doi.org/10.36721/PJPS.2024.37.1.REG.095-105.1

Formulation and evaluation of miconazole lipogel for enhanced drug permeation

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Abstract: Hydrophilic drugs could be incorporated into the skin surface by manes of Lipogel. This study aimed to prepare miconazole lipogel with natural ingredients to enhance drug permeability using dimethyl Sulfoxide (DMSO). The miconazole lipogels, A1 (without DMSO) and A2 (with DMSO) were formulated and evaluated for organoleptic evaluation, pH, viscosity, stability studies, freeze-thawing, drug release profile and drug permeation enhancement. Results had stated that prepared lipogel's pH falls within the acceptable range required for topical delivery (4 to 6) while both formulations show good results in organoleptic evaluation. The A2 formulation containing DMSO shows better permeation of miconazole (84.76%) on the artificial skin membrane as compared to A1 lipogel formulation (50.64%). In *in-vitro* drug release studies, A2 for-mulation showed 87.48% drug release while A1 showed just 60.1% drug release from lipogel. Stability studies were performed on model formulations under environmental conditions and both showed good spreadibility, stable pH, free of grittiness and good consistency in formulation. The results concluded that A2 formulation containing DMSO shows better results as compared to DMSO-free drug lipogel.

Keywords: Lipogel, miconazole, transdermal drug delivery system, permeation enhancer, evaluation.

INTRODUCTION

Skin is the largest organ of the body that acts as a barrier against toxins, microbes, and ultraviolet radiation. It inhibits the water and electrolytes lost from the body by protecting skin and efficiently processes metabolism, excretion, immunology, temperature regulation, and sensation (Hew *et al*., 2016). Structurally, the skin has three layers including epidermis, dermis and subcutaneous tissue. Between these layers, junction structure hinders the transport of biomolecules (Bader and Worsley, 2018). The significance of the outermost layer of skin known as the stratum corneum (SC) forms a barrier and provides protection (Munir *et al*., 2023a). The life of plants and animals is greatly influenced by fungal pathogens. It is reported that such pathogens affect profoundly the extinction of species, ecosystem disturbances and food security (Liu *et al*., 2018). It is of great concern to observe the high mortality rate due to fungal infections and numerous antifungal drugs are available but still, these infections lead to an increase in the rate of mortality by 50% (Kaur *et al*., 2020).

Superficial fungal infections are infections caused by pathogenic fungi that affect the epidermis, nails, hairs, and mucosa, and are limited to these areas. A most common type of superficial fungal infections is ringworm, tinea versicolor and candidiasis. Dermatophytes are usually described that involved such as tinea corporis, tinea capitis, tinea pedis and tinea unguium (Garg *et al*., 2020). There are several advantages of the topical treatment of fungal infection such as targeting infection sites, lowering the risks of systemic side effects, increases the efficacy of treatment and improved patient compliance. A variety of antifungal compounds are used in the dermatological treatment of fungal infections like polyenes, azoles, benzyl amines. Antifungal drugs are available in a conventional dosage form such as sprays, lotions creams and gels. Penetration through target tissues is the critical factor that determines the efficacy of topical antifungal treatment, due to which effective drug concentration is needed in the skin (Talaat *et al*., 2019).

The most commonly used antifungal drug families are allayl amines and azoles. For systemic candida and aspergillus infections, a new class of antifungals is used known as echinocandins. Topical use of antifungal drugs with anti-inflammatory properties and using a combination of topical and oral antifungal agents are the innovations used to enhance the effectiveness and monotherapy cure rates (Biswal *et al*., 2023). There are several approaches used to reduce the reinfection of tinea cruris (Nene *et al*., 2021). Broad-spectrum antifungal

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agent miconazole nitrate is mostly applied for the treatment and management of buccal, dermal and vaginal candidiasis (Nene *et al*., 2021).

Miconazole is an imidazole derivative that inhibits the cytochrome P450 enzyme necessary for the biosynthesis of ergosterol (Babu *et al*., 2022). It obstructs lanosterol demethylase in the sterol biosynthesis pathway and the antifungal effect of the miconazole is the induction of endogenous reactive oxygen species. Inhibiting cytochrome p-450, disruption of steroidogenic acute regulatory protein expression and suppresses steroidogenesis are the major actions shown by miconazole *in-vitro* studies (dos Santos Porto *et al*., 2022).

Miconazole belongs to the Biopharmaceutical classification system (BCS) class 2 drug and belongs to the imidazole family. Miconazole nitrate has very low solubility in water but soluble in methanol, dimethylformamide, and hot chloroform(Mousavi *et al*., 2023). The main problem in treating topical antifungal infections by miconazole nitrate is its poor skin penetration. Whereas, sufficient concentration is needed at the infection site for effective treatment. But in this study, we aimed to formulate a lipogel containing miconazole as an active ingredient to treat superficial fungal infections such as tinea pedis, tinea corporis, and tinea capitis. This study also aimed to check the stability, pH, rheological properties, spreadibility and permeation of miconazole lipogel into the skin.

MATERIALS AND METHODS

Materials

Miconazole nitrate gifted by Mass Pharma PVT. LTD., Olive oil gifted by CCL Pharmaceutical PVT. LTD., Span 80 (Sigma-Aldrich), mineral oil (Riedel-de Haen), DMSO (Guangdong Guanghua), Distilled Water, Phosphate-Buffer Saline, and Beeswax was taken from Pharmaceutical Research Lab., University of Central Punjab, Lahore, Pakistan. All the ingredients used were of analytical grade.

Formulation of blank lipogel

To prepare a lipogel, mineral oil, spam 80, beeswax, and olive oil has been selected for exhibiting successful pharmaceutical formulation. In table 1, numerous trial formulations from F1 to F16 have been conducted to check the adequate gelation. Formulation F12 shows better gelation than any other trial formulation to prepare lipogel. Moreover, it also shows better stability, appearance and homogeneity.

Preparation of drug lipogel formulations

For the preparation of the drug formulation, all the glassware was washed and dried. After cleaning melt, the beeswax at a higher temperature (80°C) and add a span 80, both of them act as a gelling agent. The 2% w/w miconazole as an active ingredient was dissolved in the heated oil phase (Mineral oil) with continuous stirring. After stirring mixture of the oil phase was added to the drug solution and add gelling agents' solution with continuous stirring. The pre-heated olive oil was added to the mixture and at last, add 5% w/w dimethyl sulfoxide in it and shake well to form continuous and homogenous lipogel. After getting clear mixture, heating was stopped and homogenization by placing at vortex mixture for about 2-3 minutes at 2000 RPM and the solution was then cooled to 25˚C to start gel formation. Two formulations were prepared and compositions of both prepared lipogel formulations were given in table 2.

Characterization of Lipogel

Organoleptic evaluation

In organoleptic evaluation, physical appearance, odor, color, of lipogels were checked.

Spreadability

For spreadability determination, the lipogel is spread in a circle of 1 cm diameter on a glass, place the second glass slide over the first glass slide. Place the 500-gram weight of the upper slide for 5 minutes and check the diameter (Bachhav and Patravale, 2009).

pH determination

For pH determination, accurately weighed 50 grams of each lipogel and place them in a beaker and measure pH by placing the cathode of the digital pH meter. The pH of the topical lipogel formulations should be varied between 4,5 to 6.5 to treat skin infections (Basha *et al*., 2011).

Viscosity measurement

For viscosity measurement, the lipogel was put into the spindle of the Brookfield viscometer and rotated for the prescribed period at the temperature of 25°C. The speed of the spindle was increased slowly by keeping the temperature constant and note the readings (Rupal *et al*., 2010).

Freeze-thaw test

Freeze-thaw studies were performed to check the stability of the pharmaceutical products at temperatures 40°C to -4°C. The changes in lipogel during freeze-thaw were observed after every 24 hours for ten cycles and observe physical stability, color, pH, liquefaction of the product (Waqar *et al*., 2023).

Stability studies

Stability studies are essential to determine the physical appearance, storage conditions, and shelf life of the pharmaceutical product. The lipogel formulation was evaluated to check the stability at temperatures of 4°C, 25°C and 40°C and evaluated periodically at 0 hours, 7.

Table 1: Various trial formulations of lipogel

Formulations	Mineral oil (gram)	Span 80 (gram)	Beeswax (gram)	Olive oil (gram)	Gel formation
F1	9	0.5		0.5	No gel formation
F2	8	1		1	No gel formation
F3	8.5	0.5		1	No gel formation
F4	6	3		1	No gel formation
F5	9.5		0.5	9.5	No gel formation
F6			0.25	9.75	No gel formation
F7			0.75	9.25	No gel formation
F8			1	9	No gel formation
F9	4.75	0.25	0.25	4.75	No gel formation
F10	4.62	0.37	0.37	4.62	No gel formation
F11	4.5	0.5	0.5	4.5	No gel formation
F12	4.37	0.62	0.62	4.37	Gel formed
F13	9.5	0.25	0.25		No gel formation
F14	9.25	0.37	0.37		No gel formation
F15	9	0.5	0.5		No gel formation
F16	8	1	1		No gel formation

Table 2: Optimized lipogel formulations

Formulations	Comp ositions (%W/W)					
	Beeswax	Span 80	Mineral oil	Miconazole	DMSO	Olive oil
A1	6.2g	6.2g	43.7g	2g	-	q.s. to 100 %
A2	6.2g	6.2g	43.7g	2g	5 g	

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO

Table 3: Organoleptic evaluation of A1 and A2 lipogel formulation

Formulations	Organoleptic profile	4° C				25° C				40° C			
		0 hour	Day 7	Day 14	Day 28	0 hour	Day 7	Day 14	Day 28	0 hour	Day 7	Day 14	Day 28
A1	Color	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW
A2		LW	LW	LW	LW	LW	LW	LW	LW	LW	LW	LW	LW
A1	Odour	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL
A2		SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
A1	Feel	G	G	G	G	G	G	G	G	G	G	G	G
A2		G	G	G	G	G	G	G	G	G	G	G	G
A1	Texture	OS	OS	OS	OS	OS	OS	OS	OS	OS	OS	OS	OS
A2		OS	OS	OS	OS	OS	OS	OS	OS	OS	OS	OS	OS
A1	Stickiness	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A2		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A1	Appearance	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE
A2		SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO, PW; pale yellow, LW; Lime yellow, OL; odorless, SP; slightly pungent, G; Good, NS; Non-sticky, OS; oily and smooth, SE; Smooth and even

Table 4: Viscosity measurements of A1 and A2 lipogel formulation

Sample	Viscosity (cP)		Speed (RPM)		% Torque		Temperature (°C)		Time Interval (Sec)	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
1	6361.8	6461.8	06	06	31.2	89.0	24.8	24.8	10.2	10.2
2	4901.5	4957.5	12	12	49.0	68.5	24.8	24.8	10.1	10.1
3	2218.3	2279.3	30	30	55.5	95.0	24.8	24.8	10.0	10.0
4	1440.6	1490.6	60	60	72.0	92.7	24.7	24.7	10.1	10.1

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO

Table 5: Stability studies on A1 and A2 lipogel formulation

Formula-tion	Test	4°C				25°C				40°C			
		0 hour	Day 7	Day 14	Day 28	0 hour	Day 7	Day 14	Day 28	0 hour	Day 7	Day 14	Day 28
A1	Spreadability	6	6	5.6	5.4	6	6	5.7	5.6	6	6	6.2	6.4
A2		5.5	5.2	5.3	5.2	5.5	5.3	5.1	4.8	5.5	5.1	4.3	5.5
A1	pH determination	5.5	5.4	5.2	4.9	5.5	5.3	5.1	5.0	5.5	5.4	5.2	5.0
A2		6.1	6.1	5.8	5.8	6.1	6.0	5.8	5.8	6.1	5.9	5.9	5.9
A1	Grittiness	No	No	No	No	No	No	No	No	No	No	No	No
A2		No	No	No	No	No	No	No	No	No	No	No	No
A1	Homogeneity	No	No	No	No	No	No	No	No	No	No	No	No
A2		No	No	No	No	No	No	No	No	No	No	No	No
A1	Consistency	4.8	4.8	4.1	4.2	4.8	4.2	4.1	4.1	4.8	4.2	4.2	4.2
A2		4.8	4.6	4.6	4.6	4.8	4.7	4.7	4.7	4.8	4.7	4.7	4.7

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO

Table 6: Freeze thaw testing of A1 and A2 lipogel formulation

Formulation	Freeze-thaw	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle 10
A1	pH	5.6	5.6	5.5	5.5	5.5	5.4	5.4	5.4	5.3	5.3
A2		6.0	6.0	6.0	6.0	6.0	6.0	5.9	5.9	5.9	5.9
A1	Liquefaction	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
A2		NL	NL	NL	NL	NL	NL	NL	NL	NL	NL

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO, NL; No Liquefaction

Table 7: Drug release studies on A1 and A2 lipogel formulations

Formulation	Test	0 hour	0.5 hour	1.0 hour	1.5 hour	2.0 hour	2.5 hours	3.0 hours	3.5 hours	4.0 hours
A1	*In-vitro* drug release	0.001	0.081	0.162	0.198	0.257	0.324	0.402	0.418	0.437
A2		0.001	0.031	0.174	0.256	0.348	0.454	0.572	0.598	0.636
A1	Percentage drug release	0.137	11.141	22.283	27.235	35.350	44.566	55.295	57.496	60.110
A2		0.137	4.264	23.933	35.2132	47.867	62.448	78.679	82.255	87.482
A1	*In-vitro* permeation of drug	0.001	0.213	0.372	0.420	0.494	0.525	0.603	0.641	0.665
A2		0.002	0.174	0.207	0.438	0.617	0.894	0.959	1.036	1.113
A1	Percentage permeation	0.0761	16.222	28.332	31.987	37.623	39.984	45.925	48.819	50.647
A2		0.1523	13.252	15.765	33.358	46.991	68.088	73.0388	78.903	84.767

A1; Miconazole lipogel without DMSO, A2; Miconazole lipogel with DMSO.

Days, 14 days and 28 days for pH, texture and viscosity for 28 days (4 weeks). Indication of instability was observed and monitored (Mahajan *et al*., 2021)

Grittiness

For checking grittiness, the lipogel was evaluated microscopically to check the existence of any particulate matter under a light microscope. Topical lipogel formulation must fulfil the requirements of particulate matter and grittiness.

Skin irritation test

The skin irritation properties of prepared lipogel samples subjected to human volunteers were checked to check signs of sensitivity problem that makes the formulation unfit for topical use. The skin irritation test lipogel formulation was checked on three volunteers and observed for signs of irritation and redness (Ghanbari *et al*., 2012).

Centrifugation

Centrifugation of the lipogel was performed to check the accelerated stability. The prepared samples of lipogel were placed in the centrifugation tube and then centrifuged for 30 minutes at 3000 rpm (Basketter *et al*., 2004, Munir *et al*., 2023b).

Homogeneity

Homogeneity of lipogel was checked after settling of gel in the container and visually inspected for observing aggregation in the formulations (Mahmood *et al*., 2021).

Consistency

The consistency of the lipogel was tested by dropping a cone from a distance of 10cm and it should fall in the center of the beaker. The distance travelled by the cone from the surface of the lipogel to the inside of the lipogel and checked after 10 seconds for the measurement of consistency (Raza *et al*., 2023).

In-vitro drug release studies

This test is performed after accurately weighing 1.05 g of lipogel sample, containing 2% (w/w) miconazole, and placed in dialysis tubes, whose one end was held by a knot. After filling the tubing with lipogel samples, the other end was also secured by a knot. The tubing was tied with the paddle of dissolution medium containing a 900ml mixture of ethanol and phosphate buffer saline of 7.4 pH. The dissolution media was prepared by (50:50) ratios of ethanol and phosphate buffer saline. The working conditions must be maintained such as temperature must be 37°C and paddle must be rotated at 50 rpm for 4 hours. After every 30 minutes, withdraw the sample near the paddle and saturate it with a fresh medium. The drug release from the lipogel was measured at λmax at 272 nm using a UV-visible spectrophotometer. The experiments were carried out in triplicates (Bhattacharya *et al*., 2012).

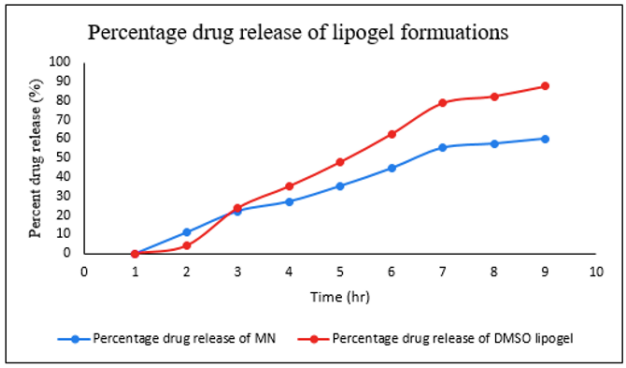

Fig. 1: Graphical representation of drug release studies of lipogel of Miconazole (without DMSO) and Miconazole (with DMSO). T-test was applied to check statistical significance, P<0.05; P<0.01

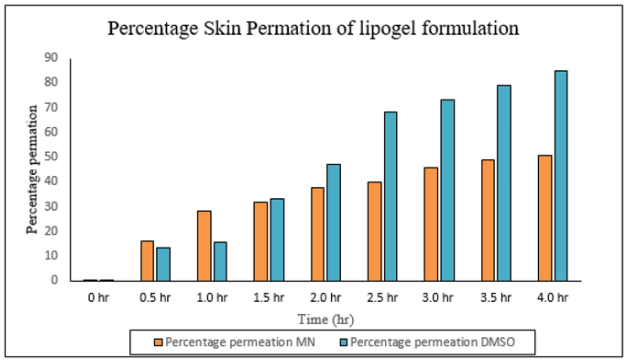

Fig. 2: Graphical representation of drug permeation studies of lipogel of Miconazole (without DMSO) and Miconazole (with DMSO). T-test was applied to check statistical significance, P<0.05; P<0.01

Skin permeation studies

The permeation studies of MCZ were measured in Franz diffusion cell. Diffusion membrane was used as the model skin. The skin was soaked in saline solution or distilled water for at least 24 hours before the permeation studies. In the case of gels, 0.3g of gel was spread and mounted on the cell. An adequate amount of sample was withdrawn from the receptor compartment and the same volume of fresh solution should replace the receptor compartment to keep the volume constant. The experiment was carried out for 3 hours (Türkmen *et al*., 2023).

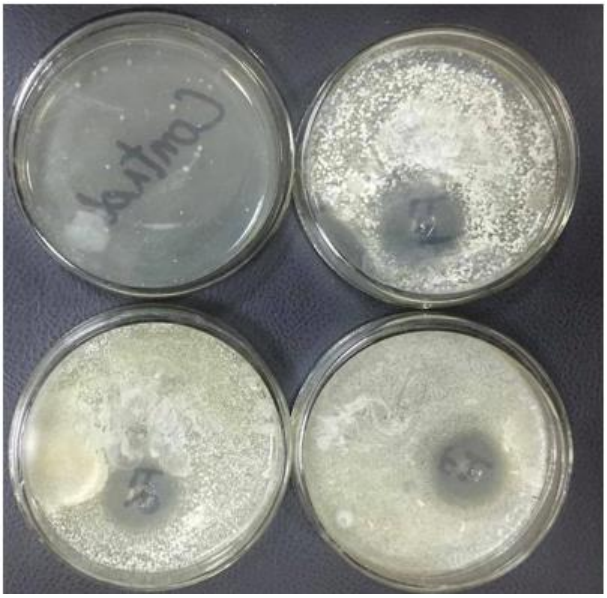

Fig. 3: Zone of inhibition of both formulations.

Antimicrobial activity of Miconazole lipogels

Sabouraud dextrose agar (65 grams) was dissolved in one litre of distilled water to prepare agar media and autoclave at 121°C for 20 minutes to sterilize the media. Candida albicans (ATCC 90028) were inoculated into sabouraud's dextrose agar plate and incubated for 48 hours before testing. After obtaining the colonies, a single colony was picked with the help of a wire loop from the medium and inoculated in the sterilized normal saline. The seeded agar medium was poured into the Petri-dishes having a diameter of 15 cm and depth of 4mm. Wells was cutted from the petri-dish with the help of a Wassermann tube having 6mm diameter. 0.4 grams of lipogels and standard formulation of miconazole was poured in the well and Petri-dishes were incubated at 25°C. Effectiveness was checked by measuring the zone of inhibition of the formulation after 3-4 days of incubation (Khalifa, 2015). The zone of inhibition created by the antifungal activity of the formulations was determined by measuring the diameter of the zone of inhibition and recorded with an accuracy of 0.1 mm (Goyal and Qureshi, 2019).

FTIR Studies
The FTIR spectra were obtained by using a Bruker FT-IR spectrophotometer with a wavelength range between 650 to 4000 cm^{-1} (Concin *et al*., 2011).

STATISTICAL ANALYSIS

Statistical analysis of One-way Analysis of Variances (ANOVA) was employed for the evaluation of the release data. Level of confidence interval was set to 95%.

RESULTS

In this study, two lipogel formulations containing miconazole were prepared with the aim to improve permeation of miconazole at the site of action with DMSO and compare permeation, *In-vitro* profile, organoleptic and surface morphology properties of both lipogel formulations.

FTIR Studies
The use of Fourier transforms infrared studies (FTIR) was performed to characterize the presence of particular chemical groups in the materials. FTIR also assists in the identification and assaying the purity of the sample materials used during formulation development. The lipogel sample of 1-2 mm thick films was analysed on FTIR by using absorbance and transmittance mode. The polymer and drug were compatible with each other and the same pattern was observed in the FTIR study of miconazole lipogel as neither emulsifier, nor gelling agent change the peak. The spectra were represented in fig. 4.

Organoleptic evaluation
The characteristics of lipogels for organoleptic evaluations such as color, odor, feel, etc. were studied at 4°C, 25°C & 40°C as physical appearance is very important for pharmaceutical formulation. Especially texture, color, and ease of application. These parameters are usually evaluated during stability studies. Lipogel formulations A1 and A2 were evaluated for organoleptic properties like feel, color and odor. The color of the A1 formulation is pale yellow whereas lime-yellow is the color of A2 lipogel. This is due to the presence of olive oil as a vehicle used in lipogel preparation. No change of color was noted after storing at different conditions for 4 to 12 weeks. A1 lipogel is odorless whereas the odor of A2 formulation is slightly pungent after 4 weeks of storage and which it might be due to the presence of DMSO having a little bit of pungency odor. Lipogel formulations A1 and A2 were difficult to wash because they contain oil phase and were difficult to wash with water. The feel of both formulations is good and the texture was smooth and oily. Lipogels show good patient compliance as they are non-sticky having no grittiness.

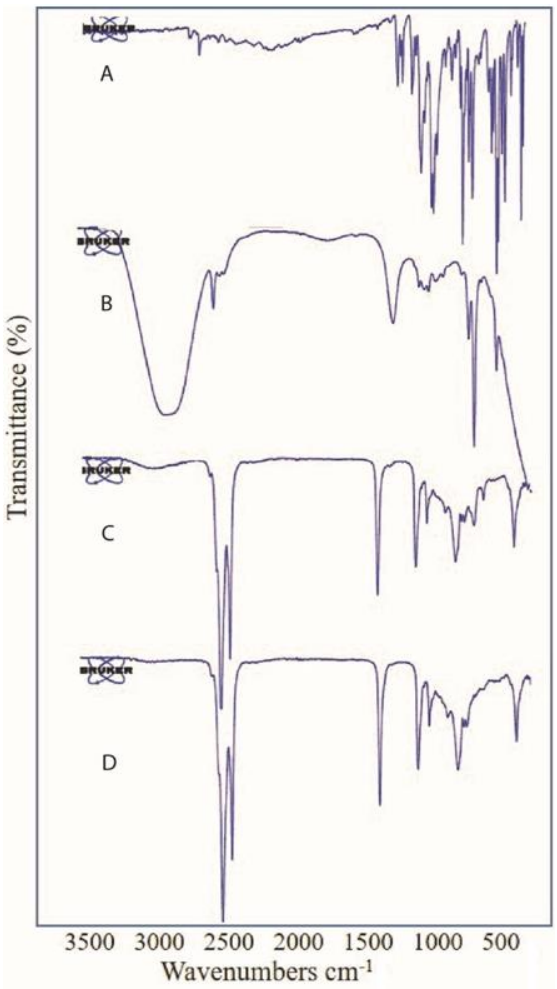

Fig. 4: FT-IR Spectra of Miconazole Nitrate (A), Miconazole ethosomes (B), Miconazole with DMSO (C), and Miconazole without DMSO (D).

Viscosity measurement
The viscosity of lipogels was checked by Brookfield viscometer after placing it in the spindle and rotate it. The viscosity values were noted at each speed while with the increase in speed viscosity of the lipogel formulations decrease down. It shows the normal behavior of semi-solid formulation subjected to high speed or shear. The temperature remains constant while measuring the viscosity of both lipogel formulations. In a previous study, changing the concentrations of gelling agents in Cutina lipogels almost the same behavior was observed (Vlaia *et al*., 2021). The rheological behavior of spermicidal gels having derivatives of polyacrylic acid and carboxymethylcellulose shows that there is a change in the viscosity because of polyacrylic acid and due to vaginal secretions (Hussein *et al*., 2020).

Spreadibility
The spreadibility of gels is essential to check the behavior of lipogel while coming out of the tube and to check how easily it will spread on the skin. Spreadibility is vital for

determining the gel behavior on the skin. The spreadibility values of lipogel formulations were checked at temperatures of 4°C, 25°C, and 40°C by placing 0.5g of gel on a glass slide, the values range from 4.5cm to 6.1 cm. The spreadibility of lipogel prepared from natural oils (olive oil and mineral oil) was in the range of 4.3 to 6.4 cm/sec, an increase in spreadibility might be because only the lipid phase was used. The results of the spreadibility of lipogels are tabulated in table 5.

Surface pH determination

The pH of the lipogels formulation was checked at different storage conditions for 4 weeks (28 days) and is in the range of pH required for skin preparations. The pH of lipogel was come out to be in the range of lipogel pH that is from 5.0 to 5.7. Also, the emulsifier used in the hydrogel was glyceryl monooleate having acidic nature. Lipogel formulations prepared for antifungal activity lie in an acidic range that's why they are appropriate for skin application.

Grittiness

The microscopic evaluation of lipogels was performed to check the presence of any particles in the formulations. No sign of grittiness on both the formulations at 4°C, 25°C, and 40°C was observed. Lipogel formulations were evaluated macroscopically and microscopically to check the presence of particulate matter. Formulations were stored at temperatures 4°C, 25°C and 40°C and also evaluated by pressing lipogel between thumb and index fig.

Homogeneity

The aggregates and lumps in the lipogels were evaluated visually after placing the lipogels in a container. The homogeneity of both formulations was checked at 4°C, 25°C and 40°C for 28 days with a one-week interval. The stored formulations at various conditions remain stable and homogenous after storing at variable conditions and even after undergoing centrifugation, freeze-thaw cycle, and stability periods. In homogeneity evaluation, no aggregates or lumps were seen in the formulations.

Consistency

The consistency of the lipogel was checked after a cone was dropped from a fixed distance of 10 cm with a rod. The distance traveled by the cone was measured after 10 seconds and values of consistency at 4°C, 25°C, and 40°C for 4 weeks were measured. The range of consistency of both formulations lies within 4.0 to 4.8, shows acceptable consistency as a topical application.

Freeze-thaw test

The prepared lipogel was evaluated using freeze-thaw test by keeping the lipogels in the refrigerator at -4°C for 6 hours and then thawing at 40°C by keeping lipogels in the oven for 18 hours. After the completion of each cycle, results were notes and collected to measure pH and liquefaction. No signs of liquefaction were observed after each cycle of the freeze-thaw test (table 6).

In a study, polyvinyl alcohol gels were undergoing repeated cycles of freezing for 8 hours at -20°C then thawing for 4 hours at 25°C shows stability and crystalline structure. The high lamellar crystals were observed with swelling of gel (Pasquali *et al*., 2010). The pH value of both formulations was in the acceptable range for transdermal drug delivery which is 4 to 6 pH. No change of color and no liquefaction means formulations are stable at hot and cold conditions. The pH values and appearance before and after freezing-thawing was not changed indicating an appropriate approach for topical application.

Stability studies

Stability studies are very important for any pharmaceutical formulation. The stability of both lipogel formulations (A1) and (A2) containing miconazole as an anti-fungal drug were checked by keeping the lipogels at 4°C, 25°C and 40°C under controlled humidity conditions (60% RH ±5%). The formulations were kept for a period of 4 to 12 weeks. The parameters during stability study were checked including physical appearance, pH, and odor for fresh samples of lipogel, at 7 days, 14 days and on 28th day.

Lipogel prepared from mineral oil, isopropyl myristate, cetyl isononanoate gelifyes, and dicaprylyl carbonate remains stable under environmental conditions for determining parameters like color, odor, appearance, and pH, etc. Whereas lipogel made from propylheptyl caprylate and decyl oleate gelifyes with hydrogenated castor oil and 12 hydroxystearic acid shows signs of instability under storage conditions (Langasco *et al*., 2016). Lipogel of isopropyl myristate and mineral oil also remains stable due to the absence of aqueous phase whereas in lipogel of propylheptyl caprylate the instability might occur due to the presence of hydrogenated castor oil which has a fatty odor and remain unstable during stability studies of the lipogel formulation. There is no effect of temperature on the color of both lipogel formulations as they remain in their original colors (pale yellow and lime yellow). The pH of both lipogel formulations lies in the normal pH range of skin 4 to 6 which helps inhibit the growth of microorganisms. Both formulations remain stable over 12 weeks and show no signs of instability.

Centrifugation

The centrifugation test was performed on both lipogel formulations stored at 4°C, 25°C, and 40°C to check the appearance, stability, and any signs of creaming or phase separation. The lipogels were centrifuged for 30 minutes at 25°C at 3000 rpm. The appearance of formulations was

observed before and after the centrifugation. Both lipogel formulations remain stable under high-speed centrifugation and do not show any signs of instability. No liquefaction in A1 and A2 formulations were observed. Lipogel formulations remain homogenous and physical appearance was also acceptable.

In-vitro drug release studies

The *in-vitro* drug release of lipogel formulations was carried out in dissolution apparatus type 2 by filling the lipogel formulation in a dialysis tube and drug concentration was measured at time intervals (0 hours, 0.5 hours, 1 hour, 1.5hours, 2 hours, 2.5hours, 3hours, 3.5 hours and 4hours) and absorbance was measured by using UV spectrophotometer at 272nm (table 7).

The formulations containing polyvinyl alcohol shows 21%, 44.8%, 9.8% and 15.9% miconazole release. The effect of gelling agents decreasing the drug release in lipogel formulation A1 which contains a slightly higher amount of beeswax and span 80. It is also possible that the percentage of drug release from lipogels shows a decrease by increasing the amount of gelling agents. So, drug release values from the formulations containing a high concentration of lipid phase and gelling agents usually lower as compared to a formulation containing lesser oil phase and gelling agents.

Skin permeation studies

The *in-vitro* permeation of both lipogel formulations was studied with the help of Franz diffusion cell under experimental conditions with continuous water flow and samples were taken after every 30 minutes till four hours with UV-spectrophotometer. The permeation behaviour of formulation A2 shows greater permeation from silicon membrane due to the presence of 5% DMSO as a permeation enhancer, whereas permeation from lipogel A1 is slightly less than A2 (fig. 2).

Skin irritation test

Skin irritation was checked on selected volunteers to observe any signs of allergy or irritation or redness after applying 1g of lipogel on the skin near wrist over 2 square inch area, no signs of redness or irritation were observed. It means lipogel formulations A1 and A2 do not cause any irritation and fulfil the requirements of patient compliance.

Antimicrobial activity of Miconazole lipogels

Antimicrobial activity (antifungal property) of lipogel formulations A1 and A2 was checked against candida species by placing the formulations in wells made in Petri-dishes and zones of inhibition were measured against the standard miconazole cream with the same concentration of miconazole. Control shows no growth in the petri-dish. Zone of inhibitions of lipogel formulations A1 and A2 were measured to be 2.6 mm for each lipogel formulations and the diameter of the standard was 2.7 mm. Minimum inhibitory concentration, minimum bactericidal concentration and bacteriostatic concentration were found to be 25mg/ml, 30mg/ml and 50mg/ml, that relates with the previously conducted study (Anumudu *et al*., 2019). There is no notable difference in the antifungal activity of A1 and A2 lipogel formulations than standard miconazole cream.

DISCUSSION

Various attempts have been made to increase the poor skin permeability of the miconazole. Some of the approaches are complexation of miconazole with cyclodextrins, a submicron emulsion of miconazole, addition of permeation enhancers in the formulation, preparation of liposomes for topical delivery of miconazole, and chewing gum containing miconazole for buccal delivery (Das *et al*., 2022).

In this study, we aimed to formulate a lipogel containing miconazole as an active ingredient to treat superficial fungal infections such as tinea pedis, tinea corporis, and tinea capitis. Numerous trial formulations from F1 to F16 have been conducted to check the adequate gelation. The FTIR spectra of both formulation and standard was presented in fig. 4. All the spectra were found in the range of 400 to 4000 cm^{-1}. According to a study, the FTIR spectrum of miconazole was observed in between 400 to 4000 cm^{-1} (Parashar *et al*., 2013). During analysis, no change in peaks was observed in the miconazole nitrate sample even when used in combination with polymers like carbapol 940.

The gel properties such as viscosity might be changed due to the effect of temperature. Skin application of lipogel does not show a definite change in viscosity but the vaginal application might show some change due to the presence of different fluids. The application of lipogel, change of temperature can lead to an increase in viscosity like from room temperature to body temperature during the initial spreading of lipogel on skin. The viscosity profile of the formulation was presented in table 4.

The spreadibility of lipogel formulations shows a different pattern where formulations A1 and A2 show a decrease in spreadibility when stored at 4°C for a period of 4 to 12 weeks. This decrease might happen due to cold temperatures that limit the spreadibility of lipogels. At 25°C both formulations show a slight increase in spreadibility due to an increase in temperature. The spreadibility of different topical gel formulations of diclofenac sodium prepared from HPMC, Carbapol 934P, and Sodium alginate was measured as 5.6, 3.8 and 3.9 cm/sec, and the spreadibility was found to be good for topical application (Khalifa, 2015).

Both lipogel formulations are stable over 12 weeks at different temperatures while pH of A1 and A2 formulations decreased from 5.5 to 5.0 and 6.0 to 5.9, respectively were found to be in the range of 5.0 to 5.7, as there is no aqueous phase present in the lipogel due to which its pH lies in the lower range as compared to the hydrogels of miconazole. Grittiness can also be observed by pressing the formulation between the thumb and index finger. It can also be detected by applying lipogel on the skin of the hand to confirm the absence of any particulate matter (KUMAR, 2020).

No signs of grittiness were observed in both A1 and A2 formulations which show that they are free from grittiness when stored for longer periods. It means both formulations fulfill the requirements of patient compliance. Under a light microscope and by visual inspection no particulate matter was seen in the miconazole lipogel which fulfills the criteria of lipogel to be fit to use as a topical preparation (Antimisiaris *et al*., 2021). Miconazole solubilize completely in both lipogel formulations and no excipient causes any aggregation that could lead to grittiness. Lipogel formulations can remain on the skin for a significant period to show better results. A study reported consistency of diclofenac sodium gel prepared from high molecular weight Carbapol 934P, Sodium alginate, and Hydroxypropyl methylcellulose (HPMC) found to be good as they lie in the range of 4 to 4.5. The consistency of gel shows its ability of ejection in uniform and desired quantity by squeezing the tube (Khalifa, 2015).

The gels of sodium alginate and tween 80 do not have any lumps, transparent and clear whereas homogeneity of gels does not affect by water content and humidity (Hassan and Peppas, 2000). The only difference is that lipogels of miconazole were yellow and were turbid while the gel of sodium alginate was transparent. No aqueous phase is used in the preparation of miconazole lipogels, that's why no change in the homogeneity was observed, and by keeping the formulation in a controlled environment, humidity also does not affect the homogeneity of lipogels. To enhance the homogeneity of the gel, a controlled radical polymerization technique is used including atom transfer radical polymerization. In a study, centrifugation of salicylic acid-loaded lipogel was carried out after 24 hours of preparation and few signs of liquefaction or cracking were observed, especially in lipogels having a lower amount of precipitates than hydrogels. Lipogels of salicylic acids remain stable after centrifugation for 3 months at a temperature of 2-5°C and no breaking and precipitation were observed after centrifugation (Fujii *et al*., 2002). In our study, no signs of instability were observed under centrifugation of lipogels of miconazole A1 and A2 formulation.

The drug release from both lipogel formulations increases with an increase in time. The percentage of drug release from lipogel formulation A2 is better than the lipogel formulation A1 that might be due to less concentration of vehicle (olive oil). The initial absorbance of both formulations is almost the same, but formulation A2 shows a dramatic increase. The maximum percentage drug release concentration from lipogel A1 is 60% whereas the maximum percentage of drug release concentration from lipogel A2 is 87%. The drug release of both formulations was presented in fig. 1.

The *in-vitro* drug release profile of lipogel containing carbopol 934, sodium hydroxide and propylene glycol can be affected by a change in the concentration of gelling agents across the cellulose membrane. The drug release from gel formulations was found to be low in which the concentration of polymers was high (Curran *et al*., 1998). Miconazole from hydrogel shows very slow release, just only 6.8% after 7 hours.

Permeation enhancers have been utilized in combination with various vehicles but their effects can be decreased. DMSO increases the permeation of drugs by altering the skin structures and thus it increases the permeation of miconazole lipogel. Miconazole, known as a lipophilic drug, have increased solubility in the presence of DMSO and thus, this enhanced solubility can facilitate the drug's penetration into biological membranes (Jadhav *et al*., 2019, Otterbach and Lamprecht, 2021). The percentage permeation from lipogel A2 is 84% whereas the percentage permeation from lipogel A1 is 50%.

The antifungal activity of clotrimazole gel was measured against candida species at different pH conditions where zones of inhibition were measured at different pH which shows that the best antifungal activity was measured at pH 4. As with the increase in the pH antifungal activity of clotrimazole was decreased (Curran *et al*., 1998). In our study, the antifungal activity of miconazole was independent of pH as lipogel was a simple formulation prepared from natural oils, and change in pH of clotrimazole gel was due to its preparation in mucoadhesive thermosensitive gel system in which concentration of excipients alters the pH of the gels and reduce antifungal activity (fig. 3).

CONCLUSION

The prepared lipogels show acceptable behaviours over the period and rheological characteristics shown by lipogel A1 and A2 were suitable for the delivery of active ingredients. The formulations A1 and A2 show homogenous behaviour, non-grittiness and non-newtonian pseudo-plastic behaviour. pH and viscosity values lies within the normal ranges. FTIR has also confirmed the presence of functional groups in the formulations. In

Freeze-thaw studies, formulations are stable in hot and cold conditions while stability studies of lipogel formulations till 12 weeks show acceptable results with no indication of instability. Olive oil is a natural permeation enhancer that increases the permeation of miconazole but DMSO it shows a further increase in drug permeation. The results of lipogel A2 show remarkable increases in drug release (miconazole) from lipogel with directly enhancing the permeation of miconazole as compared to formulation A1. However, further formulation studies should be performed by changing the vehicles, gelling agents, or by permeation enhancers, because the increasing concentration of DMSO causes carcinogenic effects.

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