



Microemulsion Based Gel of Sulconazole Nitrate for Topical Application

Topikal Uygulama İçin Mikroemülsiyon Esaslı Sulkonazol Nitrat Jeli

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ABSTRACT

Objectives: Sulconazole is a broad spectrum antifungal agent of the imidazole class used against dermatophytes and other fungi to treat skin infections. The aim of the present work was to formulate and evaluate a microemulsion-based topical sulconazole gel.

Materials and Methods: Microemulsion formulation of sulconazole nitrate was prepared by using oil, surfactant, cosurfactant and water at different ratios. This was then subjected to clarity and particle size analysis, a centrifugation test, a dilution test, and freeze thawing.

Results: The zeta potential of formulation F1 was -41.3 and stable. The pH of the microemulsion formulation was within the range of pH of skin. F1 showed a higher percentage amount of drug as compared with the other formulations. The viscosity showed that F1 was optimum. The freezing and thawing results showed there was no phase separation and the formulation was stable. *In vitro* drug release showed that the drug release from the microemulsion of F1 was higher when compared to the other formulations. It revealed F1 had the highest drug content of 95.88±0.3% and % cumulative drug release was 88.75% release in 8 h. The *in vivo* skin irritation study on rats confirmed that formulation was nontoxic and nonirritant.

Conclusion: The present study confirmed the safety of the formulated sulconazole loaded microemulsion gel for topical application.

Key words: Microemulsion, sulconazole nitrate, *in vitro* release, fungal infection

ÖZ

Amaç: Sulkonazol, dermatofitler ve diğer mantarlara karşı deri enfeksiyonlarını tedavi etmek için kullanılan imidazol sınıfının geniş spektrumlu bir antifungal ajanıdır. Mevcut çalışmanın amacı, mikroemülsiyon esaslı topikal sulkonazol jeli formüle etmek ve değerlendirmektir.

Gereç ve Yöntemler: Sulkonazol nitratin mikroemülsiyon formülasyonu, farklı oranlarda yağ, yüzey aktif madde, yardımcı yüzey aktif madde ve su kullanılarak hazırlandı. Formülasyon daha sonra berraklık ve parçacık boyutu analizine, santrifüj testine, seyreltme testine ve donma çözünme testine tabi tutuldu.

Bulgular: Formülasyon F1'in zeta potansiyeli değerinin -41,3 ve kararlı olduğu tespit edildi. Mikroemülsiyon formülasyonunun pH değeri cildin pH aralığı dahilindeydi. F1 formülasyonunun, diğer formülasyonlara kıyasla daha yüksek oranda ilaç yüzdesine sahip olduğu bulundu. Viskozite sonuçları, F1'in optimum olduğunu gösterdi. Donma ve çözünme sonuçları, faz ayrılmasının olmadığını ve formülasyonun stabil olduğunu gösterdi. *In vitro* ilaç salımı çalışmaları, F1 mikroemülsiyonundan ilaç salımının, diğer formülasyonlara kıyasla daha yüksek olduğunu gösterdi. Bu çalışma ayrıca, F1'in en yüksek ilaç içeriğine sahip olduğunu (% 95,88±%0,3) ve % kümülatif ilaç salımının 8 saat içinde %88,75 olduğunu ortaya koydu. Sıçanlar üzerinde yapılan *in vivo* deri irritasyon çalışması, formülasyonun toksik ve tahriş edici olmadığını doğruladı.

Sonuç: Bu çalışma, formüle edilen sulkonazol yüklü mikroemülsiyon jelin topikal uygulama için güvenilirliğini doğrulamıştır.

Anahtar kelimeler: Mikroemülsiyon, sulkonazol nitrat, *in vitro* salım, mantar enfeksiyonu

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INTRODUCTION

Novel drug delivery systems are meant to attain and maintain sufficient therapeutic levels of drug at the target site. In order to overcome the problems connected with traditional modes of drug administration, topical drug delivery was started. For local dermatological disorders topical drug delivery is the effective way for drug administration. Drug delivery through the skin has attracted attention because it avoids the first pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration. The topical route of administration is also utilized to produce systemic drug effects in some instances. Major attention needs to be paid to new topical formulations to ensure adequate localization of drug within the skin to enhance the local effect or to increase the penetration. Drugs applied topically, mainly for local action, include antifungal, anti-inflammatory, and antiseptic agents as well as skin emollients for protective effects, while this route can also be used for systemic drug delivery.¹

Microemulsions are thermodynamically stable multicomponent fluids consisting of oil, water, and surfactant. The droplets of microemulsions are in the range of 10-100 nm in diameter. Since the dispersed particles have diameters less than one-fourth of the wavelength of visible light, they do not refract light and that is the reason microemulsions are transparent to the eye.^{2,3}

A microemulsion when applied to the skin is expected to penetrate the stratum corneum and to exist intact in the horny layer, resulting in alteration of both the lipid and the polar pathways. The lipophilic domain of the microemulsion interacts with the stratum corneum in a different way. A drug that gets dissolved in the lipid domain of the microemulsion can directly partition into lipid of the stratum corneum.⁴

Sulconazole is a broad spectrum antifungal agent of the imidazole class used against dermatophytes and other fungi to treat skin infections such as ringworm and athlete's foot. It is lipophilic in nature and can be effortlessly formulated for topical delivery by using a microemulsion as topical vehicle for an antifungal effect with several advantages such as ease of preparation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration into the biological membrane, and increased bioavailability compared to conventional dosage form likes cream. Hence, in the present study we developed a sulconazole loaded microemulsion gel for topical delivery and investigated it in terms of physicochemical characterization, drug content, *in vitro* drug release and kinetic studies, and *in vivo* skin irritation.⁵

MATERIALS AND METHODS

Materials

Sulconazole nitrate was received as a gift sample from Ranbaxy Laboratory, New Delhi, India. Olive oil, Tween 20, and PEG 400 were purchased from HiMedia, Mumbai, India. Propylene glycol and Carbopol 934 were procured from Lobachemie Pvt. Ltd,

Mumbai, India. Triethanolamine was purchased from Rankem Fine Chemicals Ltd., New Delhi, India. All the chemicals and reagents were used of analytical grade.

Methods

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was carried out to check the compatibility between the drug and polymer. Using an IR spectrophotometer (Spectrum one model, Alpha-Bruker, Germany) IR spectra of sulconazole nitrate, Carbopol 934, and physical mixture of sulconazole nitrate and Carbopol were obtained and compared to check the interactions.

Preparation of microemulsion

Sulconazole nitrate containing o/w microemulsion was formulated by water titration method involving the following steps. First, surfactant and cosurfactant were added in fixed ratios to oil followed by sulconazole nitrate. Then bath sonication was performed for 5 min followed by heating. Later the required quantity of water was added dropwise with constant stirring until the formation of a clear and transparent liquid. A series of microemulsion formulations were prepared using Tween 20 and PEG 400 as the surfactant and cosurfactant and olive oil as the oil. In all these formulations, the amount of sulconazole nitrate was kept constant (50 mg) and the amounts of surfactant, cosurfactant, oil, and cosolvent were varied (Table 1).

Characterization of sulconazole nitrate microemulsion formulations

Globule size determination

One drop of the sample was taken on a microscopic glass slide and a cover slip was placed over it and it was observed at 45x resolution under a microscope (Zeiss, Germany). The particle size was measured using BIOVIS software.⁶

Dilution test

The formulated microemulsions were diluted with distilled water to confirm the type of emulsion and miscibility with the aqueous phase.

Centrifugation test

This is used to specify the stability of the microemulsion as to whether it is monophasic or not. Samples were centrifuged using a cold centrifuge (CM8plus, Remi Lab World, Mumbai,

Table 1. Composition of different microemulsion formulations

Formulation code	Sulconazole nitrate	Olive oil (% v/v)	S:Cos ratio	Tween 20/PEG 400 (%)	Water (%)
F1	2 g	5.0	3:1	55	40.0
F2	2 g	5.0	3:1	60	35.0
F3	2 g	7.5	3:1	45	47.5
F4	2 g	7.5	3:1	50	42.5
F5	2 g	10	3:1	55	35.0
F6	2 g	10	3:1	60	30.0

India) at 10,000 rpm for 30 min and then were examined for whether the system was monophasic or biphasic.⁷

Zeta potential determination

A Malvern Zeta Sizer (Malvern Instruments, UK) was used to measure the zeta potential of the globules on the electrophoresis and electrical conductivity of the microemulsion. Measurements were performed using small volume zeta cells.⁷

Determination of pH

The pH of the microemulsion formulations was measured using a digital pH meter (Systronics 335, Ahmedabad, India).

Determination of viscosity

The viscosity of the formulated microemulsions was determined by Brookfield viscometer DV-II model using spindle number 92 at 20, 30, 50, 60, and 100 rpm.⁷

Freeze thawing

Freeze thawing was employed to evaluate the stability of the microemulsion formulations. The microemulsion pre-concentrates of various formulations were subjected to 3 to 4 freeze thaw cycles, which included freezing at -10 °C for 24 h followed by thawing at 40 °C for 24 h. The various formulations were then subjected to centrifugation at 3000 rpm for 5 min. The formulations were then visually observed for phase separation.⁸

Preparation of drug loaded microemulsion gel

The polymers Carbopol 934P and propylene glycol were used to prepare blank gel. Among them Carbopol 934P 1% gel was optimized for its transparency and its consistency for application on skin. Carbopol 934P (1%) and propylene glycol (5%) (as humectants) were added to the required quantity of water with constant stirring and left for hydration for 4-5 h. The microemulsion was in the gel phase and it was left overnight. Finally the required quantity of triethanolamine was added to adjust the pH.⁹

Evaluation of microemulsion gel

The prepared drug-containing gel formulations were evaluated for pH and drug content. For drug content determination, microemulsion gel (1 g) was weighed and dissolved in a mixture of ethanol:phosphate buffer pH 7.4 (2:3). From this, 2 mL of the solution was diluted to 50 mL with the same medium and absorbance was measured at λ_{max} 227 nm. Blank solution was prepared in the same manner by taking gel without drug. For determination of pH, 1 g of microemulsion gel was dissolved in 100 mL of distilled water and stored for 2 h and pH was measured using a digital pH meter (Systronics 335, Ahmedabad, India).¹⁰

In vitro drug release and kinetic studies

The *in vitro* drug release study of drug from formulations was carried out using an artificial semipermeable membrane (Cellophane) with a Franz diffusion cell. The receptor compartment consisted of 50 mL of ethanol:phosphate buffer pH 7.4 (2:3), temperature was maintained at 37±2 °C and it was

stirred using a magnetic stirrer. The microemulsion gel (~10 mg sulconazole nitrate) was placed on an artificial semipermeable membrane tied to one end of an open ended glass cylinder that was then dipped into the receptor medium on a magnetic stirrer. Samples were taken from the receptor medium over 12 h at different time intervals and replaced immediately with the buffer. All the samples were analyzed at 227 nm and the cumulative amount of drug release was calculated. In order to describe the kinetics of the release process of drug in the different formulations, zero order, first order, Higuchi, and Korsmeyer and Peppas models were fitted to the dissolution data of selected formulations using linear regression analysis.¹¹

In vivo skin irritation study

The skin irritation test was carried out on healthy male albino rats (200-250 g) obtained from NUCARE-Nitte (Deemed to be University) with ethical approval from the ethical committee and the document no: is NGSMIPS/IAEC/May-2016/9. The experiments were performed in accordance with the CPCSEA guidelines. The preparation was applied to the properly shaved skin of rats and secured with adhesive tape, and its adverse effects like change in color and skin morphology were checked over 72 h. Two sets of 6 rats were used for the study. Among these 6 were used as the placebo group and the other 6 as the treatment group. The animals were observed for any sign of erythema and edema over different time intervals.^{12,13}

RESULTS

Fourier transform infrared spectroscopy

The IR spectra of pure drug sulconazole nitrate (Figure 1), Carbopol 940P (Figure 2), and a physical mixture of sulconazole nitrate with Carbopol 940P (Figure 3) were obtained and interpreted for spectral data comparison for (Figure 1).

Microemulsion formulations of sulconazole nitrate were prepared by water titration. According to the definition, a microemulsion must be clear and transparent in nature. All the formulations were transparent and clear and without any precipitation, which indicated the formation of good microemulsions.

Evaluation of microemulsion of sulconazole nitrate

Globule size determination

Microemulsions are thermodynamically stable, transparent systems having particle size <100 nm. Globule size is a very important evaluation parameter because the therapeutic effectiveness of a microemulsion depends on its globule size. The formulated microemulsions showed globule size between 243.8±0.02 µm and 679.2±0.01 µm. Formulation F1, with the highest proportions of surfactant and cosurfactant with a fixed amount of oil, showed the lowest mean particle diameter, whereas formulation F6, with the highest proportion of surfactant and a fixed amount of oil, showed the highest mean particle diameter.

Dilution test

The dilution test is based on the fact that an emulsion is only miscible with the liquid that forms the continuous phase.

Centrifugation test

The data for the formulations for stability for monophasic nature are shown in Table 2.

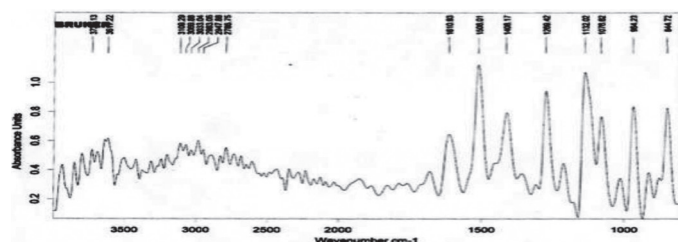


Figure 1. IR spectra of pure sulconazole nitrate

IR: Infrared spectroscopy

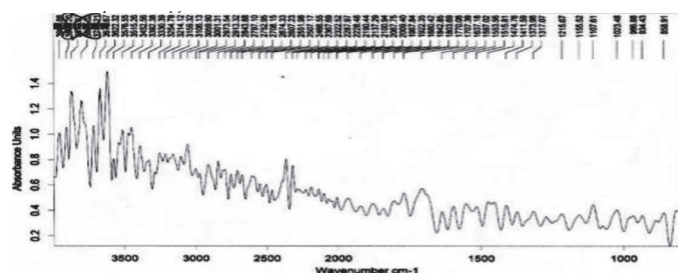


Figure 2. IR spectra of Carbopol 940P

IR: Infrared spectroscopy

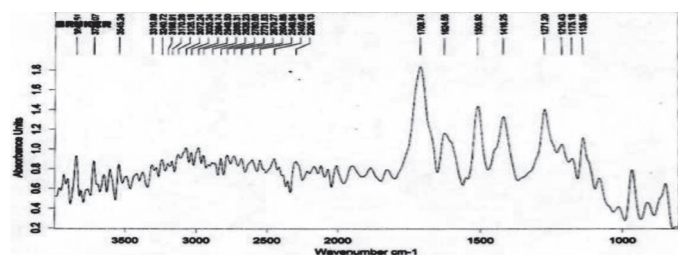


Figure 3. IR spectra of a physical mixture of sulconazole nitrate and Carbopol 934P

IR: Infrared spectroscopy

Table 2. Evaluation of the centrifugation test

Formulation code	Centrifugation
F1	Monophasic, stable
F2	Not stable
F3	Monophasic, stable
F4	Monophasic, stable
F5	Not stable
F6	Not stable

Table 3. Results of freeze thawing test

Formulation code	Freeze thawing
F1	Stable and no separation
F3	Stable and no separation
F4	Stable and no separation

Freeze thawing test

This test checks the stability and phase separation of formulations if found (Table 3).

Determination of zeta potential

The zeta potential value depends on the type and composition of the carrier used in the formulation. The zeta potential of F1 was -33.5, which indicates good stability (Table 4).

Determination of pH

F1, F3, and F4 were in the pH range of 6.32-7.0, which is within the specified range for topical formulations (Table 4).

Drug content

The percentage drug content of F1, F3, and F4 was 93.05%-95.88%. This shows that the drug is uniformly distributed throughout the microemulsion. The maximum drug content was obtained for F1 (Table 4).

Table 4. Zeta potential, pH, and drug content of F1, F3, and F4

Formulation code	Zeta potential (mV)	pH	Drug content (%)
F1	-33.5±2.3	7.0±0.01	95.88±0.3%
F3	-41.2±3.4	6.32±0.03	91.42±0.2%
F4	-38.3±2.7	6.89±0.02	93.05±0.4%

*Mean ± SD (n=3), SD: Standard deviation

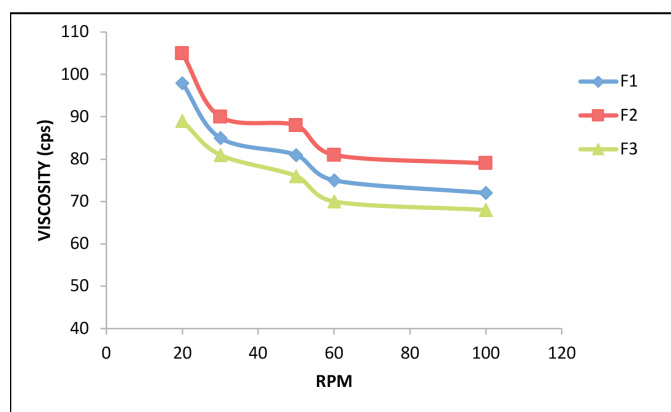


Figure 4. Rheology of sulconazole microemulsions for F1, F3, and F4

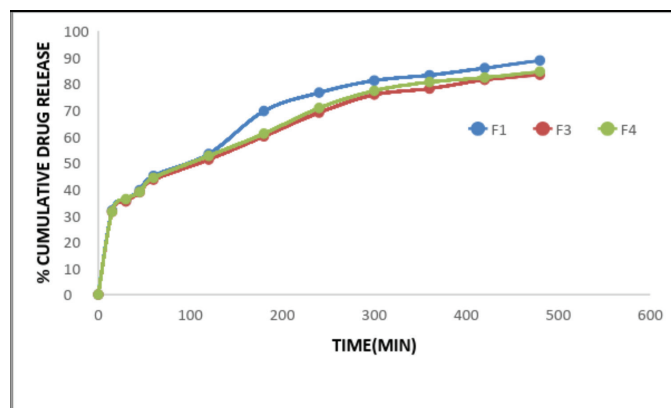


Figure 5. In vitro drug release profile of F1, F3, and F4 microemulsion gel formulations

Determination of viscosity

The viscosity of F1, F2, and F3 microemulsion formulations at 20, 30, 50, 60, and 100 rpm was measured using spindle number 92 at room temperature. The viscosity curve was plotted by taking shear rate (rpm) on the x-axis and viscosity on the y-axis (Figure 4) with the data obtained (Table 5).

Preparation of drug loaded microemulsion gel

Sulconazole nitrate microemulsion (F1, F3, and F4) containing Carbopol appeared to be transparent, clear, and smooth textured. These drug-containing gel formulations were further evaluated for pH and drug content.

Evaluation of microemulsion gel

Drug content values were in the range of 91.5-95.5% and pH ranged between 7.2 and 7.8 (Table 6).

In vitro drug release and kinetic studies

Three microemulsion gel formulations (F1, F3, and F4) were selected for the *in vitro* drug release study. The % cumulative drug release was 83.32-88.75 at the end of 8 h (Figure 5). The mechanism of drug release was calculated by fitting the dissolution data to different models like Higuchi's and Korsmeyer-Peppas. The best model was selected based on the highest regression value (Figure 6).

In vivo skin irritation study

The skin irritation study was carried out on six rats, of which three were treated with placebo and three with F1. They were observed for 72 h to check for signs of erythema and edema on the skin surface (Figure 7).

DISCUSSION

From the FTIR study, the peaks that appeared for the physical mixture indicated that the drug and the gelling agents are compatible with each other.

Table 5. Viscosity of F1, F2, and F3 at different rpm

rpm	Viscosity (cps)*		
	F1	F2	F3
20	98±0.01	105±0.01	89±0.01
30	85±0.07	90±0.01	81±0.01
50	81±0.02	88±0.02	76±0.03
60	75±0.03	81±0.03	70±0.01
100	72±0.02	79±0.01	68±0.01

*Values are mean ± SD (n=3), SD: Standard deviation

Table 6. Drug content and pH of F1 microemulsion

Test	Drug loaded microemulsion gel		
	F1	F3	F4
Drug content	95.5±0.34%	91.5±0.26%	92.5±0.36%
pH	7.21±0.01	7.72 ±0.02	7.81 ±0.02

*Values are mean ± SD (n=3), SD: Standard deviation

From the results of globe size analysis, it was observed that an increase in the ratio of the oil phase resulted in an increase in the particle size because of the decrease in the surfactant/cosurfactant proportion. An increase in the surfactant/cosurfactant ratio and decrease in oil ratio led to a decrease in mean particle size.

The dilution test showed that after dilution of microemulsion with water the microemulsions remained clear, indicating the good miscibility of microemulsions with water, which was used as the continuous phase.

From the centrifugation test, it was observed that F1, F3 and F4 were stable and monophasic liquids and they were further evaluated for pH, drug content, and zeta potential.

The freeze thawing results also confirmed that all three formulations were stable and there was no phase separation.

The zeta potential of formulation F1 was found to be good and was optimized as the best formulation. The pH of the formulated microemulsions was within the specified skin range.

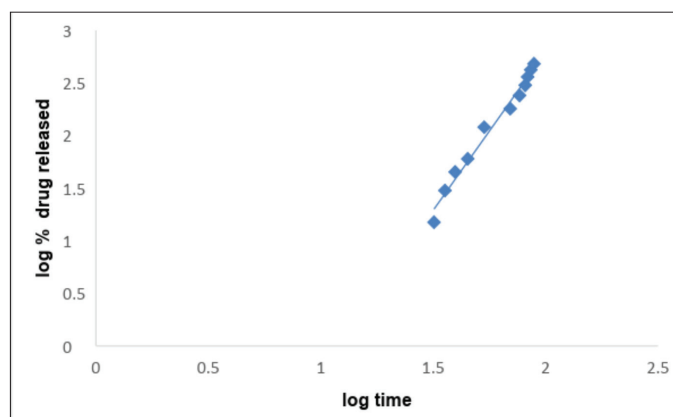


Figure 6. Korsmeyer-Peppas model for F1 microemulsion

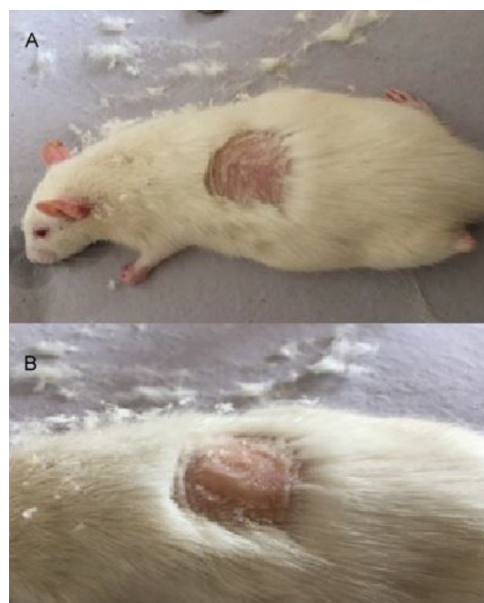


Figure 7. In vivo skin irritation test for F1 microemulsion: (A) before application, (B) after application

From the viscosity study, all three formulations showed pseudoplastic non-newtonian flow and viscosity values decreased as the shear rates increased.

From the drug release data F1 was found to show a higher percentage of drug release in comparison to F3 and F4. This may be because spontaneous formation of microemulsion with small particle size permitted a faster rate of drug release. Thus greater permeability of the dissolved sulconazole nitrate from the microemulsion gel formulation, which can lead to higher absorption through the skin, can be expected.

F1 followed the Korsmeyer-Peppas model since the regression coefficient value was higher.

The *in vivo* skin irritation study showed that there was no sign of erythema or edema after 72 h of application of the gel and F1 was found to be safe, nontoxic, and nonirritant for application on the skin.

CONCLUSION

The microemulsion based gel of sulconazole nitrate was successfully formulated for topical delivery to treat fungal infections. The formulated gel possessed good physicochemical properties, high drug content, and sustained drug release. It was also confirmed that the formulated gel is safer for topical delivery by the *in vivo* studies. Based on these results, it can be concluded that a microemulsion based gel of sulconazole nitrate is promising for topical delivery against fungal infections.

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