EVALUATION OF MICONAZOLE NITRATE PERMEABILITY THROUGH BIOLOGICAL MEMBRANE FROM DERMAL SYSTEMS

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Abstract
The development of pharmaceutical products with miconazole nitrate (MN) can bring various benefits to patients of whom fungal infections are resistant to classic antifungal formulas. In medical practice, dermal systems, in the form of polymeric films, represent an alternative to other medical products. The proposed dermal films contain hydroxyethyl cellulose (HEC) as a hydrophilic and bioadhesive matrix polymer, with the addition of polyethylene glycol (PEG) as a low-absorption promoter, so that the antifungal have a slow penetration towards the dermis. This study aims to evaluate the in vitro permeability of MN through biological membrane (pig ear skin) at pH 7.4, during its release from two polymeric films. In vitro pig ear skin permeation studies indicated that the amount of the drug released after 24 h was 40% in the case of formulation FI and 32% in the case of formulation FII from the initial dose (40 mg). The concentration of 40% MN released can be considered an appropriate antifungal dose, with the benefit of being accumulated in the stratum corneum where it is maintained for up to 4 days.

Rezumat
Dezvoltarea de forme farmaceutice cu nitrat de miconazol (MN) poate aduce numeroase beneficii pacienților care au dezvoltat rezistență clinică la formulările antifungice clasice. În practica medicală, sistemele dermice formulate ca filme polimerice reprezintă o alternativă de administrare față de alte produse medicamentoase. Filmele dermice propuse conțin hidroxietilceluloză, un polimer bioadeziv formator de matriță, în asociere cu polietilenglicol ca promotor cu proprietăți moderate de penetrare cutanată, astfel încât substanța activă să poată penetra lent în dermă. Acest studiu ar trebui să îi permită evaluării in vitro permeabilității MN prin membrană biologică (piele de ureche de porc) la pH 7,4, durat e ril de alte doisprezintă. Studiile de permeație in vitro indică că concentrația de 40% MN eliberată poate fi considerată un dozaj antifungal adecvat, cu beneficiul de a se acumula în stratum corneum, unde se menține timp de până la 4 zile după prima aplicare.

Keywords: miconazole nitrate, dermal films, hydroxyethyl cellulose matrix, Franz cell method

Introduction
The increasing number of stress conditions caused by the diversification of activities conducted in modern society, associated with the occurrence of the corresponding pollution factors and the diversity of microbial agents, in particular caused by the increased mobility of people all over the world, led, in addition to the appearance of new diseases, to the occurrence and the diversification of fungal infections. The development of pharmaceutical forms with miconazole nitrate (MN) for external use can bring various benefits to patients presenting resistance to classical antifungal forms because of the fact that the active drug lasts longer in the tissue, acting as a slow release product [7, 8]. Repeated administration leads to an accumulating effect of multiple doses in the stratum corneum, which causes the disappearance of fungal infection [6, 28]. Miconazole nitrate is an antifungal drug that inhibits the synthesis and the incorporation of ergosterol in the fungal cell’s membrane as a result of blocking sterol-14α-demethylase, a cytochrome P450 dependent
enzyme, with a key role in biosynthesis of ergoster. Accumulation of methyl-steroids affects the function of membrane phospholipids and inhibits certain enzymatic membrane dependant systems, like ATPase and enzymatic transport systems, with inhibition of growth and development of fungi. It also acts by affecting the permeability of the fungi membrane, selectively inhibiting RNA and DNA precursors and its mucopolysaccharides [6, 7, 19]. Transdermal forms offer several important advantages over conventional administration routes [2-4, 10, 14, 19, 21]. The skin acts as a barrier and the optimization of the release of the drug through this barrier is often the first step towards optimizing the efficiency of such pharmaceutical preparations [12, 23, 27]. The pharmaceutical product is placed on the skin for topical or systemic action, depending on the penetration properties (with local dermal effect through crossing the corneum layer of epidermis) and permeation capacity (with transdermal effect through absorption into the systemic circulation).

It’s known that the miconazole nitrate is retained in the skin layers up to 4 days after the first administration. The greatest challenge for such types of pharmaceutical forms with miconazole nitrate is to remain for as much as possible at the stratum corneum level. A transdermal administration can ensure an optimal concentration of the drug throughout the treatment period. The proposed dermal films contain hydroxyethyl cellulose (HEC) as a bioadhesive matrix forming polymer, with the addition of polyethylene glycol (PEG) as a low-absorption promoter so that the antifungal does not penetrate too much towards the dermis. The main reason for choosing HEC as a bioadhesive matrix forming polymer was its ability to adhere to mucous membrane and human skin, providing stability to the film [26], and the lack of toxicity [5, 15, 18]. HEC with an apparent viscosity of 4500 - 6500 mPa · s is highly water-soluble and practically insoluble in ethanol, being used as a hydrophilic gel former. HEC is a hydrophilic polymer with fast dissolution, and it forms gels at 1.5% - 2%, which exhibit good bioadhesiveness and yield capacity [5].

Although a series of mathematical assumptions have been issued describing drug diffusion through the skin, due to the complexity of the cutaneous organ, it is very difficult to describe a perfect mathematical model [20]. The unanimous acceptance is that the transportation of drugs through the skin takes place through passive diffusion. In vitro availability tests with the classical Franz cell can be performed under both static and dynamic diffusion conditions. Contact with the donor compartment is made through a diffusion membrane that simulates the transfer through the stratum corneum. According to FDA SUPAC-SS 1997, synthetic membranes with different compositions are accepted: polysulfone, cellulose acetate, cellulose nitrate or polyfluoroethylene, with a diameter close to that of the diffusion cell [11]. Nylon represents a polyamide derivative [24] very commonly used for preliminary tests. OECD Guidance (Organization for Economic Cooperation and Development) [22] considers that the use of a biological human skin membrane is the golden standard in assessing transcutaneous absorption in the design of the in vitro penetration study [9, 13]. Other types of biological membranes can be used instead, but it should be taken into account that they behave differently from human skin [16, 17].

The proposed study evaluates the in vitro permeation of the antifungal from dermal films through a biological membrane (Mb) using the Franz cell. The pig ear skin is a membrane more similar to human epidermis at pH of body fluids [1].

Materials and Methods

Materials

Miconazole nitrate (MN) was purchased from Sigma Aldrich Inc (Germany). Hydroxyethyl cellulose 250 M, NatrosolTM 250M (HEC, viscosity of 4500 - 6500 mPa · s) from Ashland (Germany), Polyethylene glycol 400 (PEG 400) from Sigma Aldrich Inc. (Germany), ethanol from Stireco LTH (Romania) (Table I).

| Ingredient                   | Abbreviation | Formula % | Function                  |
|------------------------------|--------------|-----------|----------------------------|
| Miconazole nitrate           | MN           | 5.00      | 5.00                       |
| Hydroxyethyl cellulose 250 M| HEC          | 2.00      | 3.00                       |
| Polyethylene glycol 400      | PEG<sub>400</sub> | 1.00      | 1.00                       |
| Ethanol                      |              | 10.00     | 10.00                      |
| Ultrapure water              |              | 82.00     | 81.00                      |

Films preparation technique. MN was first dissolved in alcohol with stirring at 500 rpm for 5 minutes and after we added PEG, distilled water and HEC during constant stirring. The air bubbles were eliminated from the structured gel by maintaining the fluid phase for 25 minutes in the ultrasound bath. The resulting composition was poured into circular plates (diameter of 9.8 cm) which were then kept to dry at 40°C (24 h). The obtained films were used in the study after 48 hours of preservation at 20°C, protected from humidity [13].
**Products in form of dermal systems, proposed in study.** Samples of 2.54 cm² containing 40 mg MN in polymeric matrices of HEC (2% in FI and 3% in FII), with polyethylene glycol - PEG 400 were prepared in form of films with thickness of 0.23 mm (FI) and 0.30 mm (FII), by casting and solvent evaporation technique. Preparation of biological skin used as the diffusion membrane. The biological membrane (Mb) consisted of skin from pig ears collected from a local abattoir. According to literature specifications, in order to preserve the integrity of the skin barrier function, the pig ears were removed immediately after slaughter from the carcass and washed with water [17]. After removing the hair yam, the ears were individually wrapped in aluminium foil and frozen at -26°C for a maximum of 6 months. It is known that the freezing preservation method will not influence the permeability properties of the biological skin subsequently used as the diffusion membrane in the *in vitro* tests [1, 7, 11]. The biological membranes were prepared by excising from the freshly defrosted pig ears spherical surfaces (1.8 cm diameter). The excess fat and the cartilage were carefully removed, and then the membranes were kept for 30 minutes in the buffer phosphate pH 7.4 [13].

**Determination of in vitro release and diffusion through the biological membrane profiles.** The *in vitro* permeation of MN (mg/cm²*h) was determined by Franz cell method [12] in the following conditions: cell of 14 mL, phosphate buffer at pH 7.4 with 0.045% sodium lauryl sulphate, 32 ± 0.5°C, assessing the MN from acceptor sample of 5 mL, at 273 nm (Spectrometer UVD 3200, Labomed Inc., USA). The analysed sample consisted in disk shaped films with diameter of 1.8 cm (surface of 2.54 cm²), deposited on the studied membrane (Ø 25 mm) and maintained in the donor compartment under occlusive conditions throughout the determination period.

**Data interpretation and statistical analysis.** GraphPad Prism 6 software was used running: linear regression followed by the runs tests, the Pearson correlation (r), area under the curve (AUC), unpaired t test followed by F test and Anova followed by Tukey’s multiple comparison tests. Mean and standard deviation (SD) were calculated as statistical descriptors and statistical significance was set at p < 0.05 with confidence interval of 95% [25].

In a previous study, MN permeation was assessed *in vitro* through the Franz cell from dermal films using a synthetic membrane [7]. The values of antifungal permeation through synthetic membranes (Ms - Teknokroma, 0.45 µm) were compared with those of biological membranes (Mb - pig ear skin, < 1 mm).

**Results and Discussion**

The *in vitro* permeation curves of MN (40 mg/sample) released from two types of HEC matrices as new systems intended for dermal application (FI, FII) were determined over a period of 24 h (Figure 1), by using in the Franz cell samples of 2.54 cm² deposited on diffusion membranes.

![Figure 1](image)

The *in vitro* permeation curves determined from experimental data

The permeation determined through the two membranes used is the result of two initial successive and then simultaneous processes, namely: the release of MN by dissolution and diffusion from HEC/PEG matrix, followed by the membrane crossing of MN by molecular diffusion through the membrane pores, thus resulting in a cumulative process whose rate depends on both stages (Table II), but one of which is usually more limited.

### Table II

| Linear regression of release curves | FI - Mb       | FII - Mb      | FI - Ms       | FII - Ms      |
|------------------------------------|---------------|---------------|---------------|---------------|
| Slope ± Standard Error (95% CI)   | 0.2848 ± 0.02041 | 0.2236 ± 0.01155 | 0.4335 ± 0.03616 | 0.3813 ± 0.03885 |
| Y-intercept                        | -0.3419 ± 0.19995 | -0.1989 ± 0.1129 | 1.371 ± 0.3535 | 1.655 ± 0.3797 |
| X-intercept                        | 1.20           | 0.89           | -3.16         | -4.34         |
| R square                           | 0.9653         | 0.9817         | 0.9535        | 0.9323        |
| P value                            | < 0.0001       | < 0.0001       | < 0.0001      | < 0.0001      |
| Deviation from zero                | Significant    | Significant    | Significant   | Significant   |
| P value (runs test)                | 0.2619         | 0.3452         | 0.6429        | 0.0714        |
| Deviation from linearity           | Not significant | Not significant | Not significant | Not significant |
| Equation                           | Y = 0.2848 * X - 0.3419 | Y = 0.2236 * X - 0.1989 | Y = 0.4335 * X + 1.371 | Y = 0.3813 * X + 1.655 |

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Permeation through the biological membrane. The R
square coefficient of the regression lines (Table II)
indicates a good determination (of 96 - 98%), with
the MN permeation rate of 0.28 (FI) and 0.22 (FII)
mg/cm²/h, through Mb. In quantitative terms, it was
found that MN reached a flow through Mb of 6
mg/cm² (FI) and 5 mg/cm² (FII), respectively, which
represents 40% (FI) and 32% (FII) of the initial dose
(40 mg) of the sample. On a first evaluation of the
results, 40% of MN released seems not to be a
satisfactory percentage, but if we report to the published
data in witch MN has been shown to accumulate in
the stratum corneum (6, 19).
Permeation through the biological vs. synthetic
membranes. The amount of MN permeated in 24 h
through MB was determined almost exclusively by the
contact time between the sample and the membrane,
as the Pearson r have the highest values (0.9825 -
FI, 0.9908 - FII) which shows a strong correlation
(h vs. mg/cm² MN permeated), compared with Ms.
In these later cases, the Pearson r values of 0.9765 -
FI and 0.9655 - FII indicate there are some other factors
which affect the permeation process. Comparing
all cases, it appears that the formulation FII (3% HEC)
is more suitable than FI (2% HEC) for further
studies which involve biological membranes.
Influence of polymeric matrix on permeation process.
Area under the permeation curves (AUC) was calculated
for the period of 1 h to 24 h and had the following
values (mean ± SD, n = 3): 78.61 ± 4.3 (FI-Mb),
62.44 ± 5.4 (FII-Mb) and 164.5 ± 9.2 (FI-Ms), 152.7 ±
8.5 (FII-Ms). AUC-FI vs. AUC-FII shows statistically
significant differences only for Mb (p = 0.0154; un-
paired t test, confidence interval of 95%), case in
which the variance was insignificant (Anova one way,
Tukey’s test) (Table III). The rate of MN permeation
expression by the slope of the regression
line (Table I), was significantly lower through Mb
(0.28 - FI, 0.22 - FII) than through Ms (0.43 - FI,
0.38 - FII), with time-lag (h) only in the Mb case
(1.2 - FI, 0.89 - FII), according to the calculated
data in the Table IV.

| Unpaired t test of AUC: FII vs. FI       | Mb        | Ms        |
|-----------------------------------------|-----------|-----------|
| p value                                 | 0.0154    | 0.1781    |
| p value summary                         | * significant | not significant |
| Significantly different (p < 0.05)?     | Yes       | No        |
| One- or two-tailed p value              | two-tailed | two-tailed |
| Difference between means                | -16.17 ± 3.985 | -11.8 ± 7.232 |
| 95% Confidence interval                 | -2.72 to -5.105 | -3.12 to 8.278 |
| R squared (eta squared)                 | 0.8045    | 0.3996    |
| F, Dfn, Dfd (F test)                    | 1.577, 2, 2 | 1.171, 2, 2 |
| p value                                 | 0.7761    | 0.921     |
| p value summary                         | not significant | not significant |

Table IV

| Tukey’s multiple comparisons test | Mean differences | 95.00% CI of differences | Significant/Summary |Adjusted p value |
|----------------------------------|------------------|--------------------------|---------------------|-----------------|
| FI-Mb vs. FI-Ms                  | -85.89           | -104.6 to -67.19         | Yes                 | ****            | < 0.0001|
| FI-Mb vs. FII-Ms                 | -74.09           | -92.79 to -55.39         | Yes                 | ****            | < 0.0001|
| FII-Mb vs. FI-Ms                 | -102.1           | -120.8 to -83.36         | Yes                 | ****            | < 0.0001|
| FII-Mb vs. FII-Ms                | -90.26           | -109 to -71.56           | Yes                 | ****            | < 0.0001|
| FI-Mb vs. FII-Mb                 | 16.17            | -2.527 to 34.87          | No                  | no significance | 0.0921|
| FI-Ms vs. FII-Ms                 | 11.8             | -6.897 to 30.5           | No                  | no significance | 0.2570|

Conclusions
The biggest challenge for formulators is to transport
the active substance to the desired site for a targeted
action. Compared to the synthetic membrane, the
biological membrane reduces to almost half the in
vitro permeation of MN released from polymeric
matrices consisting in HEC-PEG of 2:1 (FI) or 3:1
(FII). The differences found between the two analysed
dermal systems are insignificantly influenced by HEC
content, which means the permeation variation could
be due to the thickness difference between the membranes
(0.45 µm - Ms, < 1 mm - Mb), the PEG slightly content
variation and/or the influence of PEG on the biological
membrane, as it was used in formulations both as
plasticizer and as promoter of absorption. The
formulation FII (3% HEC) is more suitable than FI (2% HEC) for further studies which involve biological membranes, to optimize the composition by design of experiment and the factorial analysis.

Conflict of interest
The authors declare no conflict of interest.

References
1. Abd E, Yoisel SA, Pastore MN, Telaprolu K, Mohammed YH, Namjoshi S, Grice JE, Roberts MS, Skin models for the testing of transdermal drugs. Clin Pharmacol., 2016; 8: 163-176.
2. Abruzzo A, Nicoletta FP, Dalena F, Cerchiara T, Luppi B, Bigucci F, Bilayered buccal films as child-appropriate dosage form for systemic administration of propranolol. Int J Pharm., 2017; 531(1): 257-265.
3. Agut J, Palacin C, Sacristán A, Ortí J, Inhibition of ergosterol synthesis by sertaconazole in Candida albicans. Arzneim Forsch., 1992; 42(5A): 718-720.
4. Ahmed TA, El-Say KM, Mahmoud MR, Samy AM, Badawi AA, Miconazole Nitrate Oral Disintegrating Tablets: In Vivo Performance and Stability Study. AAPS Pharm Sci Tech., 2012; 13(3): 760-771.
5. Amiri A, Yu A, Webster DC, Ulven CA, Bio-Based Resin Reinforced with Flax Fiber as Thermorheologically Complex Materials. Polymers, 2016; 8(4):153-160.
6. Bergamo VZ, Donato RK, Dalla Lana DF, Donato KJ, Ortega GG, Schrekker HS, Fuentefria AM, Imidazolium salts as antifungal agents: strong antibiofilm activity against multidrug-resistant Candida tropicalis isolates. Lett Appl Microbiol., 2015; 60(1): 66-71.
7. Bîrsan M, Aposto M, Todoran N, Antonoaica P, Rusu A, Ciurba A, Development of dermal films containing Miconazole Nitrate. Molecules, 2018; 23(7): 1640: 1-12.
8. Bîrsan M, Vieriu M, Bibire N, Cojocaru I, Influence of Hydroxypropyl Methylcellulose on Flowing and Swelling Parameters in Biomucoadhesive Tablets with Miconazole Nitrate. Rev Chim (Bucharest), 2017; 68(10): 2346-2349.
9. Cojocaru V, Ranetti AE, Hinescu LG, Ionescu M, Cosmescu C, Poșoreacă AG, Cinteza LO, Formulation and evaluation of in vitro release kinetics of NaCaDTTPA decoporation agent embedded in microemulsion-based gel formulation for topical delivery. Farmacia, 2015; 63(5): 656-664.
10. De Luca L, Naturally occurring and synthetic imidazoles: their chemistry and their biological activities. Curr Med Chem., 2006; 13(1): 1-23.
11. Dinu Pirv C, Hlevca C, Ortan A, Prisada R, Elastic vesicles as drug carriers through the skin. Farmacia, 2010; 58 (2):128-135.
12. Graham P, Browne L, Capp A, Fox C, Graham J, Hollis J, Nasser E, Randomized, paired comparison of No-Sting Barrier Film versus sorbolene cream (10% glycerine) skin care during postmastectomy irradiation. Int J Radiat Oncol Biol Phys., 2004; 58(1): 241-246.
13. Guidance for Industry, Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation. 1997; Food and Drug Administration. SUPAC-SS.
14. Heeres J, Meerpoel L, Lewi P, Conzaoles. Molecules, 2010; 15(6): 4129-4188.
15. Herkenne C, Naik Y, Kalia YN, Hadgraft J, Guy RH, Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man. Pharm Res., 2006; 23(8): 1850-1856.
16. Rădulescu FS, Voicu VA, Stănescu AA, Miron DS, Shah VP, Correlation between rheology, in-vitro release and in-vivo performance of topical dosage forms: DISSO ASIA 2014 International Symposium.
17. Jacobi U, Kaiser M, Toll R, Mangelsdorf S, Audring H, Oiberg N, Sterry W, Lademan J, Porcine ear skin: an in vitro model for human skin. Skin Res Technol., 2007; 13(1): 19-24.
18. Maya Jacob J, Sabu T, Biofibres and biocomposites. Carbohydr Polym., 2008; 71(3): 343-364.
19. Marichal P, Vanden Bossche H, Mechanisms of resistance to azole antifungals. Acta Biochim Pol., 1995; 42(4): 509-516.
20. Mitragotri S, Anissimov YG, Bunge AL, Frasch HF, Guy RH, Hadgraft J, Kasting GB, Lane ME, Roberts MS, Mathematical models of skin permeability: An overview. Int J Pharm., 2011; 418(1): 115-129.
21. Morales JO, Brayden DJ, Buccal delivery of small molecules and biologics: of mucoadhesive polymers, films, and nanoparticles. Curr Opin Pharmacol., 2017; 36: 22-28.
22. OECD, Guidance Notes on Dermal Absorption. Series on Testing and Assessment, No. 156. ENV/JM/MONO (2011)36, 18-Aug-2011.
23. Panaite AD, Popa G, Pumflil D, Butnaru E, Vasile C, Tartau LM, Gafitanu C, In vitro characterization of polyvinyl alcohol/chaosan hydrogels as modified release systems for bisoprolol. Farmacia, 2018; 66(1): 44-48.
24. Simon A, Amaro MI, Healy AM, Cabral LM, de Sousa VP, Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with in vivo-in vitro correlation. Int J Pharmaceut., 2016; 512(1): 234-241.
25. Todoran N, Antonoaica P, Rusu A, Ciurba A, Bîrsan M, Rédai E, DSC and FT-IR Analysis for the Formulation of Dermal Films with Meloxicam in Biodhesive Polymeric Matrices. Rev Chim (Bucharest), 2018, 69(12): 3692-3697.
26. Touitou E, Barry BW, Enhancement in Drug Delivery. CRC Press, Taylor & Francis Group, LLC, 2019.
27. Udeanu DI, Albu Kaya MG, Ghica MV, Marin S, Marin Maria Minodora, Kaya DA, Popa L, Dinu-Pirvc C, Anti-inflammatory drug-loaded biopolymeric sponges matrices with therapeutic perspectives in burns treatment. Farmacia, 2018; 66(5): 783-790.
28. Vanden Bossche H, Dromer F, Improvisi I, Lozano-Chiu M, Rex JH, Sanglard D, Antifungal drug resistance in pathogenic fungi. Med Mycol., 1998; 36(Suppl 1): 119-128.