

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.

Rezumat

Prezenta lucrare evindețiază cinetica cedării *in vitro* a tamoxifenului din comprimate, în medii biorelevante, în scopul predicției comportamentului *in vivo*. Testele de dizolvare au fost realizate în patru medii biorelevante simulând condițiile gastrice și intestinale înainte și după ingestia de alimente. Rezultatele au indicat faptul că secreția biliară postprandială are o influență mai mare asupra solubilizării tamoxifenului decât variațiile de pH de la nivelul gastrointestinal. În consecință, experimentele au sugerat faptul că modificările fiziologice asociate ingestiei de alimente induc o creștere a biodisponibilității.

O caracteristică specială a profilelor de dizolvare a fost aceea că o parte din cantitatea de substanță activă a fost eliberată imediat din comprimat. După această fază inițială, profilul cedării tamoxifenului sugerează un proces de cedare difuzională a acestuia. Analiza datelor folosind modelele Higuchi, Peppas și Weibull a indicat că cea mai bună fitare a datelor experimentale s-a obținut prin modelul Higuchi. Modelul Weibull nu a fost aplicabil pe întreg domeniul de concentrație, însă analiza regresiei pe două faze a condus la o fitare foarte bună a datelor experimentale.

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)

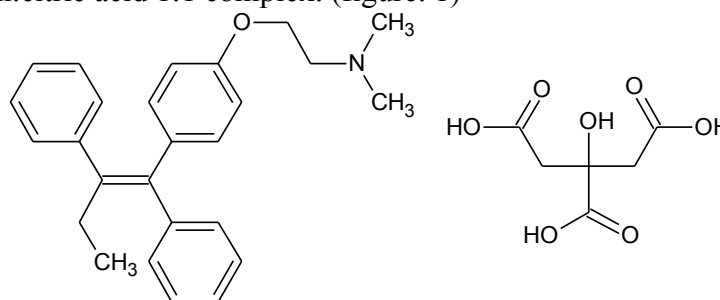


Figure 1
Tamoxifen Citrate chemical structure

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 lypophilicity ($\log P = 5.93$) [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

	SGF	FaSSGF	FeSSGF
NaCl (Sodium Chloride)	2.0 g	34,2 mM	237,02 mM
Pepsin	3,2 g	0,1 g	
CH ₃ COOH (Acetic Acid)	-	-	17,12 mM
CH ₃ COONa (Sodium Acetate)	-	-	29,75 mM
Milk/Buffer	-	-	1:1
<i>Sodium taurocholate</i>	-	80 μ M	-
<i>Lecithin</i>	-	20 μ M	-
HCl (Hydrochloric Acid)	7 ml	q.s. ad pH 1,6	q.s. ad pH 5
Water (q.s. ad)	1 L	1 L	
pH	1.2	1.6	5.0
Surface tension (mN/m)	70	42.6	52.3 $\pm$ 0.3
Osmolality (mOsm/kg)		120.7 $\pm$ 2.5	400 $\pm$ 10
Buffer capacity (mmol/L/ Δ pH)			25
Dissolution Volume (mL)	1000	500	500

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

	SIF	FaSSIF	FeSSIF
NaH ₂ PO ₄ (Monobasic sodium phosphate)	6.8 g	3.438 g	
NaCl (Sodium Chloride)	-	6.186 g	11.874 g
CH ₃ COOH (Acetic Acid)	-	-	8.65 g
NaOH pellets	-	-	4.04 g
Pancreatin	10 g	-	
<i>Sodium taurocholate</i>	-	3 mM	15 mM
<i>Lecithin</i>	-	0.75 mM	3.75 mM
NaOH (Sodium Hydroxyde)	q.s. ad pH 6.8	q.s. ad pH 6.5	
Water (q.s. ad)	1 L	1 L	1 L
pH	6.8	6.5	5.0
Surface tension (mN/m)		54	48
Osmolality (mOsm/kg)		270 $\pm$ 10	670
Buffer capacity (mmol/L/ Δ pH)		12	72
Dissolution Volume (mL)	1000	500	1000

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- μ m 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 Multisolvant 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 μ L were injected into a Hypersil Gold, 5- μ m 150 x 4 mm, (Thermo Scientific) column. The mobile phase consisted of an acetonitrile: 0.1% trifluoroacetic 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.

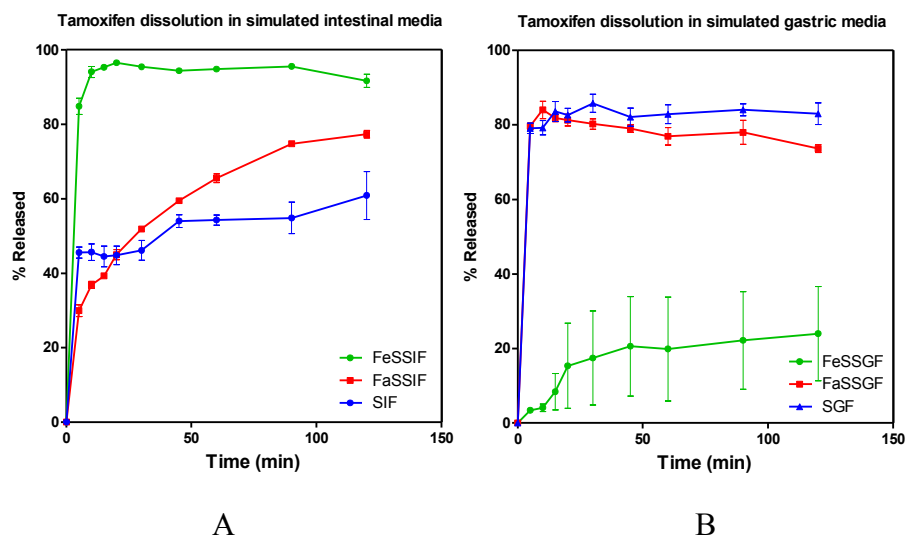


Figure 2
Tamoxifen release in simulated intestinal (A) and gastric media (B)

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 %.

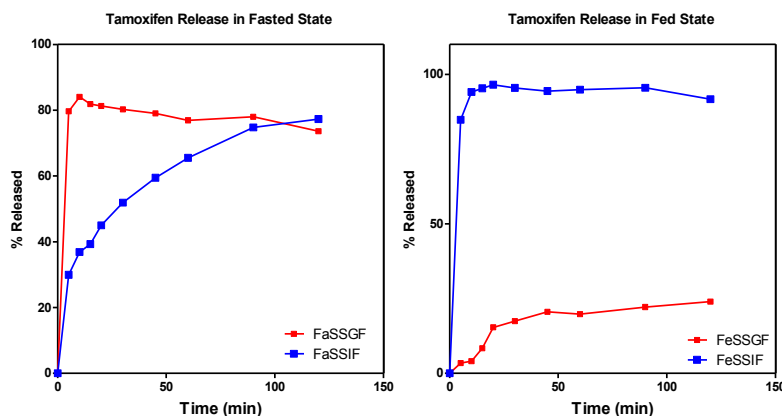


Figure 3

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

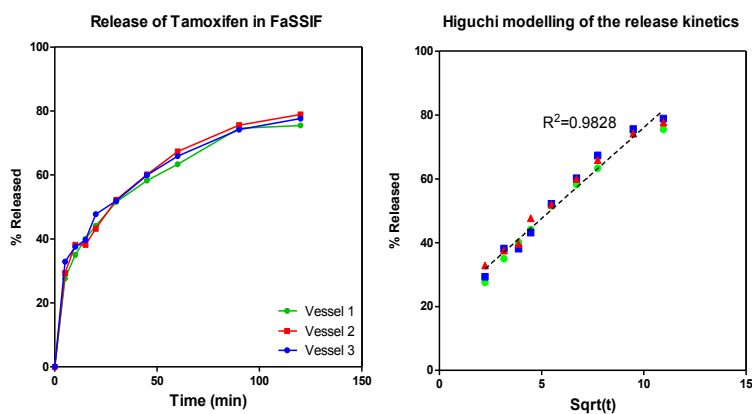


Figure 4

Release of Tamoxifen in FaSSIF. Higuchi modelling.

Most simple diffusion based model is the Higuchi law, which describe the release as a liner 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: $R(\%) = k t^\alpha$

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.

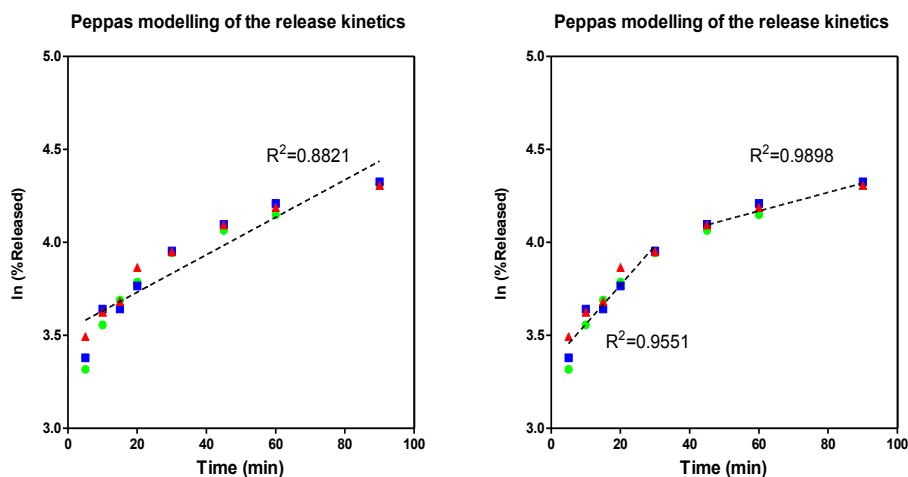


Figure 5
Peppas modelling for the Tamoxifen release kinetics

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 α and β . The model proved to be applicable in almost all dissolution curves.

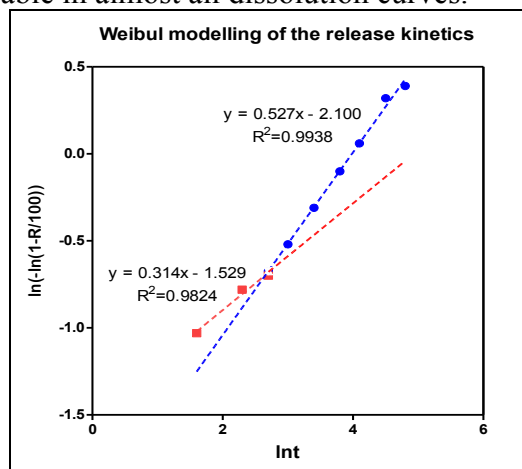


Figure 6

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

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References

1. Mircioiu C., Voicu V., Miron D., Mircioiu I: Non Standard Correlations: In vitro in vivo Correlations for Immediate Release Products: Comparison of Different Bioequivalence Experiments-*Basic and Clinical Pharmacology and Toxicology* 2005, 96, 259-261
2. Mircioiu C., Mircioiu I., Voicu V., Miron D: Dissolution – bioequivalence non-correlations. *Basic and Clinical Pharmacology and Toxicology* 2005, 96, 262-264
3. Dressman, J. B.; Amidon, G. L.; Reppas, C.; Shah, V. P. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm. Res.* 1998, 15 (1), 11–22.
4. Vertzoni, M.; Dressman, J.; Butler, J.; Hempenstall, J. Reppas, C. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. *Eur. J. Pharm. Biopharm.* 2005, 60 (3), 413–417.
5. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. B. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm. Res.* 2008, 25 (7), 1663–1676.
6. Tormey DC, Gray R, and Falkson HC. Postchemotherapy adjuvant Tamoxifen therapy beyond five years in patients with lymph node-positive breast cancer. Eastern Cooperative Oncology Group. *J Natl Cancer Inst.* 1996; 88: 1828–33.
7. Rutqvist LE, Johansson H, Signomklao T, et al. Adjuvant Tamoxifen therapy for early stage breast cancer and second primary malignancies. Stockholm Breast Cancer Study. *J Natl Cancer Inst.* 1995; 87: 645–51.
8. Galia, E.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res.* 1998, 15 (5), 698–705.
9. Tetko IV, Tanchuk VY, Villa AE. - Prediction of n-octanol/water partition coefficients from PHYSPROP database using artificial neural networks and E-state indices *J Chem Inf Comput Sci.* 2001;41(5):1407-21.
10. Amidon GL, Lennernäs H, Shah VP, Crison JR: A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* 1995, 12 (3): 413–20.
11. Nicolaides, E.; Galia, E.; Efthymiopoulos, C.; Dressman, J.B.; Reppas, C. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. *Pharm. Res.* 1999, 16 (12), 1876–1882.
12. Dressman, J. B.; Reppas, C. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. *Eur. J. Pharm.Sci.* 2000, 11 (Suppl 2), 73–80.
13. Nicolaides, E.; Symillides, M.; Dressman, J. B.; Reppas, C. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. *Pharm. Res.* 2001, 18 (3), 380–388.
14. *** United States Pharmacopeia 30 - National Formulary 25, Rockville MD: United States Pharmacopeia Convention, Inc.; 2007.
15. U.S. Department of Health and Human Services, Food and Drug Administration. Guidance for Industry, Bioanalytical Method Validation. <http://www.fda.gov/cder/guidance/4252fnl.pdf>

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