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Research Article

MUPIROCIN LOADED TRANSFERSOMAL GEL FOR TOPICAL DELIVERY

M. Arpitha and Rajeshri Dhurke*

Department of Pharmaceutics, St.Peter's Institute of Pharmaceutical Sciences, Hanamkonda, 506001, Telangana State, India

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ABSTRACT

The objective of the present study was to prepare and evaluate mupirocin loaded transfersomal gel (MTG) with an aim to improve drug retention in the skin for effective treatment of impetigo. Mupirocin loaded transfersomes were prepared by thin film hydration technique using a rotary evaporator and then incorporated in 3% w/v carbopol 934 gel bases. The prepared transfersomes were characterized for the parameters like vesicle size, shape, drug content, entrapment efficiency, and stability. MTG was characterized for spreadability, antimicrobial activity, *in vitro and ex vivo* diffusion studies and skin retention study. Transfersomes obtained were uniform, spherical in shape with the vesicle size range of 2 to 4 µm. The drug content was found to be 90% with entrapment efficiency 92-96%. *In vitro* drug diffusion for F1 formulation was found to be 90% in 7 h when compared to other formulations and followed Korsmeyer Peppas model kinetics. Almost 99 % of the drug was diffused from F1 Formulation in 6h as compared to a marketed product which released only 67% as evident from e*x vivo* diffusion studies. Optimized formulation (F1) has the highest zone of inhibition of 3.5 cm compared to marketed formulation with only 1.5 cm. From the above results, it can be concluded that MGT can prove to be a better option for topical delivery of mupirocin for effective treatment of skin diseases.

INTRODUCTION

In the last few years, the vesicular systems have been promoted as a means of sustained or controlled release of drugs. These vesicles are preferred over other formulations because of their specific characteristics such as lack of toxicity, biodegradation, the capacity of encapsulating both hydrophilic and lipophilic molecules (Kalpesh, *et al*, 2014). Transfersomes are ultra-deformable vesicles which are highly adaptable and complex aggregates possessing an aqueous core surrounded by complex lipid bilayer (Gordon LF, 1993). It is an artificial vesicle designed to exhibit the characteristics of a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potential targeted drug delivery (Fahmy, *et al,* 2005). Transfersomes penetrate through stratum corneum either by intracellular or transcellular pathways. The flexibility of transfersomes decreases the risk of vesicle rupture in the skin and allows following natural water gradient across the epidermis when applied. It protects the encapsulated drug from metabolic degradation and it can be used for both systemic and topical delivery of the drug.

Mupirocin is an antibiotic isolated from Pseudomonas fluorescens and is structurally unrelated to any other antibiotics. It is a medium potency, synthetic, non-fluorinated antibiotic used topically and primarily effective against gram-positive bacteria. It is bacteriostatic at low concentrations and bactericidal at high concentrations. It can be used for the treatment of Furuncles, Impetigo and open wounds (Ward, *et al*, 1986).

Impetigo is a primary superficial bacterial skin infection, initially vesicular or bullous and later crusted. It is caused by Staphylococcus aureus, Streptococcus pyrogens or both. Impetigo is of two types i.e., Non-bullous Impetigo and Bullous Impetigo (Luciana, 2014). A Furuncle can have one or more openings onto the skin and may occur within a hair follicle. The term carbuncle is typically used to represent a larger abscess that involves a group of hair follicles and larger area than a furuncle (Ioannides, 2003). The condition of having chronic, the recurring boil is referred to as furunculosis. An Open wound is an injury involving an external or internal break in body tissue, usually involving the skin (Julie R and Valencia H, 2018) The main aim of the present study was to prepare and

Corresponding author:* **Rajeshri Dhurke
Department of Pharmaceutics, St.Peter's Institute of Pharmaceutical Sciences, Hanamkonda, 506001, Telangana State, India

evaluate mupirocin loaded transfersomal gel as a topical formulation so as to enhance the permeability and drug retention in the layers of the drug.

MATERIALS AND METHODS

Materials: Mupirocin was procured as a gift sample from Cipla Ltd (Mumbai, India). Carbopol 934 was a kind gift from Lubrizol Advanced Materials India Pvt. Ltd, Phospholipon 90H was obtained as a gift sample from Lipoid GmbH, Germany. Span 60, triethanolamine were purchased from Merck, India. Chloroform, Methanol was purchased from S.D. Fine-Chem Ltd, Mumbai, India. All other reagents used were of analytical grade, double distilled water was used throughout the studies.

Methods: Mupirocin transfersomes were prepared by thin film hydration technique (Zhang, 2017) using Flash rotary evaporator (Heidolph, Germany). The lipid mixture consisting of phospholipon 90 H, edge activator (surfactant) and drug in different ratios were dissolved in an organic solvent mixture and evaporated by rotary evaporator under reduced pressure above lipid transition temperature to form a thin lipid film on the wall of the flask. The dried film deposited on the flask was hydrated with 6.4 pH phosphate buffer. The developed transfersomal gel was prepared by dispersing drug loaded transfersomes into 3% w/v of Carbopol 934 gel base using triethanolamine as a neutralizing agent. The compositions of transfersomal formulation are given in table 1.

Table 1 Composition of transfersomal formulation

Formulation	Mupirocin (mg)	Phospholipon 90 H (µM)	Span 60 (µM)	Chloroform: Methanol(ml)
F1	10	137.2	43.06	10
F2	10	196	75.36	10
F3	10	156.8	64.59	10
F4	10	133.28	77.51	10
F5	10	125.44	81.82	10
F6	10	235.2	21.53	10

Characterization of Transfersomal Formulation

Vesicle size and Shape Determination: The developed mupirocin loaded transfersomes was observed through an eyepiece of the optical microscope at 45X magnification and size of transfersomes were determined by using a calibration factor (C.F) (Zernike, 1942)

C.F=Stage micrometer reading / Eyepiece micrometer reading x 10

For determination of shape of the vesicles, developed formulations were observed through phase contrast microscope (HL-23, Coslab) under 45X magnification and magnified images of transfersomes were captured (Gupta, *et al,* 2012).

Determination of Zeta potential: Polydispersity index and zeta potential was measured for optimized formulation using Zeta sizer Nano ZS (Malvern Instruments Ltd, UK). The samples were diluted to a suitable concentration with filtered double distilled water. Size analysis was performed at 25 °C with an angle of detection of 90 °C. Size, polydispersity index was obtained directly from the instrument.

Drug content: The amount of drug released was analyzed by using a UV-Visible spectrophotometer (Spectro UV 2080, Double Beam Analytical Technologies, India) at 224 nm. The prepared transfersomal formulations were taken in a test tube and to this 10 ml of methanol were added to rupture the drug-loaded transfersomes to release the entrapped drug (Gupta, *et al,* 2012).

Entrapment Efficiency: Aliquots of transfersomal formulations were subjected to centrifugation using a cooling centrifuge (Remi Equipments, Mumbai, India) at 3000 rpm for about 30 min (Dhurke, *et al,* 2016). Supernatant phase was then separated carefully to determine unentrapped drug and analyzed by using UV/ Visible spectrophotometer at 224 nm. The percent drug entrapment was calculated using the formula

% Entrapment= amount of drug in sediment/ amount of drug added x 100

Characterization of Drug Loaded gel

The prepared gels were visually inspected for colour, phase separation and consistency. The pH of the gel was measured on digital pH meter (Systronic equipment Pvt. Ltd Ahmedabad, India) at ambient temperature. The Rheological analysis of the transfersomal gel of Mupirocin was performed using a Brookfield viscometer DV-II+PRO (Viscolab 3000, Mumbai, India). Viscosity was done in triplicates and mean value was calculated.

The spreadability of the gel was determined by spreadability apparatus (Mutimer *et al,* 1956). Transferosomal gel weighing 1g was placed on the slide which is fixed to the wooden block, another glass slide was placed on top of a fixed slide having the same dimension as that of ground fixed slide such that gel was sandwiched between two glass slides. The top slide consisting of the pulley was subjected to a stress of 50 g by putting weight on it. The time (in a sec) required by the top slide to travel a distance of 10 cm was noted. A shorter time interval indicates better spreadability.

In-vitro Diffusion Study

In-vitro drug release study was performed using Franz diffusion cell. Single dose equivalent transfersomal formulation was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 6.4 (15 ml). The diffusion cells were maintained at 37° C with stirring at 500 rpm (Remi equipment Pvt. Ltd, Mumbai, India). The samples were withdrawn at regular time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h and equal volume was replaced with the dissolution medium. The samples were analyzed by UV/Visible spectrophotometer at 224nm and drug flux (µg/hr/cm^2) was calculated.

To determine the mechanism of drug release from developed MTG, release kinetics was studies by using various kinetic models like, zero-order, forst-order, Higuchi kinetics, Korsmeyers- Peppas model and Hixson Crowell model.

Ex-vivo Diffusion Study

Ex-vivo diffusion study was performed using Franz diffusion cell. Porcine ear skin was placed in between receptor and donor compartments (Dhurke, *et al*, 2016, Mutimer *et al*, 1956). Transfersomal gel equivalent to 10 mg was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 6.4. The diffusion cells were maintained at 35±0. 5ºC at 500 rpm (Remi Equipment Pvt. Ltd. Mumbai, India). One mL of sample was withdrawn at regular intervals of

0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 10 h and equal volume was replaced by phosphate buffer. The samples were analyzed by U.V-Visible Spectrophotometer at 224 nm.

Skin Retention Study

Skin retention study investigates the amount of drug retained in the skin layers. After performing the above mentioned ex-*vivo* permeation study, skin mounted on the diffusion cell was removed. The formulation adhering to the skin was scraped with a spatula (Dhurke *et al*, 2016 and Baden *et al* 1970). The skin was cleaned with cotton and the skin was cut into small pieces and the skin sample was mechanically shaken with 20 ml of phosphate buffer (pH 6.4) at 37±0.5°C for 1 h for complete extraction of the drug. The filtrate was removed and the drug content in the filtrate was determined spectrophotometrically at 224 nm using a UV spectrophotometer.

Antimicrobial study

The antimicrobial activity of different formulations was carried out by the agar diffusion method. The medium used for mupirocin antimicrobial study is Muller-Hinton agar media[14]. The agar medium was prepared by dissolving 15 g of agar powder in 100 ml of distilled water and was sterilized by using an autoclave at 121°C for 15min. The plates were first sterilized in a hot air oven at 160°C for 60 min. After sterilization, the agar medium was poured into each Petri plate and gets solidified. After solidification, in each plate, 6mm in diameter were bored in the medium with borer. In each plate, the equivalent dose was placed and incubated at 25°C for 24 h (Dhurke *et al,* 2016 and Hashem *et al,* 2017). The entire study was carried out under aseptic conditions and the mean inhibition zone was calculated. The statistical analysis was performed using One-way analysis of variance (ANOVA) using Graph Pad prism software (Graph Pad Prism version 7.0.0.159, USA). One-way ANOVA followed by Bonferroni's multiple comparison tests was applied for determining the statistically significant difference in antibacterial activity between various formulations (Hashem *et al,* 2017). The value obtained for formulations at a *P* value of 0.05 was considered to be statistically significant. The characterization data were expressed as the means of three experiments±SD.

Stability Study

The optimized was stored at 4 ± 20°C (refrigeration), 25 ± 20°C (room temp) for 3 months and was visually observed for parameters like, consistency, morhology, drug content and percent drug entarpment was determined every 15 d time interval (Dhurke *et al,* 2016 and Hashem *et al,* 2017).

RESULTS AND DISCUSSION

Vesicle size and Shape Determination

Mupirocin loaded transfersomes were found to be uniform in size and spherical in shape. The size vesicles was in the range of 2-4µm as evident in Figure 1. As observed with formulation F2 and F6, when the concentration of phospholipon 90 H was increased non uniformity in vesicle size was seen, vesicles appear large in size (Figure 2). Formulation F4 and F5 with low concentration of phospholipon 90 H and high concentration of surfactant showed less vesicles formation. This may be due to the fact that the lipid available for forming vesicles was not sufficient enough. Results obtained with F3 formulation were similar to those with F1 formulation as the concentration of both phospholipon and surfactant was equally increased. Non-ionic surfactant plays an important role in size of vesicle. The mean size of vesicle increases with increase in HLB value of surfactant because the surface free energy decreases with an increase in hydrophobicity of surfactant there by the size increases.

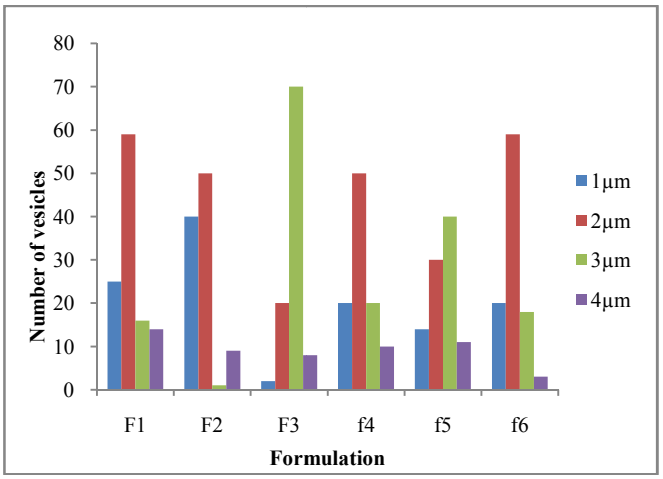

Figure 1 Vesicle size distribution of transfersomal formulation using an optical microscope

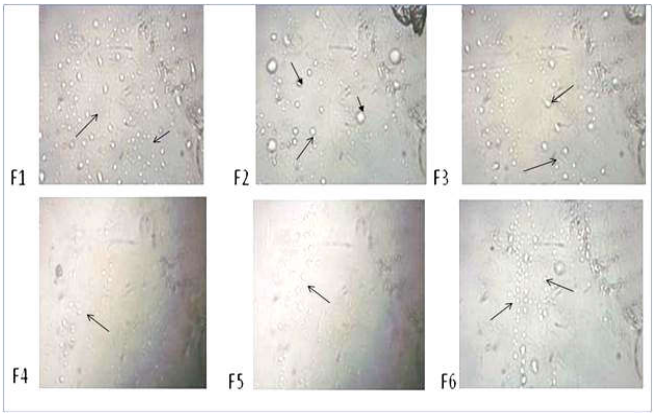

Figure 2 Phase contrast microscopy for transfersomal formulations (F1-F6)

Determination of Zeta potential

Particle size analysis of the optimized formulation shows that the size range lied between 22.78 and 2370 nm (mean 863.9 nm). The transfersomal formulation exhibited negative surface charge due to HLB value of surfactant indicating stability of the formulation. Polydispersity index of 0.3 indicates homogenety of the vesicles through out the formulation. The optimum value of zeta potential should lie between +25 and -25 mV (Figure 3). A higher zeta potential indicates higher kinetic energy and tends to move particles towards agglomeration.

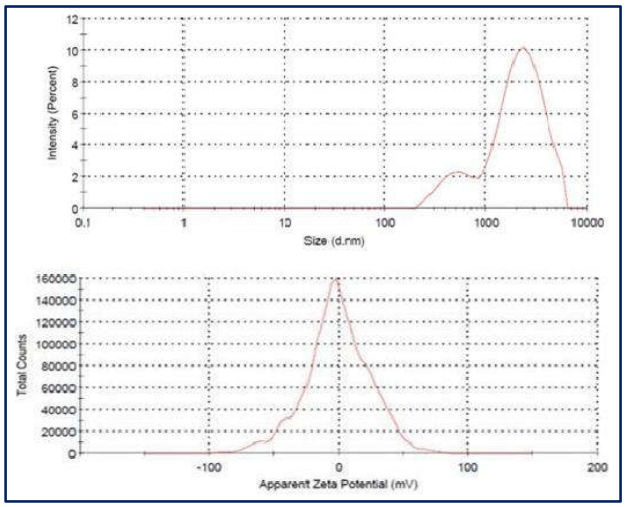

Figure 3 Zeta Potential and particle size distribution of formulation F1

Entrapment Efficiency

The entrapment efficiency of all formulation (F1-F5) was found to be in the range of 91% to 96%. The high values of entrapment efficiency would be due to the association of the surfactant molecules with the phopholipon 90H which enhances the partitioning of the drugs in the bilayer of transfersomes. In tranferosomes entrapment efficiency is dependent on surfactant concentration. The highest entrapment efficiency would be due to the interaction between surfactant and drug forming a complex which gets inserted into the transfersome bilayers. As the concentration of surfactant increases beyond certain limit vesicle size was decreased due to formation of micellar structure this results in low entrapment efficiency and low permeation efficiency.

Drug Content

The drug content of the pure drug was NLT 90% and NMT 120% (as per USP). The drug optimized formulation (F1) was found to be 96±0.75% which was within limits.

Characterization of Drug loaded gel

MTG formulations were transparent; pH was in the acceptable range of 5.5-6.5 compatible for dermatological use. There was no significant difference found between the viscosities of all six formulations it was in the range of 20,540.3±451-30,718.7±859. All developed formulations had good spreadability as the time taken by the slide to travel a distance of 10 cm was less than one min .The spreadability of the developed formulation may have improved because of carbopol 934 which results in the loose matrix of gel compared.

In-vitro diffusion study

Formulation F1 showed highest percent of drug release i.e. 90±0.64 as compared to F2 and F4 with 82± 0.36, and 88± 1.01 respectively (Figure 4). Maximum drug release was seen may be due to the narrow size range of the transfersomes which improves diffusion across the membrane Since the entrapment efficiency is high due to improved partitioning of drug might have resulted in 90 % drug release in 6 h. At high edge activator concentrations, the release of the drug was low due to the loss of vesicular structure and formation of rigid mixed micelles as seen in formulation F3, F5 and F6.

Data obtained from various release kinetics (Table 2) it is observed that formulations F1 is better fitted with zero-order rate kinetics due to high coefficient of correlation values obtained for zero-order rate kinetics. The drug is permeated by diffusion as obsreved by coefficient of correlation values obtained for Higuchi equation (0.889). On the basis of the Korsmeyer Peppas model the best fitting was obtained with 'n' value of 0.73 which indicates that the drug release mechanism is by anamolous diffusion which is intermediated by diffusion and matrix erosion.

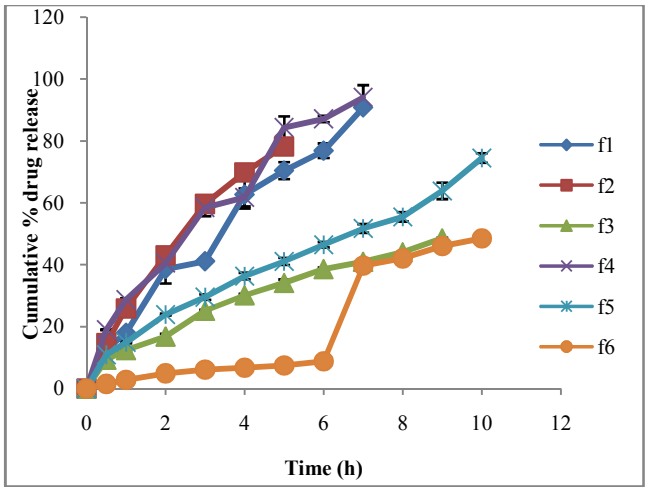

Figure 4 *In-Vitro* diffusion studies of transfersomal gel formulations

Table 2 Various kinetic models for transfersomal gel formulations

Formulation	Zero-order (r^2)	First order (r^2)	Higuchi (r^2)	Hixson-Crowell (r^2)	Korsmeyer Peppa's (r^2)	n value
F1	0.977	0.948	0.889	0.868	0.981	0.734
F2	0.918	0.888	0.913	0.753	0.977	0.774
F3	0.932	0.951	0.663	0.908	0.994	0.625
F4	0.907	0.932	0.952	0.957	0.99	0.780
F5	0.956	0.953	0.732	0.896	0.991	0.638
F6	0.787	0.759	0.566	0.399	0.641	0.460

Ex-Vivo diffusion study

The drug release of optimized formulation was found to be high compared to the marketed formulation. Optimized formulations (F1 and F2) showed 99% and 94% drug release in 6h and 5h respectively as compared to marketed formulation which showed 67% drug release in 6h (Figure 5). The reason can be explained in similar way as that of *in vitro* diffisuon studies since marketed preparation is conventional gel the particle size is large because of which the diffusion might be low.The stability data indicates that optimized mupirocin loaded transfersomal gel formulation (F1) stored at ambient temperature (i.e. room temperature) and refrigeration conditions (at 2-8 °C) showed the no signs of instability with respect to morphology. There was no change in the consistency of the gel and no precipitation of drug was seen when obseved under optical microscope. But there was decrease in the percent drug entrapment for formulations stored at room temperature to less than 60 % at the end of three months with drug content less than 50 %. Formulations stored at refrigeration temperature were found to be stable with 95% of drug entrapment and there was no significant change in drug content. The results indicated that the storage of transferosomal gel formulation at refrigeration conditions was more preferable compared to room temperature.

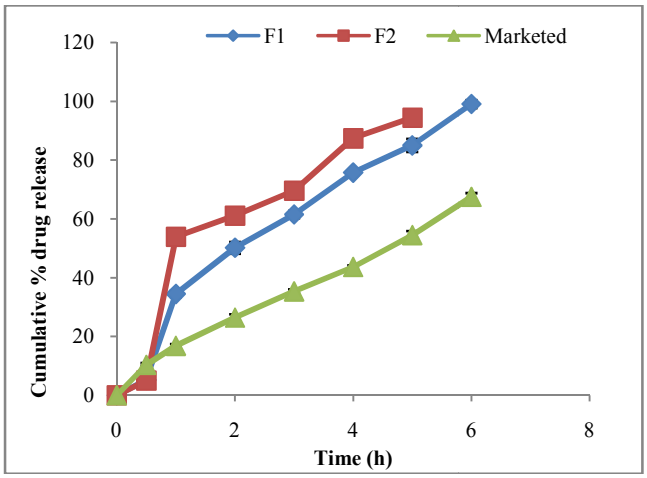

Figure 5 Ex vivo diffusion studies of optimized formulations with marketed product

Skin Retention study

The amount of drug retained in the skin for F1 and F2 formulation was 70±1.1% and 64±4% respectively while that of marketed product was just 49±0.6%. Transfersomes showed a marked increase in drug retention in the skin is due to the deformation of transfersome which increases permeation through stratum corneum which resulted in significant drug retention.

Antimicrobial Activity Studies

Zone of inhibition of mupirocin is shown in the figure 6**.** These results demonstrated that the antimicrobial activity obatined for F1 formulation was high as compared to marketed product. There is significant decrease in the number of colonies in F1 formulation as compared to marketed product where dense colony formation is observed. Formulation F2 also showed good antibacterial activity but less than F1 formulation. Enhanced antibacterial activity can be due to the narrow vesicle size of the deformable transfersomes which easily diffuses from the gel matrix throughout the media. One way ANOVA followed by Bonferroni's multiple comparison tests shows that formulation F1 has statistically significant antibacterial activity at $p<0.05$ significant level compared to marketed product and F2 formulation.

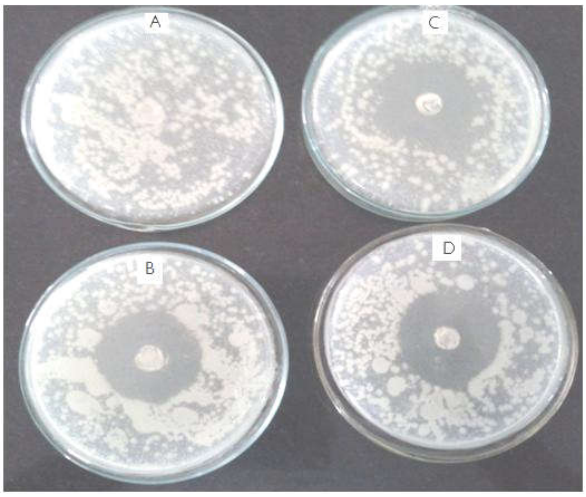

Figure 6 Zone of inhibition. A-Plain gel, B-Marketed, C-F1 formulation and D-F2 formulation

CONCLUSION

From the above studies, it was concluded that F1 formulation was found to be better as the results obtained for antimicrobial activity and drug retention in the skin was high as compared to conventional marketed product. Mupirocin loaded transfersomal vesicles can prove to be a better option for treating skin disease like impetigo due to its improved drug permeation and zone of inhibition of optimized formulation was high when compared to marketed products.

Conflict of Interest: The authors declare no conflict of interest

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