasis, approximately 150 million have severe life-threatening infections and more than 1.7 million deaths are recorded annually, while many more are undetectable due to negligence by social communities and other reasons. Fungal infection is associated with generally immunocompromised patients suffering from pulmonary disease, acquired immune deficiency syndrome, cancer, etc. Most commonly, fungal infections are topical infections like skin, nail, hair and mucosal, as well as systemic infections[1]. Superficial fungal infection is generally caused by dermatophytes belonging to *Microsporum*, *Trichophyton* and *Epidermophyton* genera. Dermatophytes generally live on keratin, hence they invade the stratum corneum and superficial tissue of skin. *Candida albicans*, a ubiquitous fungal pathogen, is a major causative agent of candidiasis infection. It generally infects skin, vagina, intestine and oral mucosa. Various classes of antifungal agents like azoles (triazoles and imidazoles), griseofulvin, echinocandins, allylamines and polyene antibiotics are used in the management of candidiasis.

Topical formulations are generally best for the local administration of drug, ensuring direct access and prolonged retention rate at the target. Moreover, it contributes in minimizing systemic side effects and prevents pre-systemic metabolism, improving the efficacy of the treatment, increasing topical bioavailability and improves patient compliance, as self-medication is possible. On the contrary, this formulation may suffer from poor skin penetration due to stratum corneum and poor ungual or dermal bioavailability, uneven distribution of drug at different level, skin irritation, allergy, and uncontrolled elimination of drugs from the formulation. Therefore, novel approaches are required to overcome limitations of conventional topical formulations. In the past decade, nano, colloidal particulate approaches like lipid vesicular systems, nano & micro emulsions, solid lipid nanoparticles and polymeric particles have been intensively studied for delivering the drug precisely with greater skin penetration and low irritancy.

Miconazole nitrate (MN) is an ideal candidate for the treatment of superficial candidiasis[2]. It inhibits fungal enzyme 14α-sterol demethylase which results in interruption of ergosterol synthesis, a leading component of fungal cell membrane. It interferes with the membrane bound enzymes and barricade system of membrane. It is generally used to treat topical fungal infections like ringworm of the body, groin, and feet (athlete's foot), as it remains for prolonged time in the stratum corneum.

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Marketed preparations suffer from poor skin penetration and long-term treatment is required. Hence, alternate approaches are necessary to enhance skin penetration for MN topical formulations.

For enhancing the local effect of MN to the topmost level of skin, it must be loaded with skin penetration enhancer to increase the skin absorption at target sites. Lipid vesicular drug delivery systems are more appropriate as lipid characteristics mimic the phospholipid bilayer of cell membrane. Moreover, they can easily deform due to elasticity and plasticity, without rupturing or losing the drug and improves the skin penetration[3]. Ufosomes are lipidic vesicular systems comprising of unsaturated fatty acid and ionic surfactant can encapsulate the lipophilic drug[4]. Oleic acid, a type of fatty acid acts on selective extracellular lipid in the stratum corneum. This leads to temporary disorganization of cells and forms principal regulatory channels, and enhances skin penetration. It has been reported that fatty acids can form vesicular systems in the aqueous phase[5]. These vesicles are deformable vesicles which can easily diffuse through skin cells without rupturing. Further, the formulation of ufosomes is cost-effective as fatty acids are easily available and inexpensive.

Therefore, the present study aimed to formulate the oleic acid and Tween® 80 based ufosomes of MN using ethanol injection method. 3^2 factorial design was utilized to study the effect of excipients on the vesicular size and drug entrapment efficiency. Based on the desirability, the optimized formulation was prepared and characterized for *ex vivo* skin permeation, *in vitro* diffusion and drug release kinetic studies were performed.

MATERIALS AND METHODS

MN was gifted by FDC India Private Ltd., Mumbai, India. Oleic acid, Tween® 80, Carbopol® 934, monosodium phosphate, disodium phosphate, phosphoric acid and sodium hydroxide were purchased from Lab Chem Corporation, Vadodara, Gujarat, India. All other reagents used were of analytical grade.

Ufosomes preparation[6,7]

Ethanol injection method was employed for the preparation of ufosomes. Briefly, the organic phase was prepared using oleic acid, Tween® 80 and drug in ethanol and stirred on the magnetic stirrer, till the clear solution was obtained. The resulting organic phase the was injected into the aqueous phase containing saline phosphate buffer (pH 6.8) using 18 G syringe. Spontaneous ufosomes formed after organic solution came in contact with the aqueous phase. The resulting solution was kept under stirring for 15 to 30 minutes at room temperature. Prepared solution was probe sonicated (Sonics Vibram -15 mm) for 5 minutes with 2 second pause to reduce the vesicular size and aggregation. Sonicated solution was centrifuged at 4 ^{0}C for 60 minutes at 8000 rpm (Beckman Ultracentrifuge). The supernatant was discarded and the sediments were collected using micro spatula in the Petri dish. Sediment was washed with water using mannitol as a cryoprotectant.

Experimental design

For studying the effect of variables on performance of ufosomes system, a 3^2 factorial design was utilized in the present study. Based on preliminary batches and prior literature review, the appropriate limits for the independent variables were chosen. The amount of oleic acid (X1) and Tween® 80 (X2) were selected as independent variables. The effect of these variables on dependent variables like vesicular size (Y1) and % entrapment efficiency (Y2) were evaluated (Table I). A conclusion is considered significant, depending upon the estimated p-value ($p < 0.05$). Design-Expert® software 11.0 trial version was used for formula optimization. The formula was optimized by keeping X1 and X2 within the limits employed in the current work, while Y1 and Y2 were kept at minimum and maximum levels, respectively. Other process variables like amount of ethanol, drug and the PBS were kept at constant, as shown in Table I. The significance of independent factors was determined using Analysis of Variance (ANOVA) method. Based on the high desirability value for the expected goals, the formula composition was calculated. For the determination of the design space of the desired responses, the overlay plot was constructed. The suggested formulations were made, and % relative error was calculated.

Characterization of ufosomes

Particle size analysis

The particle size and poly dispersibility index were measured using a Zetasizer Ver. 7.11 (Malvern Instruments) at 25 ^{0}C by the wet method. All measurements were performed in triplicate.

% Entrapment efficiency

Entrapment efficiency of the formulations was calculated by extraction method. In brief, the ufosomes were transferred to eppendorf tube and centrifuged at 12000 rpm for 4 h at 4 ^{0}C. The sediment containing ufosomes was collected. The sediment was mixed with methanol and bath was sonicated for 15 minutes. The resultant methanolic solution was kept overnight in shaking

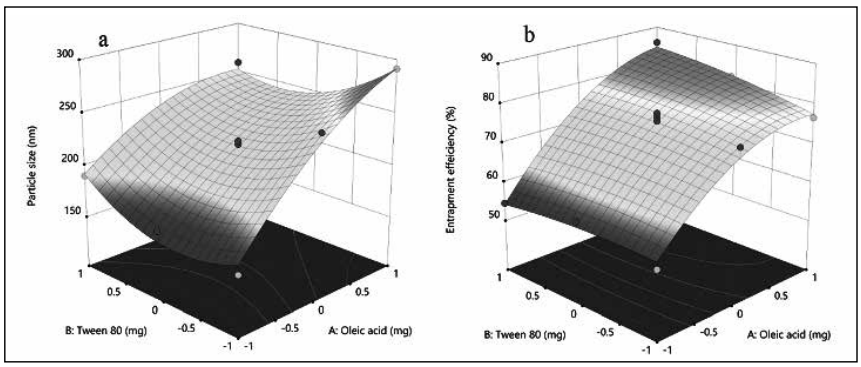

Fig.1: Response surface graph (a) particle size; (b) % Entrapment efficiency

water bath (RT; 100 rpm) for complete extraction of the entrapped drug. The resultant solution was centrifuged at 12000 rpm for 1 h at 4 ^{0}C to separate methanol and lipid layer. The methanolic supernatant was collected, diluted appropriately and the concentration of entrapped drug was determined. Entrapment efficiency was calculated using the following formula.

$$\text{Entrapment efficiency (\% EE)} = \frac{\text{Entrapped drug in ufosomes}}{\text{Total amount of drug}} \times 100$$

Morphological characterization

Thermal analysis was performed for pure MN and lyophilized powder. 5 mg of each sample was placed into aluminum crucible and sealed. The thermograms were obtained by differential scanning calorimetry (DSC Q10, TA, USA) at a heating rate of 10 °C min^{-1} under atmosphere of dry nitrogen using plain Al_2O_3 as a reference.

Surface morphology of the optimized ufosomes was studied by using scanning electron microscope with a high-resolution field emission source (Thermo Fisher Scientific). High resolution images of ufosomes were visualized at an accelerated voltage of 20 keV.

X-ray diffraction (XRD) was carried out in symmetrical reflection mode using Cu Kα line as the source of radiation, and the wavelength was set at 1.5405 Å. Samples of pure MN and lyophilized ufosomes were scanned at 2θ between the angle range 3° to 50° with scanning rate of 0.02 ° min^{-1} .

In vitro diffusion studies

In vitro diffusion studies were performed using Franz diffusion cells. Cellophane membrane 150 (HiMedia dialysis) having 0.22 µm pore size were mounted on Franz cells and stabilized by keeping overnight in contact with PBS[8]. Ufosomal formulation in Carbopol® gel (0.5 %) and marketed formulation (Miconaz 2% gel, Innovax Pharmaceuticals) were placed on the membrane (equivalent to 20 mg) in donor chamber. The receiving chamber was filled with approximately 12.5 mL of PBS (pH

Table I: Composition and evaluation of batches prepared as per the applied design

Batch No.	Oleic acid (mg) X1	Tween® 80 (mg) X2	Particle Size (nm) Y1	% Entrapment efficiency (%) Y2	PDI
P1	500	200	218.9	73.78	0.267
P2	200	400	190.4	54.58	0.458
P3	500	200	216.9	75.78	0.267
P4	200	100	157.2	54.14	0.183
P5	900	200	242.2	81.7	0.154
P6	900	100	291.6	76.64	0.242
P7	900	400	259.8	85.84	0.172
P8	500	200	211.9	71.78	0.267
P9	500	200	223.9	77.78	0.267
P10	500	400	234.7	74.18	0.177
P11	200	200	165.49	57.54	0.424
P12	500	100	255.31	75.58	0.265
P13	500	200	220.9	76.78	0.267

Table II: Analysis of variance for response surface model for dependent variables

Source	Sum of squares	Degrees of freedom (df)	Mean square	F value	p – value
Particle size					
Model	16018.82	5	3203.76	48.15	<0.0001
Residual	465.71	7	66.53	-	-
Corrected total	16484.53	12	-	-	-
% Entrapment efficiency					
Model	1189.87	5	237.97	41.48	<0.0001
Residual	40.16	7	5.74	-	-
Corrected total	1230.02	12	-	-	-

7.4) as the receiving phase. The cell was thermostated at 37 ^{0}C, and magnetically stirred at 100 rpm. 1 mL aliquot was collected from the receiving phase at predetermined time intervals. The amount of MN diffused to the receiving phase was quantified using the UV- spectrophotometric method.

Ex vivo skin permeation

The skin permeation was tested using goat skin. The skin was obtained from a local slaughterhouse, and stored at 4 ^{0}C in the phosphate buffer till use. About 3 × 3 cm^2 sized of patches of intact skin were cut and both the sides were rinsed with isopropyl alcohol for removing underlying subcutaneous fat followed by final rinse with sterile PBS (pH 7.4). The skin samples were placed between the donor and receiver compartments of the Franz cell, with the stratum corneum side facing the donor compartment. The skin was allowed to stabilize for 60 minutes before use. The skin permeation was evaluated under similar conditions used for *in vitro* diffusion, using the same protocol. However, the experiments were conducted for 4 h (not 12 h) due to sensitivity of the extracted skin samples to the temperature used for the test.

Skin retention

Skin was carefully removed using Franz diffusion cell after performing skin permeation investigations. The remaining formulation was cleaned with a cotton swab saturated through 7.4 pH phosphate buffer. The cleaned skin piece was crushed. 50 mL of 6.8 pH phosphate buffer containing the meshed mass was shaken for 1 h at 37 °C in a water shaker bath to extract the medication completely. The resultant solution was filtered after extraction, and drug content was quantified using UV spectrophotometer. Skin retention (%) was calculated using the following formula:

$$\% \text{ skin retention} = \frac{\text{Amount of drug remained in skin}}{\text{Total amount of drug}} \times 100$$

Halo-zone test

The cup plate method was employed to test the *in vitro* antifungal activity. *C. albicans* culture was cultivated in Sabouraud agar (SDA) media plates for 24-30 h. After the growth of fungus, three wells, each 10 mm in diameter were drilled in each plate. Adequate amounts of ufosome dispersion, plain drug and marketed preparation were filled, respectively. Incubate the plate for 48 h and observe the zone of inhibition.

RESULTS

Experimental design

13 experimental runs were obtained using Design Expert ® version 11. The actual value of independent variables along with the obtained dependent variables are presented in Table I. Analysis of variance is used for studying correlation between variables. The highest order model with significant terms (Prob < F is less than 0.005) is quadratic model (suggested by the software) and are applicable to justify the effect of independent variables on dependent variables.

Particle size

Vesicle size and particle size distribution of the lipid based vesicular preparation are the most important parameters which need to be monitored during preparation. As evident from reports, it can be said that the size and poly dispersibility of the vesicles directly affect their *in vitro* or *in vivo* performance. The particle sizes of different batches of ufosomes were in a range of 157 to 291.6 nm. The obtained polynomial equation

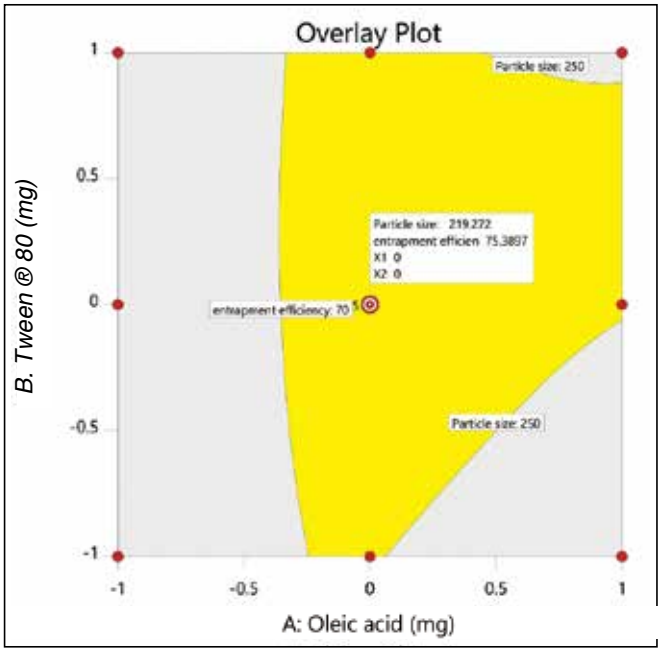

Fig. 2: Overlay plot

was as follows:

$$Particle\ size = 210.27241 + 46.75167X_1 - 3.20167X_2 - 16.25X_1X_2 - 17.35845X_1^2 + 23.80155X_2^2$$

The positive values of independent variables in the polynomial equation clearly reveals that selected variables are strongly influencing the particle size. The negative value of coefficient of X1 states that oleic acid concentration has an inverse effect on particle size. The coefficient of X1 and interaction between two variables was found to be significant at the level of $P < 0.05$. From the obtained result, it could be concluded that Tween® 80 concentration has minimum effect on the particle size. Surfactant is responsible for controlling the rate of aggregation of lipid vesicles. Negative value of co-efficient indicates that, small amount of surfactant can control the rate of flocculation.

Further, the F value is near about 48.15, indicating the significance of the model (Table II).

% Entrapment efficiency

For the effectiveness and efficacy based of the vesicles, entrapment efficiency is important. More amount of drug entrapped can easily cross the stratum corneum through fatty acid vesicles and improve the efficiency of dosage form. The % entrapment efficiency of different batches of ufosomes was in the range of 54.14 to 85.84 %. The minimum and maximum size values correspond to formulations F4 and F7, respectively.

The obtained polynomial equation for % entrapment efficiency is

$$\%\ \text{Entrapment Efficiency} = +75.38966 + 12.98667X_1 + 1.37333X_2 + 2.19X_1X_2 - 6.29379X_1^2 - 1.03379X_2^2$$

From the equation, it can be concluded that amount of oleic acid has a positive effect on the drug entrapment. This is because of the molecular interaction between the fatty acid and MN. Further, the F value is near about 41.48, indicating the significance of the model (Table II).

Response surface methodology

Contour plots was constructed using Design-Expert® version 11 for the visualization of the effects of independent variables and their interactions on the dependent variables. The effect of independent variables along with their interactions on particle size and % entrapment efficiency are depicted in the Figs. 1a & 1b. This shows non-linear relationship between the independent variables. From the contour plot, it was concluded that higher concentration of surfactant is not affecting the particle size of the oleic acid vesicles. However, the concentration of oleic acid influences the particle size as well as drug entrapment.

Confirmation of the model

Higher degree of encapsulation of drug and lower particle size improve the efficiency and skin penetration of the prepared dosage form. Based on this hypothesis, overlaid contour plot with defined conditions of desired

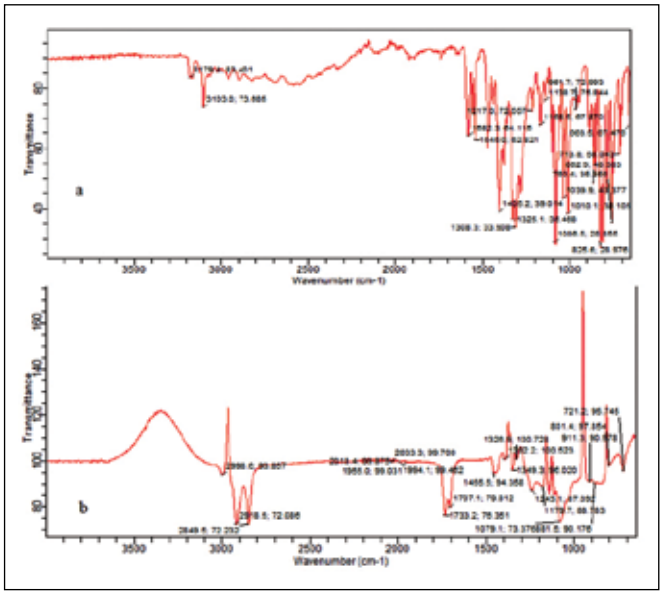

Fig. 3: FTIR studies (a) – Plain drug; (b) Ufosomes

Table III: Check pint batches

Batch No.	Oleic acid (mg)	Tween® 80 (mg)	Particle size			% Drug entrapment efficiency		
			Actual value	Predicated value	% Relative error	Actual value	Predicated value	% Relative error
1.	160	216.9	211.9	211.373	0.6	71.78	70.37	1.44
2.	352.5	22.4	242.2	242.268	0	81.7	81.73	0

PS and %EE (entrapment efficiency) was constructed by keeping the independent constraints in the range (Fig. 2). The yellow area (design space) corresponds to conditions resulting in a particle size in the range between 100 nm to 250 nm and % EE range between 70 to 85 %. The predicted and observed values of particle size and % entrapment efficiency of the check point batches are shown in the Table III. The calculated % relative error was less than 5 %.

Selection of the optimized batch

Graphical method was used for the selection of optimized batch. The desired constraints were applied, like minimum particle size and maximum drug entrapment for construction of overlay plot (Fig. 2). Overlaid plot was generated which showed the design space (yellow region) for the desired constraints. This plot indicates that as the oleic acid concentration increases, particle size and entrapment efficiency also gets increased. In the presence of high concentration of Tween® 80, particle size decreases but no effect is observed on drug entrapment. Hence, 200 mg oleic acid and 100 mg Tween® 80 were selected for the optimized batch.

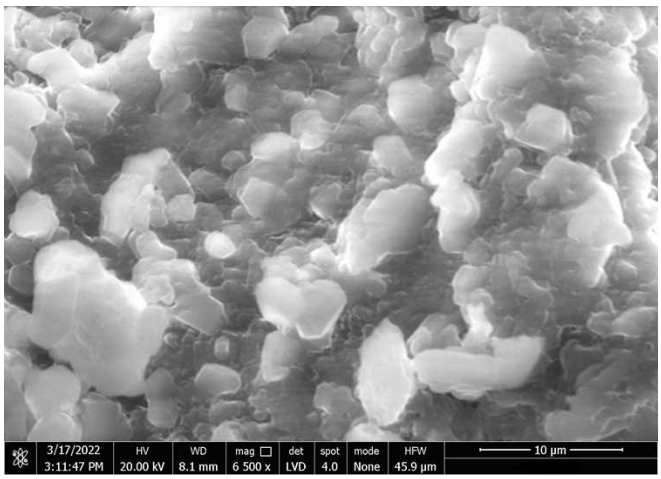

Fig. 4: SEM of Ufosomes

Characterization of ufosomes

FT-IR spectra of pure MN shows imidazole C-N, aromatic and aliphatic C-H stretching, C=C aromatic

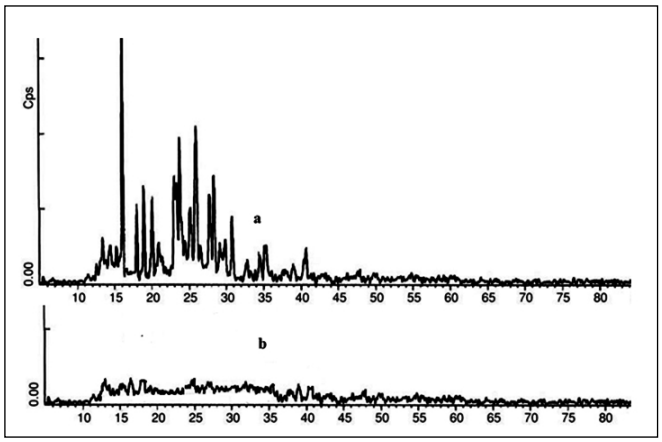

Fig. 5: XRD diffraction (a) plain drug; (b) Ufosomes

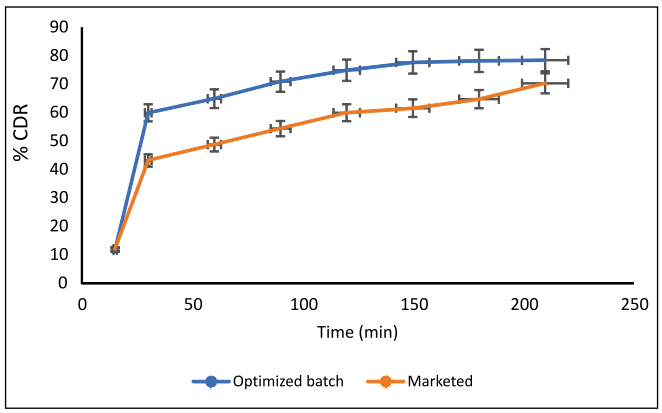

Fig. 6 : *In vitro* transmembrane diffusion

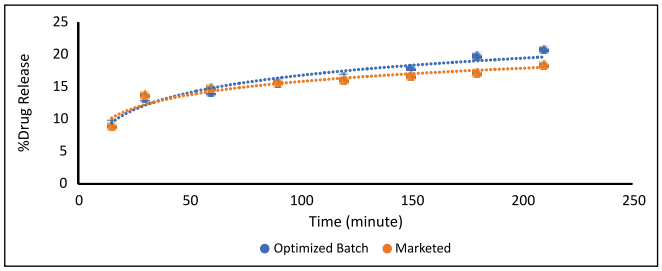

Fig. 7: *Ex vivo* skin permeation study

and C-Cl halogen attached at benzene ring stretching at 3179.4, 3103.0, 3103.0, 1582.3 and 1582.3 cm^{-1}, respectively. The obtained characteristics peaks were matched with the standard values. These peaks were also present in the FT-IR spectra of mixture containing MN and excipients. Fig. 3 shows the FT-IR spectra of drug and ufosomes.

SEM image of optimized ufosomes is shown in Fig. 4. The observed image shows the irregular spherical shapes in the agglomerates of the prepared vesicles. DSC study helps in understanding of crystalline and melting properties of the material. In the ufosomes, the shifting of endothermic peak was observed near the melting point of oleic acid. X-Ray diffractograms of miconazole nitrate and the optimized ufosomes are depicted in Fig. 5. The XRD pattern of pure MN demonstrates sharp and narrow peaks at diffraction angles 13.345°, 15.22°, 18.927°, 20.857°, 23.00°, 23.296°, 27.745°, 29.834° , 35.34° and 40.63°, which shows a typical crystalline pattern. The absence of characteristic peaks in the XRD pattern of optimized ufosomes was noted.

In vitro transmembrane diffusion

Franz diffusion cell was used for the study of % drug release. Results show that 60% of drug is released within 30 minutes. On comparison with marketed preparation, optimized ufosomes show better drug release, as shown in Fig. 6. Moreover, the ufosome formulation follows the drug release kinetic Higuchi model (R^2 = 0.938) and achieve sustained release characteristics.

Ex vivo skin permeation and skin retention of vesicular dispersion

Ex vivo skin permeation studies on goat skin were conducted using the Franz diffusion cell. Fig. 7 shows the results of permeation tests. The graph shows, that the amount of medicine permeated from commercial gel was less than ufosomes. The total amount of medicine permeated in 4 h from commercialized gel was 18.318 % and from optimized gel, it was 20.793 %. The amount of medication retained in skin after 24 h was 43.56 %, but for commercialized gel, it was found to be 14.3 %.

Halo – zone test

Antifungal activity study was performed against *C. albicans* using cup plate method, where the developed formulation showed a large zone of inhibition in comparison to the marketed formulation.

DISCUSSION

For superficial fungal infection, drug molecules must be present in the upper layer of skin for the prolong period. The solubility, stability and effectiveness of the dosage form can be dependent on the drug - excipient interaction, amorphization of crystalline molecules and proper storage.

In this study, the FT-IR spectra shows all the characteristics peaks of functional groups of MN in the ufosomal preparation. This shows the compatibility of the excipients with MN. For the skin penetration, particles having particle size less than 200 µm can easily diffuse through skin cells. Moreover, BCS class 2 drugs are lipophilic in nature and trigger more skin penetration. Unsaturated fatty acids like oleic acid have good skin penetration power by temporary disorganization of the stratum corneum. Oleic acid can entrap a larger amount of drug by bridge formation. Surfactant also minimizes the aggregation of prepared ufosomes by minimizing the interfacial tension by forming a thin film around the vesicles. Applied experimental design also showed similar kinds of results and it can be said that oleic acid improves drug entrapment and surfactant concentration up to critical micelle concentration, minimizing the aggregation of particles.

SEM data shows the spherical shape of the vesicles which can be easily deformed into the skin and shows better penetration. Thermal analysis XRD and DCS also reveals that inclusion of MN into the ufosomess and transition of crystalline form to amorphous form. This can improve the drug release.

Lipid vesicles of size less than 200 nm can easily penetrate through skin. When the lipid or oily phase come in contact with water, hydration of lipid takes place. The difference in water content between skin and formulation leads to penetration[9]. Generally, topical diffusion study is carried out to differentiate the concentration of drug release from the formulation between stratum corneum and epidermis. In transdermal diffusion study results show better drug delivery from ufosomes compared to marketed gel because ufosomes show high drug release and this might be the reason for more diffusion of drug through membrane. In the SEM results, many irregular spherical densely packed pores were observed. This makes possible for ufosomes to form tiny droplets to line along stratum corneum and increases the absorption area and thus facilitates more drug penetration[10].

Due to the presence of the single bond in unsaturated fatty acid like oleic acid, deformable vesicles were more easily prepared which can easily penetrate skin without rupturing. This can be observed in the *ex vivo* skin permeation and skin retention studies. Moreover, drug release is also improved, and greater zone of inhibition was observed compared to the marketed preparation.

CONCLUSION

Miconazole nitrate loaded ufosomes were successfully formulated using oleic acid and Tween® 80 surfactant by ethanol injection method. The selected lipid and surfactants are compatible with the drug. Optimized batch of ufosomes has particle size around 250 nm and entrapment efficiency of 70 % . DSC and XRD results showed conversion of crystalline form into amorphous form. Morphologically prepared vesicles were of rough spherical shape. Optimized batch showed better drug release and followed sustained release kinetics. They show better skin penetration and retention compared to marketed preparation. That confirmed the ability of ufosomes to accumulate high amount of lipophilic drug and improve the topical bioavailability.

REFERENCES

1. Gupta M., Sharma V. and Chauhan N. S.: Promising novel nanopharmaceuticals for improving topical antifungal drug delivery., Nano-and Microscale Drug Delivery Systems, Elsevier; Amsterdam, 2017, pp- 197-228.
2. Zhang L. W., Fu J. Y., Hua H. and Yan Z. M.: Efficacy and safety of miconazole for oral candidiasis: a systematic review and meta-analysis. **Oral Dis.**, 2016, 22(3), 185-195.
3. Al-Maghrabi P. M., Khafagy E. S., Ghorab M. M. and Gad S.: Influence of formulation variables on miconazole nitrate–loaded lipid based nanocarrier for topical delivery. **Colloids Surf. B: Biointerfaces**, 2020, 193, 111046.
4. Morigaki K. and Walde P.: Fatty acid vesicles. **Curr. Opin. Colloid Interface Sci.**, 2007, 12(2), 75-80.
5. Bahadır E. B. and Sezgintürk M. K.: A comparative study of short chain and long chain mercapto acids used in biosensor fabrication: A VEGF-R1-based immunosensor as a model system. **Artif. Cells Nanomed. Biotechnol.,** 2016, 44(2), 462-470.
6. Verma S., Bhardwaj A., Vij M., Bajpai P., Goutam N. and Kumar L.: Oleic acid vesicles: a new approach for topical delivery of antifungal agent. **Artif. Cells Nanomed. Biotechnol.**, 2014, 42(2), 95-101.
7. Kremer J., Van der Esker M. W., Pathmamanoharan C. and Wiersema P. H.: Vesicles of variable diameter prepared by a modified injection method. **Biochem.,** 1977, 16(17), 3932-3935.
8. Kravchenko I.: Influence of cholesterol and its esters on skin penetration *in vivo* and *in vitro* in rats and mice. **Ukrainica Bioorganica Acta,** 2011, 1, 17-21.
9. Nagaich U.: Deformable vesicles: Alternatives in topical drug delivery. **J. Adv. Pharm. Technol. Res.,** 2016, 7(4), 117.
10. Csongradi C., Du Plessis J., Aucamp M. E. and Gerber M.: Topical delivery of roxithromycin solid-state forms entrapped in vesicles. **Eur. J. Pharm. Biopharm.,** 2017, 114, 96-107.