DISSOLUTION OF TAMOXIFEN IN BIORELEVANT MEDIA. A TWO PHASE RELEASE MODEL

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Abstract

The present paper evaluates the release kinetics of tamoxifen from tablets, in biorelevant media as a prediction of the in vivo behavior.

Four biorelevant media have been chosen to model the composition of the gastric and intestinal contents before and after meal intake. Results indicated that the effects of bile secretion are greater than that of food-induced pH variations. As a consequence, the effect of the changes in the gastrointestinal content associated with food ingestion was predicted to be an increase in the bioavailability. A special characteristic of all dissolution curves was that a part more or less significant of tamoxifen was released immediately. After this initial phase the rate of the process looked rather like a diffusion controlled release. Analysis of data using Higuchi, Peppas and Weibull models indicated the best fitting was obtained in the case of Higuchi square root law. Weibull model was not applicable for the entire domain of concentrations a two phase regression analysis being the single applicable model.

Keywords: Tamoxifen, biorelevant dissolution media, release kinetics modelling
Introduction

Dissolution testing was implemented as a tool for quality testing of solid pharmaceutical forms, mainly tablets and capsules. More recently, an expansion of the role of dissolution as predictor of in vivo absorption was attempted. Since the new objectives were much more complex, the compendial dissolution tests proved to be frequently non adequate, due to an ignorance of physiological phenomena, a series of non-correlations being reported. [1, 2]

As part of a drive to develop predictive in vitro models to forecast the in vivo performance of drugs and drug products, two biorelevant dissolution media simulating conditions in the proximal small intestine, Fasted-State Simulated Intestinal Fluid (FaSSIF) and Fed-State Simulated Intestinal Fluid (FeSSIF), were proposed in 1998. [3]

Together with the medium simulating the preprandial gastric conditions (FaSSGF) proposed by Vertzoni et al. in 2005, [4], and postprandial gastric conditions (FeSSGF) presented by Jantratid et al. [5], a core group of four biorelevant media simulating both stomach and proximal small intestine of humans in the pre- and postprandial states has been established. These media can be used to investigate the release characteristics of drugs and drug products in the stomach and small intestine, particularly in terms of food effects.

The aim of the present study is the development of biorelevant dissolution tests for pharmaceutical formulations containing a BCS class II drug, Tamoxifen, and modelling its release kinetics in terms of successful predicting their in vivo performance.

Tamoxifen, commonly used for adjuvant therapy of breast cancer, is a key drug for chemoprevention of breast cancer in high-risk women [6, 7] Chemically, Tamoxifen Citrate is the trans-isomer of a triphenylethylene derivative, and is formulated in oral pharmaceutical products as a Tamoxifen: citric acid 1:1 complex. (figure. 1)

![Tamoxifen Citrate chemical structure](image.png)
Tamoxifen is a weak base, therefore ionization will occur in the gastric environment, leading to a predicted rapid dissolution in the stomach. As the drug is emptying from the stomach to the duodenum, the degree of ionization is significantly reduced due to the elevated pH, with possible precipitation of the drug. [8]. Its high lipophilicity (logP= 5.93) [9] makes the presence a food-effect highly probable. This leads to a complicated intestinal absorption model, controlled by many factors, including the extent of supersaturation, pH, fluid volume, viscosity, and bile salts concentration, therefore biorelevant simulation of gastrointestinal conditions is essential to adequately predict its in vivo behaviour.

Tamoxifen belongs to BCS Class II (high permeability, low solubility) [10] providing dissolution rate-limited absorption. Substances that belong to class II possess poor aqueous solubility but are easily transported across the GI mucosa. Correlation of in vivo results with dissolution tests is likely to be best for those drugs, because in this case the dissolution rate is the primary limiting aspect to their absorption. [11, 12, 13]

Materials and Methods
Tamoxifen citrate was obtained from Actavis. Tamoxifen Sandoz® 10 mg tablets, lot BP 8573, was purchased commercially.

Physiological compounds - granular Lecithin, (Acros Organics) Pepsin (Fluka) and Sodium taurocholate 97% (Sigma) were used for the preparation of biorelevant media. Acetic acid, sodium acetate trihydrate, sodium chloride (NaCl), sodium dihydrogen phosphate monohydrate and NaOH pellets were all of analytical grade and purchased from Merck KGaA (Darmstadt, Germany). 37% hydrochloric acid (conc. HCl) was obtained from Riedel-de Haën. Long-life heat-treated and homogenized milk (UHTmilk) containing 3.5% fat was purchased commercially.

Media Preparation
Compendial Dissolution Media
Compendial dissolution media for comparative approach consisted in the followings [14]:
- Simulated Gastric Fluid (SGF) pH 1.2 buffer (Table I)
- Simulated Intestinal Fluid (SIF) pH 6.8 buffer (Table II)

Biorelevant Dissolution Media
A set of four biorelevant media was used, proved to be representative for the fasted stomach (FaSSGF), the postprandial stomach
(FeSSGF), (Table I) fasting state conditions in the small intestine (FaSSIF) and simulated postprandial conditions in the small intestine, (FeSSIF). (Table II)

### Table I.
Composition of the Compendial and Biorelevant Gastric Dissolution Media

<table>
<thead>
<tr>
<th></th>
<th>SGF</th>
<th>FaSSGF</th>
<th>FeSSGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (Sodium Chloride)</td>
<td>2.0 g</td>
<td>34.2 mM</td>
<td>237.02 mM</td>
</tr>
<tr>
<td>Pepsin</td>
<td>3.2 g</td>
<td>0.1 g</td>
<td></td>
</tr>
<tr>
<td>CH₃COOH (Acetic Acid)</td>
<td>-</td>
<td>-</td>
<td>17.12 mM</td>
</tr>
<tr>
<td>CH₃COONa (Sodium Acetate)</td>
<td>-</td>
<td>-</td>
<td>29.75 mM</td>
</tr>
<tr>
<td>Milk/Buffer</td>
<td>-</td>
<td>-</td>
<td>1:1</td>
</tr>
<tr>
<td>Sodium taurocholate</td>
<td>-</td>
<td>80 µM</td>
<td>-</td>
</tr>
<tr>
<td>Lecithin</td>
<td>-</td>
<td>20 µM</td>
<td>-</td>
</tr>
<tr>
<td>HCl (Hydrochloric Acid)</td>
<td>7 ml</td>
<td>q.s. ad pH 1,6</td>
<td>q.s. ad pH 5</td>
</tr>
<tr>
<td>Water (q.s. ad)</td>
<td>1 L</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.2</td>
<td>1.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Surface tension (mN/m)</td>
<td>70</td>
<td>42.6</td>
<td>52.3 ± 0.3</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>120.7 ± 2.5</td>
<td>400 ± 10</td>
<td></td>
</tr>
<tr>
<td>Buffer capacity (mmol/L/ΔpH)</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolution Volume (mL)</td>
<td>1000</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

### Table II.
Composition of the Compendial and Biorelevant Intestinal Dissolution Media

<table>
<thead>
<tr>
<th></th>
<th>SIF</th>
<th>FaSSIF</th>
<th>FeSSIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaH₂PO₄ (Monobasic sodium phosphate)</td>
<td>6.8 g</td>
<td>3.438 g</td>
<td></td>
</tr>
<tr>
<td>NaCl (Sodium Chloride)</td>
<td>-</td>
<td>6.186 g</td>
<td>11.874 g</td>
</tr>
<tr>
<td>CH₃COOH (Acetic Acid)</td>
<td>-</td>
<td>-</td>
<td>8.65 g</td>
</tr>
<tr>
<td>NaOH pellets</td>
<td>-</td>
<td>-</td>
<td>4.04 g</td>
</tr>
<tr>
<td>Pancreatin</td>
<td>10 g</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sodium taurocholate</td>
<td>-</td>
<td>3 mM</td>
<td>15 mM</td>
</tr>
<tr>
<td>Lecithin</td>
<td>-</td>
<td>0.75 mM</td>
<td>3.75 mM</td>
</tr>
<tr>
<td>NaOH (Sodium Hydroxyde)</td>
<td>q.s. ad pH 6.8</td>
<td>q.s. ad pH 6.5</td>
<td></td>
</tr>
<tr>
<td>Water (q.s. ad)</td>
<td>1 L</td>
<td>1 L</td>
<td>1 L</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Surface tension (mN/m)</td>
<td>54</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>270±10</td>
<td>670</td>
<td></td>
</tr>
<tr>
<td>Buffer capacity (mmol/L/ΔpH)</td>
<td>12</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Dissolution Volume (mL)</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>
Dissolution Studies

Drug release experiments were performed with USP Apparatus 2 (Paddle), DT 800H, Erweka, Germany. Each vessel was filled with 500mL/1000 mL of media, and an agitation speed of 75 rpm was used for all dissolution studies. Experiments were run in triplicate. Samples (5 mL) were removed after 5, 10, 15, 20, 30, 45, 60, 90, and 120 min using a glass syringe, then filtered through a 0.45-µm Teflon® filter and immediately diluted with methanol. Quantification of Tamoxifen was achieved by using a validated HPLC method [15].

HPLC Analysis

The analyses were carried out using a Waters 600E Multisolvent Delivery System, a Waters 717 Autosampler and a Waters 486 Tunable Absorbance Detector (Waters, Milford, MA, USA). The detector was set at 275 nm.

Samples of 5 µL were injected into a Hypersil Gold, 5-µm 150 x 4 mm, (Thermo Scientific) column. The mobile phase consisted of an acetonitrile: 0.1% trifluoracetic acid mixture = 60:40 (v/v) delivered at 1.0 mL/min flow rate.

Results and Discussion

Dissolution

Effect of dissolution medium composition on rate and extent of release

Effect of pH. Solubility of Tamoxifen decreases with increasing of the pH value. Consequently, its solubilization is decreased by pH changes during the transfer from gastric to intestinal fluid. Dissolution in simulated gastric fluid (pH 1.2) was practically immediate and complete. In simulated intestinal fluid (pH 6.8), almost 45 % of the Tamoxifen was released immediately and further the released quantity increased slow, approximately linear, to 60 % in two hours. It was not clear that this value represents saturation solubility or not.

Effect of bile salts. Presence of bile salts increases the solubility of Tamoxifen following its inclusion in micelles. Since sodium taurocholate concentration is greater in intestinal fluids than in gastric fluids, the final solubility of Tamoxifen by the intermediate of micelles, increase at the transfer from gastric to intestinal conditions.
Tamoxifen dissolution in simulated intestinal media

Tamoxifen dissolution in simulated gastric media

Figure 2

Tamoxifen release in simulated intestinal (A) and gastric media (B)

**Estimation of food effects following modifications of characteristics of gastrointestinal media.**

The above results allow an estimation of food effects on in vivo dissolution. Food effects are related to pH variation and increase of surface active agents concentration as a result of bile secretion, in gastrointestinal tract.

At gastric level, food leads to significant increase of pH, influence of surface active agents being minimal due to their small amounts in both fasted and fed state. Experiments showed a release much lower in gastric fed conditions (20 % versus 80 % in fasted condition)  

At intestinal level, food is slightly lowering the pH and substantially increasing bile salts concentration, consequently the Tamoxifen release is enhanced (over 80% release in 5 minutes) mainly as an effect of its mixed micelles inclusion.

Since bile salts and pH have antagonistic effect on solubilisation the result of their interaction cannot be predicted theoretically. Experimental it was obtained that food increases the rate and extent of release. Extent increased from 80% to 95 %.
In vitro simulated influence of food on Tamoxifen release

Since absorption is reduced at gastric level, food intake could promote an increased bioavailability.

Modelling of in vitro release kinetics in biorelevant media.

Release of Tamoxifen in all media was very rapid, saturation solubility being reached in less than 15 minutes. The only profile which seems to represent a release kinetics is dissolution in FaSSIF medium.

Intervariability of curves is very low (Figure. 4) Since, frequently, dissolution of the first 60% quantity of substance follow a diffusion mechanism, modelling of Tamoxifen release kinetics in FaSSIF was attempted by comparing goodness of fitting for different models describing diffusion.

Higuchi modelling of the release kinetics

Figure 3

In vitro simulated influence of food on Tamoxifen release.

Figure 4

Release of Tamoxifen in FaSSIF. Higuchi modelling.
Most simple diffusion based model is the Higuchi law, which describe the release as a lineer dependence of square root of time. Fitting of the experimental data appears to be successful ($r^2 = 0.98$). Unexplained is the fact that the obtained linear regression had not a lag-time. Much more difficult to understand is the fact that apparently, after the tablet-solvent contact, a part of the Tamoxifen is immediately transferred into solution. This behaviour appeared as a constant in all experiments whatever the release medium. Apparently there are two types Tamoxifen in the tablet – one immediately available and one bound more strongly and the ratio between the two fractions appeared to be strongly dependent on the release medium. It would be reliable to think as explication to a rapid interaction between solvent and matrix of the tablet, mainly povidone.

A more flexible model, based also on the hypothesis of diffusion controlled release is Peppas model:  
\[ R(\% \text{Released}) = k t^{\alpha} \]

The model is valid mainly in case of release from a polymeric matrix. It is expected that, if Higuchi model is applicable, Peppas is also applicable since for $\alpha = 1/2$ square root law is obtained.

![Figure 5](image)

Peppas modelling for the Tamoxifen release kinetics

It can be seen that the fitting is worse than in case of Higuchi model and the slope corresponds to $\alpha = 0.01$, a value significant different from $1/2$. (Figure 5). The explanation is difficult to find but is possible to be connected to the same suddenly release at the beginning of the dissolution.
A most flexible model is the empiric model based on the Weibull distribution: 

\[ R(t) = 1 - e^{-\alpha t^\beta} \]

with two parameters \( \alpha \) and \( \beta \). The model proved to be applicable in almost all dissolution curves.

![Weibull modelling of the release kinetics](image)

**Figure 6**

Weibull modelling for the Tamoxifen release kinetics

Once again the result is unexpected. Fitting with a single line is a poor solution. Instead of a single line, a two phase linear regression proved to be very successful (Figure 6). Testing the hypothesis that the slopes of the two lines indicated with \( p < 0.01 \) that this are significantly different. In fact all above analysis suggested a two phase evolution of the release phenomenon.

**Conclusions**

The release kinetics of Tamoxifen is strongly dependent on the composition of release media, critical factors being apparently the pH and concentration of physiological surface active agents.

Results suggested that effect of bile salts is in all cases greater than the effect of pH. As a resultant of these effects it was predicted that at intestinal level, the main site of absorption, food would increase solubility.

In all cases, a two phase dissolution behaviour was obtained: an initial sudden release followed by a slower phase.

In FaSSIF medium release appeared to follow a diffusion mechanism, data being well described by Higuchi square root law.

Application of Peppas and Weibull models in describing the entire process failed, a two phase line regression being a probable consequence of the two phase release mechanism.

In comparison with compendial media, the biorelevant media showed different dissolution profiles and a more predictive power.
Acknowledgements
This paper is partly supported by the sectorial operational Programme Human resources Development (SOP HRD), financed from the European social Fund and by the Romanian Government, under the contract number POSDRU/89/1.5/S/60782.

References

Manuscript received: April 12th 2011