



New Formulation and Characterization of Topical Films of Tioconazole and Evaluation of Their Antifungal Therapy

Siddique Akber Ansari^{1*}

¹Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, KSA.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i46A32882

Editor(s):

(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.

Reviewers:

(1) E. S. Sushmitha, RRMCH, MGR University, India.

(2) Forman Erwin Siagian, Universitas Kristen Indonesia, Indonesia.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/75418>

Original Research Article

Received 05 August 2021

Accepted 11 October 2021

Published 15 October 2021

ABSTRACT

Purpose: New formulation of Scleroglucan (Sclg) films loaded with tioconazole, a medication typically applied for dermal treatments prepared. The feasibility of that treatment relies on the penetration of medications through the target layers of the skin in effective concentrations.

Methods: Dynamic and mechanical characterization and swelling studies of the novel delivery system were analysed. An aqueous solution of Sclg (Cp=1% w/v) and glycerol (2% w/v) was prepared and kept at room temperature under magnetic stirring for 72 hrs. Tioconazole previously solubilised in Labrasol, was added to the polymer/glycerol solution. 4 ml of solution was poured in a plastic plate. The films were dried at 40°C for 1 hr and then allowed to dry at room temperature (about 25°C) for a week. Translucent films were obtained.

The fungal strain used to test the film are CO23 sensitive to drugs, CO23 RFLC resistant to fluconazole, CO23 RFK resistant to micafungin, ATCC standard strain.

Results: The water uptake of the films significantly increased up to 24 hrs. The optical microscope films images show that the presence of the drug did not significantly influence the appearance of the samples. The *in vitro* studies demonstrated the perceptible fungal activity of the new formulation against *Candida albicans* infections.

Conclusion: The patches showed antimicrobial activity against all tested strains. An evident inhibition zone diameter, about 40 mm, for the strains sensitive to azoles (CO23 RFK and CO23) in comparison to strain resistant to fluconazole (CO23 RFLC) was observed. After 48 hours the inhibition zone diameters were reduced of about 6-7 mm in comparison to those observed after 24 hours of incubation.

Keywords: Scleroglucan; tioconazole; glycerol; labrasol; *Candida albicans*.

1. INTRODUCTION

Fungal contaminations on the skin are most regularly facing infections around the worldwide. Such huge numbers of individuals have experienced fungal infections in all countries. The development of fungal contaminations can be quick and caused serious effect due to the damage of immune functions [1,2]. Topical treatment is commonly used as an interesting alternative for the treatment of the cutaneous infections because of its advantages, for example, focusing of medications to the site of infection and reducing the risk of systemic side effects [3,4]. Nowadays, antifungal medications are mostly used as traditional cream and gel preparations in topical treatment [5]. The feasibility of that treatment relies on the penetration of medications through the target layers of the skin at the effective concentrations. The physicochemical characteristics of drug molecules and the definite formulation are effective factors in topical drug delivery. Therefore, a number of formulation strategies have been examined for delivering antifungal compounds through targeted sites of the skin.

Dermatophytosis is the causative agent responsible for tinea and onychomycosis diseases. Cutaneous *Candida* infection is superficial fungal infection typically involving the skin [6,7]. Even, *Candida* may penetrate and enter the blood and even more to deep tissue, a condition that prompts to life-threatening systemic candidiasis [8]. This is most common when the immune system is weakened [9]. Topical treatment of fungal infections has a number of advantages, including targeting of the infection site, decrease of the risk of systemic side effects, improvement of the efficacy of treatment and high patient compliance.

Nowadays, the formulation may play a most significant role for penetration of drugs into skin [10,11]. Topical delivery of drugs for the treatment of fungal infections of skin encompasses new carrier systems for approved and investigational

compounds. Delivery of antifungal compounds into skin can be enhanced with the carriers including colloidal systems, vesicular carriers, and nanoparticles.

Furthermore, researchers have established and proposed various formulation techniques to improve the solubility and bioavailability of poorly water-soluble compounds [12]. One of the most common approaches involves the use of a solubilizing agent, such as Labrasol (saturated polyglycolized C₈-C₁₀ glyceride) [13] mixed with other pharmaceutical excipients, such as hydrophobic oils, lipids, and co-solvents, to form micro emulsions or emulsion systems [14-16]. The drugs are held tightly in the more hydrophobic cores of the emulsion system, thus avoiding drug precipitation.

1.1 Topical Delivery of Antifungal via Skin

Human skin is a well-organized membrane and, it has three main layers, *epidermis*, *dermis* and *hypodermis*. *Stratum corneum*, the outermost layer of *epidermis*, is shaped by dead and keratinized cells, and it is an outstanding barrier to penetration of drugs through the skin [17]. Drugs should penetrate into skin layers to confirm effective drug concentrations following topical administration. In topical administration, the entering of drugs to systemic circulation is prevented or minimized. Thus, the systemic adverse effects of drugs are avoided [18]. Moreover, topical preparations have improved patient compliance due to their non-invasiveness and they can be self-administered [19,20].

2. METHODOLOGY

2.1 Materials

ScI_g was provided by Cargill (Minneapolis, USA). Glycerol (analytical grade) and Labrasol was bought by Gattefosse. Tioconazole, was purchased by Sigma. For the sample preparations distilled water was used.

2.2 Methods

2.2.1 Preparation of sclg films

Sclg was cleaned by dialysis. An aqueous solution of Sclg (cp=1% w/v) and glycerol (2 % w/v) was prepared and kept at room temperature under magnetic stirring for 72 hrs. Tioconazole previously solubilised in Labrasol, was added to the polymer/glycerol solution. 4 ml of solution was poured in a plastic plate. The films were dried at 40°C for 1 hr and then allowed to dry at room temperature (about 25°C) for a week. Translucent films were obtained.

Films were prepared with three different amounts of tioconazole, 5µg, 10µg and 20µg (Table 1). In Fig 1 the preparation of polymeric films by the casting solvent evaporation technique is reported. The original size of the films was 3x3cm². For the swelling studies and for the fungal activity tests 1x1 cm² samples were used.

2.2.2 Dynamo-mechanical analysis

The mechanical properties were investigated with a software-controlled dynamometer, TA-XT2i (Stable Micro Systems, UK), equipped with a 5-kg load cell. The accuracy of the force measurement was 0.0025 %, and the distance resolution was 0.0025 mm.

The film was placed in a holder with a cylindrical hole ($d= 8.50$ mm) and the diameter of the spherical probe was 6.35mm. The pre-test and post-test speeds were fixed at 2.00 mm/s, the test speed at 1.00 mm/s and the trigger force at 0.098 N.

The following parameters were calculated:

$$1) \text{ Puncture strength at break} = \frac{F_{\max}}{ACS}$$

Where F_{\max} is the maximum applied force at film break, ACS is the cross-sectional area of the film above the hole of the film holder, $ACS = 2r\delta$, where r is the radius of the hole and δ is the thickness of the film.

$$2) \text{ Elongation (\%) at break} = \left[\frac{[(r^2 + D^2)]^{\frac{1}{2}} - r}{r} \right] \times 100$$

Where r is the radius of the film exposed in the cylindrical hole of the film holder and D represents the displacement of the probe from the point of contact to point of puncture. The maximum load and the maximum displacement of films were measured, and then converted to puncture strength, and elongation at break.

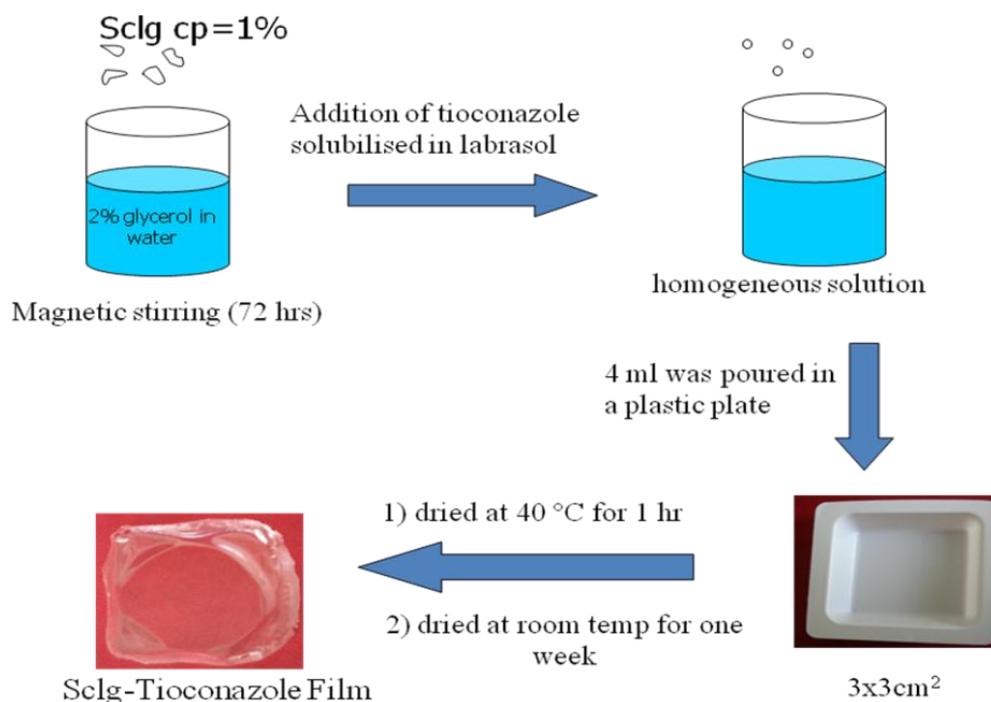


Fig 1. Schematic representation of Sclg film preparations

2.2.3 Susceptibility testing method

The antimicrobial activity of the patches was assessed against the test organisms *C. albicans* ATCC 24433, and the strain CO23 of *C. albicans*, which was isolated from a subject with vulvo-vaginal candidiasis, originally susceptible to micafungin (FK463, MIC 0.025 µg/ml) and fluconazole (FLC MIC 0.25 µg/ml). The strains CORFK and CO23 RFK were made resistant to FK463 or to FLC by ten growth passages in step wise-increasing concentrations in solidified agar, according to the National Committee of Clinical Laboratory Standards CLSI.;(2007).

2.3 Evaluation of Prepared Films

2.3.1 Film thickness measurements

The thickness of the films was assessed by means of a posiTector 6000 digital thickness meter purchased by DeFelsko (USA). The thickness of the films was measured at five different positions and the obtained values always lay within 10% of the mean.

2.3.2 Water Uptake Studies

The Sclg films showed an extraordinary swelling. The water uptake behaviour was followed as a function of time. The experiments were carried out in distilled water at room temperature. At fixed time intervals the excess of water was removed from the films with soft filter paper for 5 sec, and then the corresponding weights were determined. All experiments were carried out in

triplicate and the obtained values always lay within 10% of the mean.

2.4 Folding Endurance

The film folding endurance were manually measured. A strip of film was repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance. The folding endurance of prepared films was measured in triplicate and the obtained values always lay within 10% of the mean.

3. RESULTS AND DISCUSSION

3.1 Water Uptake Studies

The water uptake results are reported as a function of time in Fig 2. The water uptake of the films significantly increased up to 24 hrs and after 30 hrs the films completely dissolved in the swelling medium.

In Fig 3 the films images recorded by means of an optical microscope show that the presence of the drug did not significantly influenced the appearance of the samples.

3.1.1 Physical and dynamo-mechanical characterization of Sclg films

Different formulations of Sclg films were prepared, as listed in Table 1.

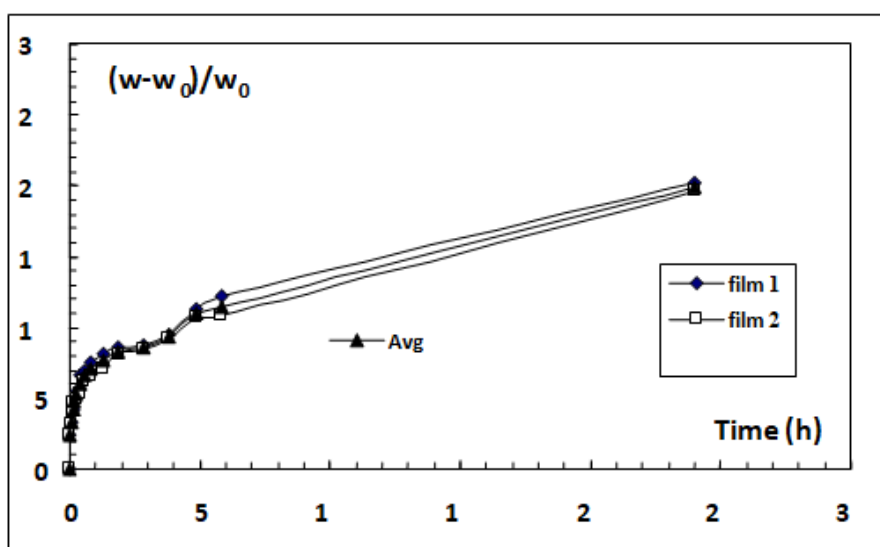


Fig. 2. Water uptake $(w-w_0)/w_0$ of Sclg films as a function of time

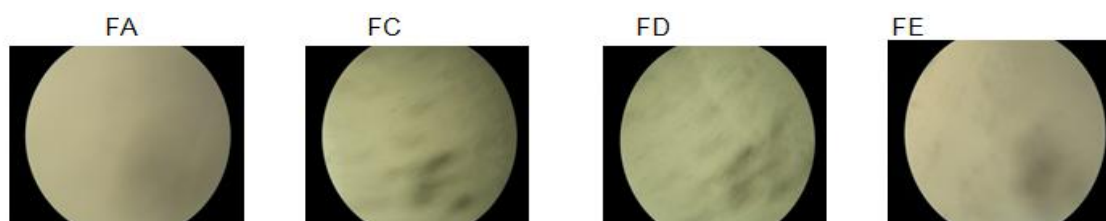


Fig 3. Optical microscope images of Sclg films prepared with different amount of the tioconazole drug. FA, FC, FD and FE (see Table I)

Table 1. List of Sclg film formulations

Formulations code	Sclg % (w/v)	Glycerol % (w/v)	Labrasol (µl)	Tioconazole (µg)	Distilled water (ml)	Surface area of Film (cm ²)
FA	1	2		4	3x3
FB	1	2	5		4	3x3
FC	1	2	5	45	4	3x3
FD	1	2	5	90	4	3x3
FE	1	2	5	180	4	3x3

In principle, an adequate film mechanical strength is required in order to withstand mechanical stress (e.g., filling and packaging processes, pushing through blister package, handling by patient).

Puncture strength is a measure of film toughness and is directly proportional to the resistance to break or fracture. Sclg films were capable to elongate the double of their initial size. In Table II the physical parameters evaluated for the Sclg films are reported.

As an example, the mechanical properties of FA and FE formulations are shown in Fig 4.

3.1.2 C. albicans activity tested on Sclg films loaded with tioconazole

Petri plates were set up by pouring 20 ml of Sabouraud medium that was allowed to solidify, and then dried for 30 min in a biological safety cabinet with vertical laminar flow. 10 µl of a standardized inoculum suspension of 2.5x10³ CFU/ml was poured and uniformly spread over the plates. Patches with different amounts of tioconazole (5, 10, 20 µg) were placed in the

center of Petri plates. The Petri dishes were then incubated at 30 °C for 24 and 48 hrs. The antifungal activity was measured as the diameter of the inhibitory zone by a caliper and expressed in mm (disk diameter included) (Fig 5).

The sensitivity to the different patches was classified by the diameter of the inhibition zone. An inhibitory zone with diameter less than 10 mm corresponds to lack of activity.

The inhibitory zones of samples containing 5, 10 and 20 µg of tioconazole were measured. As reported in Table III, the patches showed an antimicrobial activity against all tested strains. An evident inhibition zone diameter, about 40 mm, for the strains sensitive to azoles (CO23 RFK and CO23) in comparison to strain resistant to fluconazole (CO23 RFLC) was observed. After 48 hours the inhibition zone diameters were reduced of about 6-7 mm in comparison to those observed after 24 hours of incubation. Patches without drug showed no activity; in some cases no significant differences of inhibition zone between the patches with different concentrations of tioconazole (5 and 10 µg) were observed.

Table 2. List of physical characteristics of Sclg films

Formulations code	Thickness (mm)	Film Weight (mg)	Folding Endurance (-)	Puncture strength (MPa)	Elongation (%)
FA	0.110	153.6	87	10.73	95.71
FB	0.106	148.1	85
FC	0.102	147.3	83
FD	0.104	150.4	89
FE	0.102	150.7	95	6.48	72.23

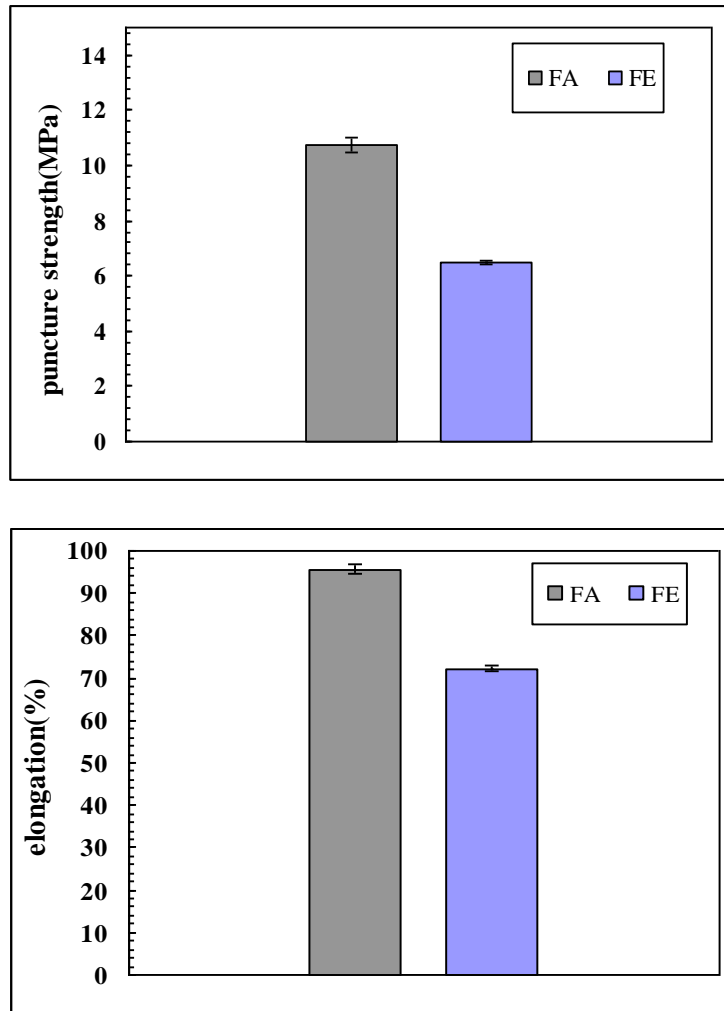


Fig 4. Mechanical properties (puncture strength, and elongation) of ScIg (FA) and ScIg-tioconazole (FE) films

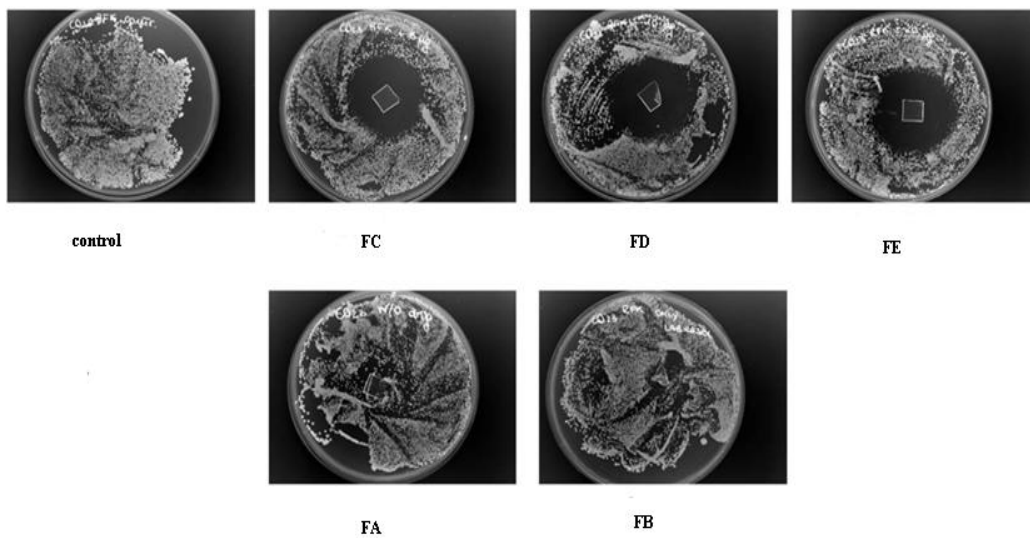


Fig. 5. Pictures of different ScIg films loaded with tioconazole and incubated for 24 hrs

Table 3. Fungal activity of ScIlg films loaded with tioconazole

<i>C. albicans</i>	Sample	Inhibition zone (diameter expressed in mm after 24h)	Inhibition zone (diameter expressed in mm after 48h)
Co23	Control	+++	+++
Co23	FC	30 ±2.8	20±1.4
Co23	FD	38±1.4	26.5±3.18
Co23	FE	44±2.8	28.5±2.8
Co23	FA	+++	+++
Co23	FB	+++	+++
CO23 RFLC	Control	+++	+++
CO23 RFLC	FC	18±0.70	+++
CO23 RFLC	FD	22±1.41	15.5±2.12
CO23 RFLC	FE	24±0.0	18±0.70
CO23 RFLC	FA	+++	+++
CO23 RFLC	FB	+++	+++
Co23RFK	Control	+++	+++
Co23RFK	FC	40±1.41	33
Co23RFK	FD	40±0.70	34
Co23RFK	FE	42±2.8	35±2.12
Co23RFK	FA	+++	+++
Co23RFK	FB	+++	+++
ATCC 24433	control	+++	+++
ATCC 24433	FC	22±0.70	+++
ATCC 24433	FD	30±0.70	23±0.70
ATCC 24433	FE	30±2.8	24±0.0
ATCC 24433	FA	+++	+++
ATCC 24433	FB	+++	+++

*CO23 sensitive to drugs; CO23 RFLC resistant to fluconazole; CO23 RFK resistant to micafungin
ATCC standard strain; +++ strain growth*

4. CONCLUSIONS

A new formulation, a Scleroglucan (ScIlg) films loaded with tioconazole. The films were prepared with ScIlg and glycerol used as a plasticizer, and Labrasol a surfactant used for increasing the solubility and bioavailability of the low-soluble drug. Films were prepared with three different amounts of tioconazole, 5µg, 10µg and 20µg . The in vitro studies demonstrated the perceptible fungal activity of the new formulation against Candida Albicans infections. The inhibitory zones of samples containing 5, 10 and 20 µg of tioconazole were measured. The patches showed an antimicrobial activity against all tested strains. An evident inhibition zone diameter, about 40 mm, for the strains sensitive to azoles (CO23 RFK and CO23) in comparison to strain resistant to fluconazole (CO23 RFLC) was observed. After 48 hours the inhibition zone diameters were reduced of about 6-7 mm in comparison to those observed after 24 hours of incubation. Patches without drug showed no activity; in some cases no significant differences of inhibition zone between the patches with

different concentrations of tioconazole (5 and 10 µg) were observed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Ameen M. Epidemiology of superficial fungal infections. *Clin. Dermatol.* 2010;8(2):197-201.
Available:<https://doi.org/10.1016/j.clindermatol.2009.12.005>.
2. Havlickova B, Czaika V, Friedrich M. Epidemiological trends in skin mycoses worldwide. *Mycoses.* 2008;4:2-15.
Available:<https://doi.org/10.1111/j.1439-0507.2008.01606.x>
3. Williamson DA, Carter GP, Howden BP. Current and emerging topical Antibacterials and antiseptics: agents, action, and resistance patterns. *Clin Microbiol Rev.* 2017;30:827-860.
Available:<https://journals.asm.org/doi/10.1128/CMR.00112-16>.
4. Domenico Aurora DM,Roberta G,Claudio C,Chiara B,Caterina F,Paolo . Topical antibiotics in the dermatological clinical practice: Indications, efficacy, and adverse effects.*Dermatol.Therap.*2020;33(6).e13824.
Available:<https://doi.org/10.1111/dth.13824>
5. Boonme P, Kaewbanjong J,Amnuaitit T, Andreani T, Silva M, Amélia B,Souto E. *Curr. Pharm. Design.*2016;22(27):4257-4263
6. Zhang AY,Camp WL, Elewski BE.Advances in topical and systemic antifungals. *Dermatol. Clin.* 2007; 25(2):165-83.
7. Gupta AK, Foley KA,Versteeg SG. New Antifungal Agents and New Formulations Against Dermatophytes .*Mycopathologia.* 2017; 182(1-2):127-141.
Available:<https://doi.org/10.1007/s11046-016-0045-0>
8. Joshua P, Bryan C,Brad S.Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Med Mycol.*2007;45(4): 321-46.
DOI: 10.1080/13693780701218689
9. Sharma R, Pathak K. Polymeric nanosponges as an alternative carrier for improved retention of econazole nitrate onto the skin through topical hydrogel formulation. *Pharm. Dev. Technol.* 2011;16(4):367-76.
Available:<https://doi.org/10.3109/10837451003739289>
10. Lee CM, Maibach HI. Deep percutaneous penetration into muscles and joints. *J Pharm Sci.* 2006 ;95(7):1405-13.
Available:<https://doi.org/10.1002/jps.20666>
11. Gungor S,Erdal Sedef, Aksu B. New formulation strategies in topical antifungal therapy. *Journal of Cosmetics. Dermatological Sciences and Applications* . 2013 ; 3(1A):56-65.
12. Samuel H, Yalkowsky.Solubility and solubilization in aqueous media. *J. Am. Chem. Soc.* 2000;122(40):9882.
Available:<https://doi.org/10.1021/ja0047424>
13. Strickley RG. Solubilizing excipients in oral and injectable formulations. *Pharm. Res.* 2004;21:201-230.
Available:<https://doi.org/10.1023/B:PHAM.0000016235.32639.23>
14. Barakat NS. Enhanced oral bioavailability of etodolac by self-emulsifying systems: in-vitro and in-vivo evaluation. *J. Pharm. Pharmacol.* 2010;62:173–180.
Available:<https://doi.org/10.1211/jpp.62.02.0004>
15. Kommuru TR, Gurley B, Khan MA, Reddy IK.Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: Formulation development and bioavailability assessment. *Int. J. Pharm.* 2001;212:233–246.
Available:[https://doi.org/10.1016/S0378-5173\(00\)00614-1](https://doi.org/10.1016/S0378-5173(00)00614-1)
16. Kim HJ, Yoon KA, Hahn M, Park ES, Chi SC. Preparation and in vitro evaluation of self-microemulsifying drug delivery systems containing idebenone. *Drug Dev Ind Pharm.* 2000; 26:523–529.
Available:<https://doi.org/10.1081/DDC-100101263>
17. Flowers FP. Transdermal and topical drug delivery: from theory to clinical practice. *Ann. Pharmacother.* 2004;38(4):726-727.
Available:<https://doi.org/10.1345/aph.1D555>
18. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects.*Drug Deliv.* 2006;13:175–87.
Available:<https://doi.org/10.1080/10717540500455975>

19. Guy RH, Transdermal drug delivery. *Handb. Exp. Pharmacol.* 2010;11:399–410.
Available:https://doi.org/10.1007/978-3-642-00477-3_13
20. Tanner T, Marks R. Delivering drugs by the transdermal route: Review and comment. *Ski. Res. Technol.* 2008;14(3):249-60.
Available:<https://doi.org/10.1111/j.1600-0846.2008.00316.x>

© 2021 Ansari; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<https://www.sdiarticle4.com/review-history/75418>