

IN VITRO DRUG RELEASE FROM TOPICAL ANTIFUNGAL PHARMACEUTICAL FORMULATIONS

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Abstract

The paper presents the evaluation of the diffusion profiles obtained for ciclopirox olamine, naftifine hydrochloride and terbinafine hydrochloride from liquid and semisolid topical dosage forms, using a vertical diffusion cells system (VDC). The aim was to evaluate the *in vitro* kinetics of the drugs' release, including both formulation characteristics and the drugs' physico-chemical properties in the analysis of the results. The *in vitro* drug release parameters are highly dependent on the formulation factors, a considerable influence being assigned to the drug solubility within the pharmaceutical vehicle, but also to the viscosity and its role in the overall diffusion resistance. The apparent ranking of the diffusion coefficient values for each drug product confirmed the theoretical biopharmaceutical assumptions, but the biological relevance is dependent upon the experimental conditions (mainly the nature of membrane interface and/or receptor media).

Rezumat

Lucrarea prezintă evaluarea profilelor de difuzie obținute pentru ciclopirox olamina, naftifin clorhidrat și terbinafin clorhidrat din forme farmaceutice lichide și semisolide, utilizând un sistem de celule vertical de difuzie. Scopul a fost de a evalua cinetica de cedare *in vitro*, incluzând caracteristici de formulare și proprietățile fizico-chimice ale substanțelor în analiza rezultatelor. Parametrii de cedare *in vitro* sunt dependenți de factorii de formulare, o influență considerabilă fiind atribuită solubilității substanței active în vehiculul farmaceutic, dar și vâscozității, prin rolul acestora în rezistența difuzională globală. Ordinea aparentă a coeficienților de difuzie obținuți pentru fiecare produs farmaceutic a confirmat considerentele biofarmaceutice teoretice, dar relevanța biologică este dependentă de condițiile experimentale (în special de natura interfeței membranare și/sau a mediului receptor).

Keywords: ciclopirox olamine, naftifine hydrochloride, terbinafine hydrochloride, vertical diffusion cell system (VDC).

Introduction

The vertical diffusion cells (VDC) are one of the regulatory recommended experimental equipment for the evaluation of *in vitro* drug release profiles in case of semisolid dosage forms [1]. Since the official adoption of the Scale-Up Post-Approval Changes Guidance in 1997 by the United States Food and Drug Administration [2], the 1% hydrocortisone cream formulation has been imposed as an adequate reference for the Performance Verification Test (PVT) [3]. It can also be considered a key starting point in the development of specific quality control procedures. The variability of experimentally-determined or predicted physico-chemical properties for the drugs currently administered by transdermal route, as well as the particularities of drug formulations designed for assuring the accurate biocompatibility, stability and bioavailability of the therapeutic agents, are some of the most important factors to be considered in the design of the *in vitro* drug release tests.

Several experimental parameters have been studied as potential high-variability or low-performance sources for the results generated by implementing the *in vitro* drug release tests [4]. The temperature, the composition of the receptor media and the nature of the membrane, subject to various pre-treatment procedures intended to increase the biological relevance of the quality control tests, displayed a significant impact on the release profiles. While for the temperature, the $32\pm0.5^{\circ}\text{C}$ value represents a commonly adopted parameter for the simulation of the *stratum corneum* conditions, the selection of the membrane interface is still controversial. It has been previously reported that hydrophilic membranes, combined with a hydro-alcoholic media [5] revealed considerable differences between lipophilic and hydrophilic topical formulations. Nevertheless, for the use of hydrophilic membrane pre-soaked in either isopropyl-myristate with or without ethoxylated aliphatic amines it is reasonable to consider a significant change of kinetic profile, since the interface ceases to behave as an inert support of the drug formulation. In this particular case, a supplementary, lipophilic compartment will mediate the diffusion process, while accurate evaluation of the adsorbed fraction is difficult to be performed.

The biopharmaceutical profile of antifungal drugs for topical administration is frequently dominated by low aqueous solubility, necessary for the distribution across the biological barriers. From the formulation standpoint, this characteristic imposes in several instances the presence of high quantities of aliphatic alcohols, acting also as absorption promoters. Currently, the evaluation of lipophilic (especially cream) topical drug

products represents the mainstream in the regulatory-driven development of the official *in vitro* drug release guidance [1]. Therefore, limited data are reported for liquid formulation with local administration, although special designs or adaptors for VDC (vertical diffusion cells), e.g. with 2 and 6 mL volumes [6], are available.

The implemented experimental protocol aimed the comparison of the diffusion profiles for liquid and semisolid topical dosage forms containing ciclopirox olamine, naftifine hydrochloride and terbinafine hydrochloride. The aim was to evaluate the kinetics of drug release in conditions recommended for the PVT, but considering both formulation and drug physico-chemical characteristics in the analysis of the results.

Materials and methods

The *in vitro* release profiles of the active ingredients were evaluated using a 6 VDC system (Hanson Microette System, Hanson Research, USA). The individual volume was determined by accurately weighing the empty and water-filled assembled cells, at 25°C (9.47 ± 0.12 mL). For the receptor media, a hydro-alcoholic system (30% ethanol absolute in purified water, v/v) was prepared and degassed by filtration using 0.45 µm regenerated cellulose membranes under vacuum, followed by stirring for 2 min at room temperature (a higher value of the temperature was considered to alter the media composition, by partial evaporation of the alcoholic component). The temperature of the system was set to 32°C, using a Lauda Ecoline Staredition E100 / 090 thermostat. The membranes composed of hydrophilic polysulfone (Tuffryn[®], PALL Life Sciences HT-450, 0.45 µm average pore diameter, batch no. T72556) were soaked in the receptor media for 30 minutes. The same period of time was used for equilibration of the hydro-alcoholic mixture within each cell, under vigorous stirring (1000 rpm).

Naftifine hydrochloride was purchased from Effepi S.R.L., Italy; all the other analytical standards were purchased from Sigma Aldrich. The purified water was generated by a SGW Ultraclear UV Plus[™] system.

The drug products (identified in table I) were applied on the membrane using Teflon wafer (15 mm internal diameter, corresponding to an exposed membrane surface of 1.767 cm²) and either occlusive glass disk or 6 mL low-viscosity liquids adaptor (accommodating 2 mL of drug product) (Table I).

Table I

The description of the antifungal drug products used in the experiment

Active ingredient	Commercial name	Dosage form, concentration	Batch number	Expiry date
Ciclopirox olamine	Batrafen [®] solution	Topical solution, 10 mg/ml	0F005A	02.2014
	Batrafen [®] cream	Cream, 10 mg/g	0F013A	02.2012
	Stieprox [®] shampoo	Medicated shampoo, 1.5%	377L	10.2011
Naftifine hydrochloride	Exoderil [®] solution	Topical solution, 10 mg/ml	10175	03.2015
	Exoderil [®] cream	Cream, 10 mg/g	B0435409	11.2014
	Naftifina Antibiotice	Cream, 10 mg/g	042 0008	06 2011
Terbinafine hydrochloride	Lamisil [®] DermGel	Gel, 1%	K01202B	04.2013
	Lamisil [®] cream	Cream, 1%	K01798A	06.2015
	Terbisil [®] cream	Cream, 1%	G03001B	03.2012

All experiments were performed in triplicate. The stirring rate was set to 400 rpm. The manual sampling procedures (0.5 mL waste and 0.5 mL sample) were performed at 15, 30, 60, 90, 120, 150 and 180 min. The quantitative evaluation of each active ingredient was performed after dilution (1:9) with the receptor media, using a Jasco UV-Vis V-530 spectrophotometer (equipped with Spectra Manager software for Windows 95/NT, version 1.54.03) at 297.0 nm for ciclopirox olamine, 252.8 nm for naftifine hydrochloride and 223.4 nm for terbinafine hydrochloride (correlation coefficient, $R^2 > 0.9999$).

The *in vitro* drug profiles were analyzed using the Higuchi model for solutions [7]. The cumulative amount released per unit of surface area (Q) for a specific sampling time was calculated using the concentrations determined in the corresponding (C_n) and previous (C_i) samples concentrations, the individual cell volume (V), the sampling volume (v) and the diffusion surface (S), according to the formula [4]:

$$Q = (C_n \cdot V + \sum_{i=1}^{n-1} C_i \cdot v) / S$$

The prediction of solubility and water / n-octanol partition coefficient were calculated using ADMET Predictor software, version 5.0.0012.

Simulation Plus Inc. Marvin software was used for drawing, displaying and characterizing chemical structures (Marvin version 5.3.8, 2010; www.chemaxon.com).

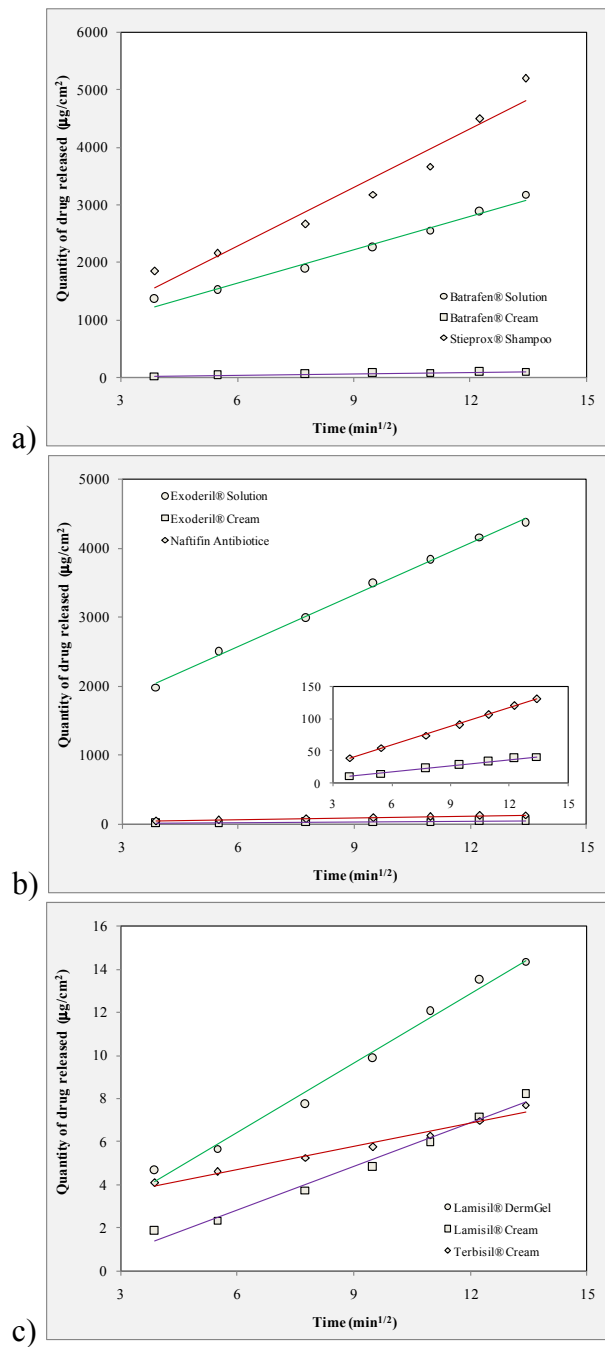
Results and discussion

The use of a simple hydro-alcoholic mixture combined with a hydrophilic membrane generates discriminatory *in vitro* drug release profiles. The diffusion coefficient, calculated as the slope of the linear regression of the quantity released per unit area as a function of the square root of time display the highest values in case of solution formulation, either alcoholic or aqueous with considerable amounts of tensioactive agents (shampoo).

Table II
The linear regression parameters for the *in vitro* drug release profiles

Commercial name	Slope ($\mu\text{g}/\text{cm}^2/\text{min}^{1/2}$)	Intercept ($\text{min}^{1/2}$)	Correlation coefficient
Batrafen [®] Solution	193.86	-2.30	0.9922
Batrafen [®] Cream	8.05	1.50	0.9997
Stieprox [®] Shampoo	340.06	-0.22	0.9740
Exoderil [®] Solution	250.10	-4.24	0.9986
Exoderil [®] Cream	3.23	0.75	0.9982
Naftifina Antibiotice	9.61	-0.08	0.9992
Lamisil [®] DermGel	1.08	0.14	0.9944
Lamisil [®] Cream	0.67	1.92	0.9907
Terbisil [®] Cream	0.36	-6.66	0.9896

It is to be mentioned that the use of a reduced volume of the alcoholic solutions of ciclopirox olamine and naftifine hydrochloride (2mL, compared to the 6mL maximum volume) was selected based on preliminary tests. The low volume allows a rapid heating to 32°C, while the remaining 4mL represents a reasonable volume for expansion of volatile components, while not exerting a pressure on the drug product (transforming the diffusion processes in a filtration).

**Figure 1**

In vitro drug release profiles obtained for the considered antifungal drug products (quantity released per unit area as a function of square root of time)
 a) ciclopirox olamine; b) naftifine hydrochloride; c) terbinafine hydrochloride.

The diffusion coefficients in case of the two alcoholic solutions were $193.86 \mu\text{g}/\text{cm}^2/\text{s}^{1/2}$ for ciclopirox olamine and $250.09 \mu\text{g}/\text{cm}^2/\text{s}^{1/2}$ for naftifine hydrochloride, while the predicted aqueous solubility was lower for the later compound (table III). It is reasonable to consider that the main driving force for the *in vitro* release process is the concentration gradient, combined with drug solubility within the formulation itself. The shampoo formulation is susceptible to alter the solubility of the active ingredient within the receptor compartment, by the concomitant diffusion of various components from the complex mixture of tensioactive agents (the value of diffusion coefficient reaching $340.06 \mu\text{g}/\text{cm}^2/\text{s}^{1/2}$, with cumulative amounts of ciclopirox olamine released of 5.2 mg in 3 hours).

The role of solubility in the formulation as the release rate controlling parameter in case of liquid (hydrophilic) formulations is taken by the lipophilicity, although, in some instances, a relationship between the two parameters can be described. A linear correlation was observed between predicted water / n-octanol partition coefficient (logP), and the diffusion coefficient values obtained in the case of cream formulations ($R^2 > 0.967$).

Table III

The predicted values of aqueous solubility and partition coefficients for the considered antifungal drugs

Compound	Aqueous solubility ($\mu\text{g}/\text{ml}$)	logP	MW	logKp (cm/s)
Ciclopirox	1000	2.35	207.27	-5.90
Naftifine	4.65	5.43	287.41	-4.20
Terbinafine	6.31	6.09	291.44	-3.75

where: logP – water/n-octanol partition coefficient; MW – molecular weight; logKp – permeability coefficient through the skin.

Potts RO and Guy RH [8] reported the following dependence of the permeability coefficient through the skin (K_p , cm/s) on logP and molecular weight (MW):

$$\log K_p = -6.3 + 0.71 \cdot \log P - 0.0061 \cdot MW$$

The relation, established on a group of 90 compounds, suggests the complexity of the *in vivo* diffusion processes. In fact, the optimal logP values for accurate delivery through the skin are between 1 and 3 (criteria met only by ciclopirox), since higher values indicate a possible strong binding to phospho-lipidic interfaces. The correlation logKp and diffusion coefficient ($R^2 > 0.977$) is probably due to the reduced influence of the MW [9] (table III). Nevertheless, it seems highly probable that the *in vivo* diffusion process, especially for terbinafine hydrochloride, will display

higher rate than *in vitro* predicted, although the lipophilicity reduces the extent by limitations on distribution (substrate binding) phase.

Conclusions

The *in vitro* drug release parameters obtained for the liquid and semisolid drug products containing antifungal drugs are highly dependent on the formulation factors, a considerable influence being assigned to the drug solubility within the pharmaceutical vehicle, but also to the viscosity and its role in the overall diffusion resistance.

The apparent ranking of formulations based on the diffusion coefficient values (solutions > hydrophilic semisolids > lipidic semisolids) confirms the theoretical biopharmaceutical assumptions, but this fact could be also due to the hydrophilic experimental conditions that concerns the membrane interface and the receptor compartment.

For quality control purposes, solubility in both donor and receptor phase, correlated or not with logP values, must be included as criterion for development of valuable tests in case of VDC. One should also consider the changes induced by the formulation factors to the composition of the receptor (surfactants, aliphatic alcohols) and furthermore, on the sink-conditions.

Acknowledgements

This work was supported by Romanian UEFISCDI grant PNII 42-135/2008. One of the authors (FSR) acknowledges the financial support given through the CNCSIS-UEFISCDI, project number PNII-RU 138/2010. Portions of these results were generated by ADMET Predictor™ software provided by Simulations Plus, Inc., Lancaster, California, USA.

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Manuscript received: September 25th 2010