THE SOLUBILITY OF OXICAMS, ANTIFUNGAL AZOLES AND SELECTED PSYCHOTROPIC DRUGS IN SIMULATED GASTRO-INTESTINAL FLUIDS AND THE CONSEQUENCES ON THEIR BIOPHARMACEUTICAL CLASSIFICATION

FLAVIAN ȘTEFAN RĂDULESCU¹, VICTOR VOICU², DALIA SIMONA MIRON³*, MIRELA ADRIANA MITU⁴, ANDREEA LETIȚIA ARSENE⁵

University of Medicine and Pharmacy „Carol Davila” Bucharest, Faculty of Pharmacy, 6th Traian Vuia street, 020956, Bucharest, Romania
¹Department of Drug Industry and Pharmaceutical Biotechnologies, ²Department of Clinical Pharmacology, Toxicology and Psychopharmacology, Faculty of Medicine, ³Department of Pharmaceutical Physics and Informatics, ⁴Department of Pharmaceutical Technology and Biopharmaceutics, ⁵Department of Biochemistry.
*corresponding author: dalia_simona_m@yahoo.com

Abstract
The role of solubility in four simulated gastro-intestinal fluids in classification of drugs with different biopharmaceutical profiles was investigated. The results underline the different impact of composition variables (mainly pH and concentration of the endogenous tensioactive agent) on the in vivo absorption process, since either dissolution rate or solubility limitation are generally assumed. The most promising candidates for biowaver procedure seem to be the oxicam anti-inflammatory drugs, while safety precaution restricts further extension to psychotropic drugs. The supersaturation process is confirmed for azole antifungals, with major influence of the pH values recorded for ketoconazole.

Keywords: simulated gastro-intestinal fluids, biopharmaceutical profiles, antifungal azoles, oxicams, psychotropic drugs.

Rezumat
A fost evaluat rolul solubilităţii în patru fluide fiziologice simulate pentru clasificarea unor medicamente cu profile biofarmaceutice diferite. Rezultatele subliniază impactul diferit al variabilelor de compoziţie (în principal pH-ul și concentraţia agentului tensioactiv endogen) asupra proceselor de absorbtie in vivo, întrucât în general sunt asociate limitări ale vitezei de dizolvare sau de solubilitate. Cel mai probabil, procedura de exceptare de la starea in vivo se poate aplica în cazul oxicamilor cu efect anti-inflamator nesteroidian, în timp ce extinderea conceptului la agenţii psihotropi este limitată de considerente de siguranţă. Procesul de suprasaturare a fost confirmat pentru azolii antifungici, valorea pH-ului având o influenţă majoră în special pentru ketoconazol.

Keywords: simulated gastro-intestinal fluids, biopharmaceutical profiles, antifungal azoles, oxicams, psychotropic drugs.
Introduction

The role of simulated gastric and intestinal fluids in the development of predictive in vitro – in vivo correlations is illustrated by several applications in drug candidate selection or forecasting drug product performance, within biowaiver framework [1,2]. Particular interactions and specific impact of pH, tensioactive agent nature and concentration have been described. The starting points for their composition have been represented by the simulated gastric or intestinal fluids, as recommended by the United States Pharmacopeia (USP), critically adjusted by adding several endogenous components. Although not adopted by the regulatory authorities as official monographs, the experimental models using fast or fed state simulated fluids, generate supportive data and provide accurate information for the development of solid oral dosage forms [3]. The compendial perspective on gastro-intestinal fluids changed over time, as more information become available on pH, volume and composition of secretion, motility and differences between the animal species considered as alternative models. For example, the 1996 change of pH for the simulated intestinal fluid (USP-SIF) from 7.5 to 6.8 generated several modifications within the solubility and permeability based classification systems [3]. The food effect on the pharmacokinetic profile of several drugs is difficult to predict, as the gastric acid media used for the compendial in vitro dissolution test does not include a tensioactive substance, while the role of several food components, involved in increased drug binding or dissolution is not simulated [4]. Nevertheless, it is to be pointed out that the current bioavailability / bioequivalence approaches of the regulatory authorities, including the in vitro evaluation requirements as described in the Scale Up Post Approval Changes framework, do not necessarily include structural or therapeutic class approaches. The water solubility and the permeability estimated by simple lipid-water partition coefficient are displaying an important, but sometimes overestimated role in the overall bioavailability profile of a given xenobiotic. Their limit values used for classification purposes are subject to continuous debate. Also, the supersaturating phenomenon, specific to weak basic drugs such as antifungal azoles, cannot be accounted for within currently protocols used mainly as quality control tools, since they do not simulate the dynamics of qualitative and quantitative composition of the gastrointestinal fluids. Lately, for the most promising class concerning potential extension of biowaiver concept (exemption from in vivo clinical studies based on in vitro model), that is class II drugs with low solubility high permeability profiles, two subclasses have been
described, based on dissolution rate limited or solubility limited characteristics [1].

The current paper presents a concise, rapid experimental protocol for the evaluation of the solubility parameters of several drugs in gastric and intestinal, fast and fed state simulated fluids, according to currently recommended composition [5-7]. The experimental and estimated values of solubility were correlated, in an attempt to point out particular profiles and to elucidate the potential source of errors.

Materials and methods

Drugs from three major therapeutic classes have been selected, mainly based on their known biopharmaceutical properties and on structural similarities: psychoactive agents (clozapine, haloperidol, olanzapine, paliperidone, risperidone), oxicams with non-steroidal anti-inflammatory profiles (meloxicam, piroxicam, tenoxicam) and weak basic antifungal azoles ( clotrimazole, itraconazole, ketoconazole, miconazole). The intestinal biorelevant dissolution media applied for simulation of fast or fed state (FaSSIF, FeSSIF) composition were prepared according to the formula of Dressman J. [6]. For the gastric level, the current recommendations of Jantratid E. et al [4] were implemented, with reduced milk content for the fed state simulated gastric fluid (FeSSGF) (Table I). The ratio between the acetate buffer and milk was increased from 1 to 3, with a provisioned lower bounded fraction to protein or lipid components.

<table>
<thead>
<tr>
<th>Media</th>
<th>Component / parameter</th>
<th>Concentration/value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FaSSGF</strong> (Fast State Simulated Gastric Fluid)</td>
<td>Sodium taurocholate</td>
<td>80 µM</td>
</tr>
<tr>
<td></td>
<td>Lecithin</td>
<td>0.75 mM</td>
</tr>
<tr>
<td></td>
<td>Pepsin</td>
<td>0.1 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride</td>
<td>34.2 mM</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>FeSSGF</strong> (Fed State Simulated Gastric Fluid)</td>
<td>Sodium chloride</td>
<td>237.02 mM</td>
</tr>
<tr>
<td></td>
<td>Acetic acid</td>
<td>17.12 mM</td>
</tr>
<tr>
<td></td>
<td>Sodium acetate</td>
<td>29.75 mM</td>
</tr>
<tr>
<td></td>
<td>UHT milk 3.5% : acetate buffer</td>
<td>1:3</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>5.0</td>
</tr>
</tbody>
</table>
The shake-flask method was applied by adding 500 µL of each media to an excess of drug substance, using 1.5 mL Eppendorf polypropylene vials (all determinations were performed in triplicate). The vials were vigorously mixed for 30 seconds at 5000 rpm, using Ika® Vortex Genius 3 Shaker and maintained for 4 hours at 37°C, on a Lauda Ecoline Staredition E100 / 090 thermostat bath. The duration was selected based on both reported transit times (0 to 4 hours, largely variable depending on various physiological and pathological factors) and stability of the mentioned media (8 hours in physiological temperature and 72 hours in ambient conditions). The samples were centrifuged for 10 minutes at 14000 rpm at the same biorelevant temperature and 200 µL from the supernatant were diluted with 1800 µL methanol. Spectrophotometric methods were implemented for quantitative evaluations of drug dissolved, with calibration probes prepared in methanol and processed blank media as reference. For ketoconazole, clotrimazole and miconazole, first derivative spectra were obtained, due to the lack of a specific absorption maximum in the ultraviolet domain.

The prediction on theoretical values of FaSSGF, FaSSIF and FeSSIF were performed with ADMET Predictor software, version 5.0.0012, Simulation Plus Inc.

All the standards and reagents were of analytical grade. The purified water was generated by a SGW Ultraclear UV Plus™ system.

Results and discussion

The experimental data revealed the different biopharmaceutic behaviour of the analyzed sub-groups of drug substances. In the case of antifungal drugs, the major fact is the dependence of solubility on the pH, the influence of the tensioactive agents being rather reduced. In the case of ketoconazole, the lower pH value in the case of fasted state gastric fluid leads to solubility values almost 41 times higher compared to fast state, mainly due to the low pH (4.5 mg/mL, for pH=1.6 and 0.11 mg/mL, for pH=6.0) (figure 1). The lower solubility profile in the intestinal region confirms the potential of precipitation at intestinal level, one of the reasons of the non-correlation of in vitro - in vivo data for antifungal azoles [8,9]. The compendial methods for in vitro release require the acidic pH for the dissolution media. For itraconazole, one of the typical representatives of class II drugs, the low solubility conclusion is valid independent of the fast or fed state. For clotrimazole and miconazole, an intermediate behaviour is concluded, with a minimum solubility recorded in the FaSSIF (maximum pH value is 6.5)
Solubility (µg/mL)

0  500  1000  1500  2000
Itraconazol  Clotrimazol  Ketoconazol  Miconazol

Figure 1
Solubility of antifungal azoles in simulated gastro-intestinal fluids (µg/mL)
(FaSSIF – fast state simulated intestinal fluid; FeSSIF – fed state simulated intestinal fluid; FaSSGF – fast state simulated gastric fluid; FeSSGF – fed state simulated gastric fluid)

In the case of psychotropic drugs, the solubility profile presents a maximum in the fed state simulated gastric fluid, correlated with weak basic characteristics (figure 2). It is interesting that the 9-hydroxy metabolite of risperidone, paliperidone, theoretically more hydrophilic since it is the product of a metabolic transformation, presents a higher solubility in all the four simulated media, while maintaining an accurate permeability across biological barriers. It displays an approximately 2.6 times higher solubility in the intestinal environment with a minimum decrease of the n-octanol water partition coefficient (0.1 units).

Figure 2
Solubility of psychotropic drugs in simulated gastro-intestinal fluids (µg/mL)
The complex composition of the simulated fluids (especially the presence of endogenous tensioactive agent [10]) induces theoretically an almost complete dissolution of the maximum strength for all psychotropic drugs, while the high permeability is assumed as they penetrate the high-specialized blood-brain barrier in order to express their pharmacodynamic effect.

The oxicams seem to be the favorites for the biowaiver procedures. Since their solubility displayed a reduced dependence on either pH or surface tension of the media (figure 3), it is reasonable to assume a low contribution of these physiological factors on intra and inter-individual variability. Therefore, the impact of the formulation performance on the overall bioavailability characteristics is major, and the predictive power of \textit{in vitro} dissolution test results is considerable.

For the correlation between the experimental and predicted values of solubility, it should be noted that the software used didn’t provide data of FeSSGF solubility. For all the other media, the calculation procedures seem to overestimate the solubility in several instances (figure 4). Nevertheless, considering the reduced number of elements, the best correlation was reported for psychotropic drugs in simulated intestinal fluids ($r^2 = 0.765$ for FaSSIF and 0.873 for FeSSIF), probably due to the fact that some of the structures are included in the training-set of the prediction software.
Figure 4
Correlation between the experimental and predicted values of solubility in simulated gastro-intestinal fluids
(FaSSIF – fast state simulated intestinal fluid; FeSSIF – fed state simulated intestinal fluid; FaSSGF – fast state simulated gastric fluid)
Conclusions
A concise, rapid experimental protocol for the evaluation of the solubility parameters for several drugs in gastric and intestinal, fast and fed state simulated fluids was developed. The results suggest that, concerning the drugs from the class II of the Biopharmaceutical Classification System, the most promising candidates for biowaver procedure are the oxicam anti-inflammatory drugs. Safety issues, but also possible involvement of active transport systems and their potential modulation by several excipients (permeability phase), restricts further extension to the analyzed psychotropic drugs. The supersaturation process is confirmed for azole antifungals, with major influence of the pH values recorded for ketoconazole.

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References

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