KETOCONAZOLE IN TOPICAL PHARMACEUTICAL FORMULATIONS. 
THE INFLUENCE OF THE RECEPTOR MEDIA ON THE IN VITRO DIFFUSION KINETICS

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Abstract
The paper describes the evaluation of the in vitro release profiles of ketoconazole from various semisolid and liquid formulations using a vertical diffusion cells system and two types of receptor media, differing by the solubilizing capacity for the active pharmaceutical ingredient (hydro-alcoholic mixture and phosphate buffer pH=5.4, 50 mM). The experimental results indicated the impact of both composition of the receptor media and nature of the pharmaceutical dosage form (the hydrophilic or lipophilic character, the content of tensioactive agents and their solubility in the receptor media, the process of incorporation of the drug into the vehicle) on the diffusion profiles.

Keywords: ketoconazole, vertical diffusion cells, solubility, in vitro release.

Introduction
The United States Pharmacopeia \cite{1} defines the sink conditions by means of the volume three times higher than the one generating a saturated solution, for a defined quantity of drug substance. It is to be mentioned that
the sink conditions are not mandatory, while the use of available tools to achieve them (e.g. high quantities of surfactant, high volume of dissolution media, pH values without any physiological relevance etc.) must be justified. The usefulness of the in vitro release methodology has been established mainly for the solid dosage forms, either as quality control (QC) test or predictive methodology for the in vivo drug product performance.

Several active pharmaceutical ingredients (API) have been the subject of non-correlations reports between in vitro dissolution rate or extent and the in vivo bioavailability, especially in the case of immediate release formulations [2,3]. The API solubility profile has been frequently incriminated to be one of the main causes, since the current compendial recommendations specify a percentage released at the end of the test. The use of classical, 900-1000 mL dissolution vessels imposes in the case of tablets, for example, the change of several parameters in order to increase the solubility. As a general observation and key support for the use of tensioactive agents, the in vivo dissolution at the level of gastro-intestinal tract is better than in vitro predicted, based only on pH values for various regions. For the semi-solid dosage forms with local administration, the qualitative and quantitative composition of the formulation has a different and far more complex influence on the general release kinetics of API. From the point of view of in vitro release assessment, one of the most important differences is that the current draft guidance allows the use of various quantities of alcohol at the level of the receptor media [4]. This fact is based on the nature of the biological interface mediating the drug transfer at the administration site (mainly lipidic, if one considers the stratum corneum as the limiting barrier), but also on the difficulties reported in the use of the tensioactive agents in combination with the vertical diffusion cell design (degassing procedure of the receptor media, increase incidence of air bubble formation on the membrane during the test).

Concerning the receptor media, most of the currently reported tests, mainly on experimental formulation (not QC tests), employ a phosphate buffer system with pH=7.2 to 7.4, by similarity with the blood or plasma compartment. Nevertheless, in several instances, its use generates low levels of API released, despite the extended duration of the test, the general diffusion profile being solubility-limited.

The paper presents the analysis of the in vitro drug release profiles generated by vertical diffusion cell systems in the case of three ketoconazole marketed drug products and four experimental formulations, using two receptor media (hydro-alcoholic mixture and phosphate buffer system pH=5.4) as extreme solubility conditions.
Materials and methods

A manual system of 6 vertical diffusion cells (Hanson Research, Chatsworth, CA) was used for the evaluation of ketoconazole release profiles (the description being provided in figure 1). The system was composed of a 6 cells drive, a speed controller, a media replace backer and a circulating bath (Lauda Ecoline Staredition E100 / 090 thermostat). Due to the nature of the formulations, both dosage wafers for semi-solid dosage forms (15 mm internal diameter, accommodating approximately 300 mg of product) and liquid adaptors (for a volume of 6 mL) were used.

The individual volume of each cell (12 mL), determined by weighing with purified water, was found within ± 10 mL of the nominal value. The two receptor media (absolute ethanol:purified water mixture (1:1, v:v) and phosphate buffer pH=5.4, 50 mM) were degassed by filtration through 0.45 µm cellulose acetate membranes at room temperature.
The diffusion membranes were of cellulose ester type (Teknokroma® membrane filters code TR-200240, 0.45 µm average pore diameter, batch no. 133895), soaked in the corresponding receptor media for 30 minutes.

Three available pharmaceutical formulations with ketoconazole (cream, shampoo and capillary lotion) were purchased from the local market. Supplementary, four experimental formulations with ketoconazole were prepared, using 1% Carbomer 940 as gel forming agent (see Table II). For Gel F1 and Gel F2 (representing two levels of concentrations for API), the polymer was soaked and hydrated in water for 24 hours, neutralized with triethanolamine and mixed with a ketoconazole solution prepared in a mixture of hydrochloric acid 2N, absolute ethanol and a non-ionic surfactant, polyoxyethylene sorbitan monooleate. In the case of Gel F3, the above-mentioned solubilizing mixture was used for the hydration of the macromolecule, while Gel F4 was generated by simple dispersion of ketoconazole in the formulation, having the same qualitative and quantitative composition.

### Table I

The description of drug products and experimental formulations of ketoconazole

<table>
<thead>
<tr>
<th>No.</th>
<th>Abbreviation</th>
<th>Product</th>
<th>Ketoconazole content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N-Cream</td>
<td>Nizoral® Crema, Janssen Pharmaceutica NV, batch no.9FB1P00</td>
<td>2 %</td>
</tr>
<tr>
<td>2</td>
<td>N-Shampoo</td>
<td>NizoralTM Shampoo, Janssen Pharmaceutica, batch no.9JB3R00</td>
<td>2 %</td>
</tr>
<tr>
<td>3</td>
<td>Tis-lotion</td>
<td>Keto-Tis gel, Tis Pharmaceutic, batch no. 79 08 2009</td>
<td>2 %</td>
</tr>
<tr>
<td>4</td>
<td>Gel F1</td>
<td>Carbomer 940 gel 1%, neutralized with triethanolamine (ketoconazole was dissolved in ethanol-Polysorbate 80-hydrochloric acid 2N solution mixture and dispersed in the hydrophilic matrix)</td>
<td>1 %</td>
</tr>
<tr>
<td>5</td>
<td>Gel F2</td>
<td>Carbomer 940 gel 1% (the polymer was allowed to soak and hydrate into the ketoconazole solution prepared as for Gel F1 or F2, and neutralised with triethanolamine)</td>
<td>2 %</td>
</tr>
<tr>
<td>6</td>
<td>Gel F3</td>
<td>Carbomer 940 gel 1% (ketoconazole suspended in the hydrophilic matrix)</td>
<td>1 %</td>
</tr>
<tr>
<td>7</td>
<td>Gel F4</td>
<td>Carbomer 940 gel 1% (ketoconazole suspended in the hydrophilic matrix)</td>
<td>1 %</td>
</tr>
<tr>
<td>8</td>
<td>Solution</td>
<td>Ketoconazole solution in the receptor media, 3 mL</td>
<td>40 µg/mL</td>
</tr>
</tbody>
</table>

For the evaluation of possible adsorption processes of ketoconazole on the cellulose ester membranes, two solutions of ketoconazole in each receptor media were prepared (concentration of 40 µg/mL), serving also as references with reduced viscosity resistance in the overall diffusion process.
The temperature was maintained at 32.5°C during the experiments. The stirring rate was set at 400 rpm. The sampling procedures (2 aliquots of 0.5 mL) were performed manually, using 1.0 mL Hamilton syringe at 15, 30, 60, 90, 120, 150 and 180 minutes after introducing the drug product (the determinations were conducted in triplicate). The quantitative determination of ketoconazole was performed after dilution (1:9 or 1:29) 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 221 nm.

Ketoconazole (purity ≥ 98%), ethanol (CHROMASOLV®, absolute, for HPLC), anhydrous monobasic sodium phosphate and dibasic sodium phosphate dihydrate were purchased from Sigma Aldrich. The purified water was generated by a SGW Ultraclear UV Plus™ system (conductivity < 2 µS/cm).

Results and discussion

The in vitro drug release profiles displayed a high influence of both formulation and nature of the receptor media (figure 2). As a general observation, the hydro-alcoholic media generated higher amounts of ketoconazole released, compared to the aqueous buffer system, except for the shampoo drug product. It should be mentioned that usually, hydrophobic or anhydrous formulations are preferred as topical vehicle for the antifungal azoles [5,6], since the clinically effective doses of these therapeutic agents request either drastic acidic condition (e.g. pH values of 1.2, as mentioned in the tablet dissolution monograph, simulating the gastric environment) or higher volume of fluid for complete solubility and therefore, accurate thermodynamic activity. However, it is the same pH-solubility pattern that generates the incompatibility with neutral conditions mandatory for hydrophilic gel structuring. In this particular case, although the shampoo formulation is designed for short time contact with the skin and only after the dilution with water, the obtained release profile indicates a concomitant diffusion of the tensioactive components into the receptor compartment and therefore, a dynamic change of the solubility for ketoconazole. It is possible that this particular diffusion process of the solubilizing agents could explain the different rates for the two receptor media, almost double for the phosphate buffer (1409 and 718 µg/cm²/min¹/₂, respectively). The cumulative amount released over the 180 min interval overpasses the theoretical solubility.
Figure 2

*In vitro* diffusion profiles of ketoconazole (quantity released per unit area as a function of square root of time)

Thus, the correlations with the *in vivo* processes are difficult to establish. Actually, for the high content of excipients with a considerable influence on the solubility of the active ingredient, the biological mechanism of action as absorption promoters could in fact be limited to the alteration of the barrier properties, while assuring the molecular dispersion status within the donor vehicle.
For the cream formulation, diffusion-controlled kinetics is displayed only in the case of the phosphate buffer receptor media (diffusion coefficient value of $2.67 \mu g/cm^2/min^{1/2}$, correlation coefficient $R^2$ of 0.99). The hydro-alcoholic mixture generates probably a receptor media back-diffusion process (due to the high percentage of the ethanol component), inducing a two region profile ($1.48 \mu g/cm^2/min^{1/2}$, for the first 90 min interval, and almost 10 times higher for the next region). Together with the shampoo and lotion formulations, this represents a clear case where the experimental design should consider not only the solubility profile of the API, but also the nature of the pharmaceutical formulations [7,8]. It can be also stated that when in vitro release profiles are compared, the similarity conclusion should consider the pharmaceutical equivalence as mandatory.

On the other hand, the experimental formulations represent a case of identical qualitative and quantitative composition, while the preparation methodology was varied in order to induce different thermodynamic activity for ketoconazole. The phosphate buffer system displayed no discriminatory character, the release profiles being essentially similar due to the solubility limitations. The hydro-alcoholic mixture revealed kinetic similarity between the two concentrations of API (similar diffusion coefficient, with almost double diffused amounts, for Gel F1 and F2), while discriminating between the methods of incorporation. The linearity of cumulative drug released per unit area as a function of square root of time (Higuchi equation) is observed only for Gel F3. In the other cases, the kinetics is neither of zero nor of first order (figure 3).

![Figure 3](image)

The comparative presentation of the cumulative release (%) of ketoconazole from the four experimental formulations in hydro-alcoholic media
The experimental data were fitted only with the Korsmeyer-Peppas equation for Gel 4 (ketoconazole dispersed in the hydrophilic matrix), usually implemented for evaluation of drug release pattern from polymeric systems:

$$\frac{M_t}{M_\infty} = k \cdot t^n$$

where the $\frac{M_t}{M_\infty}$ represents the fraction of drug released (%) at time $t$, $k$ is a constant dependent on structural and geometrical characteristics, and $n$ is the release exponent, correlated with the release kinetics and mechanism [9]. The exponent value of 1.78 (correlation coefficient $r^2 > 0.997$) indicates a Super Case II kinetics.

The solutions prepared in the two receptor media displayed similar diffusion kinetics (it concerns both rate and extent), while the mass balance of the system proved no significant quantitative adsorption of ketoconazole on the membrane surface. The diffusion coefficient values (approximately 2 $\mu$g/cm$^2$/min$^{1/2}$) are mainly the result of the diffusional resistance displayed by the membrane, the overall process being actually a filtration. The protocol seems to be a more accurate approach in order to evaluate the adsorptive phenomenon. The current protocols recommend the filtration of a solution of API through the membrane using an appropriate holder, without considering the particular kinetics of delivery of the drug from the formulation to the potentially retentive interface. The current approach indicates the amount adsorbed, concomitantly simulating all the implemented parameters (receptor media, temperature, stirring rate).

It seems reasonable to assume that the pharmaceutical equivalence must be mandatory when comparing two products for the assessment of their in vitro release similarity. Various inactive components of the drug products, even in reduced quantities, could alter the solubility and therefore, the kinetic pattern, during the test. For a more accurate evaluation of the profile, especially in the case of pilot formulations, a more detailed sampling schedule in the first hour could reveal critical information on drug product quality and on the mechanism of release.

**Conclusions**

The hydro-alcoholic media displayed a discriminatory character, concerning both formulation and the method of incorporation of the active ingredient into the pharmaceutical vehicle. The use of physiologically relevant phosphate buffer pH=5.4 limited the diffusion process, since it provides a low solubility for ketoconazole.
For the evaluation of the possible adsorption of the active ingredient to various interfaces of the experimental device, applying a solution of the drug in the receptor media while using the special adaptors and preserving all the other experimental parameters could be a better approach than the simple filtration.

Acknowledgements
This work was supported by Romanian UEFISCDI grant PNII 42-135/2008. One of the authors (RFS) acknowledges the financial support given through the CNCSIS-UEFISCDI, project number PNII-RU 138/2010. The authors also acknowledge the kind support of the representatives of ABL&E-Jasco Romania S.R.L.

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