

RESEARCH CONCERNING THE DEVELOPMENT OF A BIORELEVANT DISSOLUTION TEST FOR FORMULATIONS CONTAINING NORFLOXACIN.

I. MODELLING OF *IN VITRO* RELEASE KINETICS

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Abstract

The paper presents a study of norfloxacin release kinetics from solid oral formulations in compendial and biorelevant media simulating the composition of the gastric and intestinal contents before and after meal intake in terms of establishing the predictive kinetic models to be further correlated with *in vivo* pharmacokinetic profiles. Norfloxacin release kinetics was strongly dependent on the dissolution medium as well as on formulation. Results indicated a very rapid and complete dissolution in compendial simulated gastric fluid (SGF) and in acetate buffer (pH 4.0), while in biorelevant media the release followed a different release kinetics, probably connected with the variability of solubility of norfloxacin in the proximity of the isoelectric point. Though bile salts were expected to increase the solubility of norfloxacin by the intermediate of micelles, this result appeared to be significant only under fed state simulated conditions (100% release versus 32% under fasting conditions). Experimental data was fitted using Higuchi, Peppas, Noyes-Whiney and Weibull models, in order to establish the most relevant predictive kinetic models.

All analysis suggested a two phase evolution of the release phenomena. This change in behaviour was thought as a consequence of both norfloxacin solubility changes during the release and its including in micelles by means of bile salts and lecithin.

Rezumat

Lucrarea prezintă un studiu privind cedarea *in vitro* a norfloxacinei din forme farmaceutice orale în medii compendiale și biorelevante simulând compoziția gastrică și intestinală pre- și postprandială și stabilește modele cinetice predictive pentru a fi corelate ulterior cu profilele farmacocinetice *in vivo*. Cinetica de cedare a norfloxacinei a fost puternic dependentă atât de mediul de dizolvare utilizat, dar și de formularea celor două produse testate.

Rezultatele au indicat o dizolvare rapidă și completă în mediile compendiale – fluid gastric simulat (SGF) și tampon acetat (pH 4.0), în timp ce în mediile biorelevante cedarea a avut un profil diferit, probabil datorat variabilității solubilității norfloxacinei în proximitatea punctului izoelectric. Deși era de așteptat ca sărurile biliare să determine o creștere a solubilității norfloxacinei prin intermediul includerii substanței active în micle, acest efect a fost semnificativ numai în condiții postprandiale (cedare 100%, față de doar

32% în condiții preprandiale). Analiza datelor s-a realizat utilizând modelele Higuchi, Peppas, Noyes-Whiney și Weibull, în scopul de a stabili modelul cu cea mai mare putere de predicție a datelor experimentale.

Analizele au sugerat o evoluție în două faze a cedării *in vitro*. Această schimbare a comportamentului a fost evaluată ca fiind o consecință atât a modificărilor de solubilitate a norfloxaciniei pe parcursul cedării, precum și a includerii în micelle coloidale.

Keywords: norfloxacin, biorelevant dissolution media, release kinetics modelling

Introduction

Norfloxacin is a synthetic chemotherapeutic antibacterial agent belonging to the flouroquinolones class, usually prescribed in urinary tract infections and gonorrhea [13]. Norfloxacin is 30–40% absorbed after oral administration [16]. Concurrent ingestion of food has been shown not to interfere with its absorption to a clinically significant degree. However, milk, yoghurt or antacid drugs significantly decrease the extent of absorption, through the formation of poorly absorbed chelate complexes [7].

Owing to the presence of both carboxylic and amino substituents on the nucleus, norfloxacin is an amphoteric molecule. Consequently, four different chemical species coexist in solution, corresponding to positive, zwitterionic, neutral and anionic forms; their relative abundance depending on the pH of the solution (Figure 1). These different molecular species have distinct physicochemical properties, with respect to water solubility and lipophilicity.

Following these transformations, norfloxacin exhibits a “U”-shaped pH–solubility profile with high solubility at pH values below 5 and above 10, presenting minimum solubility close to neutrality, in the proximity of the isoelectric point. Although the ionized forms are highly soluble, neutral forms are more lipophilic and have higher membrane permeability; consequently norfloxacin is primarily absorbed in the duodenum and jejunum [18].

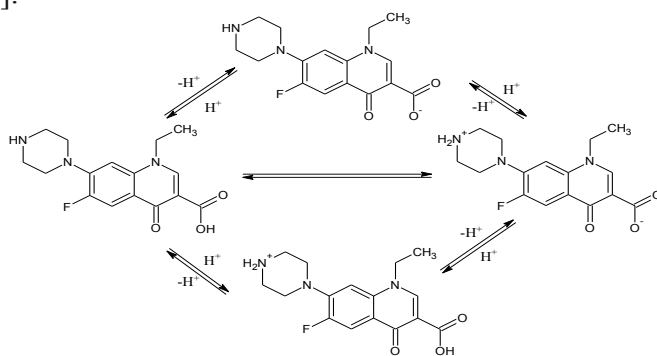


Figure 1

Protolytic equilibria of norfloxacin

The pH–solubility profile emphasized a minimum solubility of 0.31mg/mL at pH 7 [2]. According to the FDA (Food and Drug Administration), an active pharmaceutical ingredient (API) is defined as highly soluble if its dose: solubility (D/S) ratio at 37°C is below 250 mL, over the pH range 1.0–7.5 [19]. Since norfloxacin highest strength immediate release solid dosage form is 800 mg, the API has to be classified as low soluble, the D/S ratio exceeding by far the 250 mL limit at pH values close to neutrality [3].

Norfloxacin is absorbed by passive diffusion and there is a dose proportionality of AUC (area under the curve) in humans from 200 to 800mg [17]. Human pharmacokinetic data for norfloxacin show a fraction absorbed of only 30–40%, indicative of a poorly permeable compound [1,21]. The apparent permeability of norfloxacin either in non-everted intestinal sacs or Caco-2 cells was also described as low, confirming it is a BCS (Biopharmaceutical Classification System) low permeability drug [14].

A major concern in recent years was to identify bioavailability (BA) problems of drug formulations based on the results of appropriately designed dissolution experiments. Many of the compendial dissolution media described in International Pharmacopoeias proved to be frequently non adequate, due to an ignorance of physiological phenomena.

In the present paper, release kinetics of norfloxacin from solid formulations in physiological relevant media was investigated. Modelling of experimental data was performed in order to establish most relevant predictive kinetic model, to be further correlated with *in vivo* pharmacokinetics profiles.

Materials and Methods

Norfloxacin reference standard was purchased from Sigma (Sigma-Aldrich, St. Louis, USA). Two different immediate-release oral formulations containing 400 mg norfloxacin were 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 (UHT milk) containing 3.5% fat was purchased commercially.

Media Preparation

Compendial Dissolution Media for comparative approach consisted in the following [22]: Simulated Gastric Fluid SGF (Table I); pH 4.0 acetate buffer (USP30 dissolution medium for Norfloxacin tablets); Simulated Intestinal Fluid SIF pH 6.8 buffer (Table II).

Biorelevant Dissolution Media

A set of four biorelevant media was used, presented in recently published articles and 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) [4,20,6].

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
Sodium taurocholate	-	80 μ M	-
Lecithin	-	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	-	
Sodium taurocholate	-	3 mM	15 mM
Lecithin	-	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 500 mL/1000 mL of media, and an agitation speed of 50 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 Norfloxacin was achieved by using a validated HPLC method.

HPLC Analysis

The analyses were carried out using a Waters Liquid Chromatograph (600E Multisolute Delivery System, 717 Autosampler, 486 Tunable Absorbance Detector, Waters, Milford, MA, USA). The detector was set at 282 nm. The chromatographic separation was achieved on a Hypersil Gold, 5- μ m 150 x 4 mm column (Thermo Scientific) using as mobile phase an isocratic mixture of acetonitrile: methanol: potassium dihydrogen phosphate buffer pH 2.0 containing 5mM tetrabutylammonium hydroxide (4:16:40 v/v/v) delivered at 1.0 mL/min flow rate. Five microliters of each sample were injected onto the chromatographic column.

Results and Discussion

In vitro dissolution study

Norfloxacin release kinetic is strongly dependent on the dissolution media (figure 2).

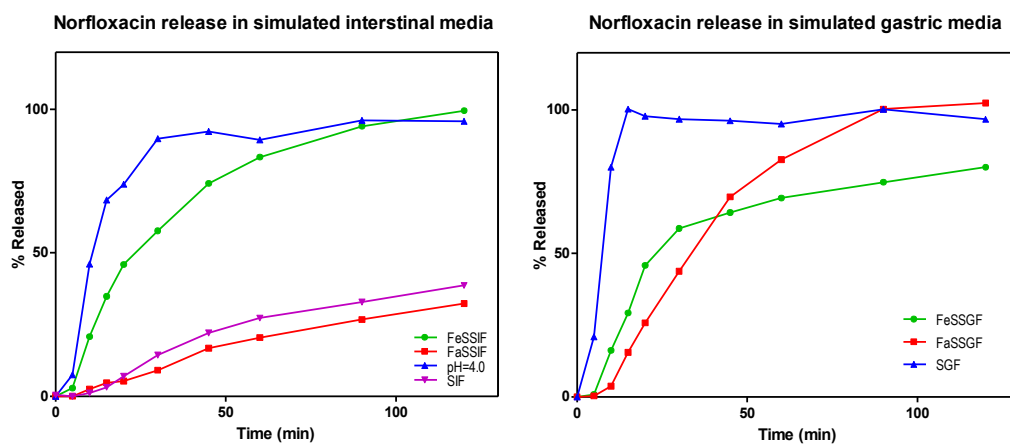


Figure 2

Norfloxacin release in simulated intestinal and gastric media

Norfloxacin solubility in gastro-intestinal (GI) tract is decreased by pH changes during the transfer from gastric to intestinal fluid. The dissolution in simulated gastric fluid (pH 1.2) was practically immediate and complete, whereas in simulated intestinal conditions (pH 6.8) the release was slow and incomplete (approximately 40% after two hours, value that could imply saturation of the dissolution medium). Differences between dissolution in simulated intestinal fluids are connected with the variability of norfloxacin solubility in the proximity of the isoelectric point, where the behaviour is critical: small pH variations lead to significant alterations in solubility. The food effect is related to pH variation and increase of surface active agents concentration as a result of bile secretion in gastro-intestinal tract.

At gastric level, food leads to a significant increase of pH, the influence of surface active agents being minimal due to their small amounts in both fasted and feed state.

Experiments emphasize a slightly lower release rate in gastric fed conditions than in fasted condition (80% *versus* 100 %), following the increase in pH up to 5 (value that fall into the 5 – 10 low solubility critical pH domain).

At intestinal level, food is slightly lowering the pH and substantially increasing bile salts concentration, consequently norfloxacin release in FeSSIF is enhanced (100% release, corresponding to maximum solubility) mainly as an effect of its mixed micelles inclusion. In fasting conditions, the released quantity is minimum (some 30 %). Since bile salts and pH have antagonistic effect on solubilisation, the result of their interaction cannot be predicted theoretically.

Release profiles stated that substances simulating food, increased significantly the rate and extent of release. The extent increased from 30% to 100 %.

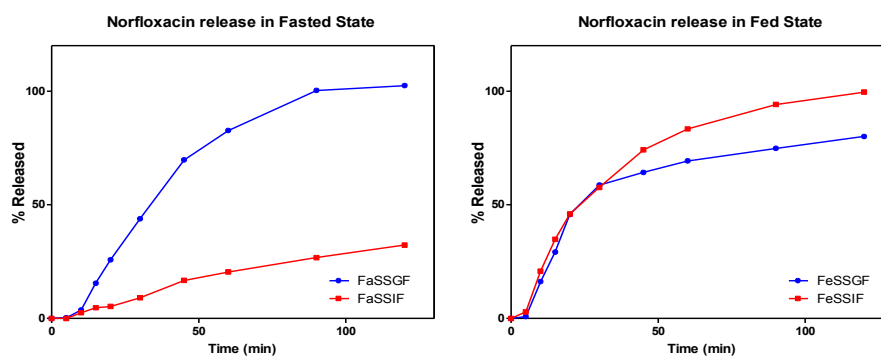


Figure 3
In vitro simulated influence of food on norfloxacin release

The presence of bile salts increases the solubility of norfloxacin following its inclusion in micelles. Since taurocholate concentration is greater in intestinal than in gastric fluids, the final solubility of norfloxacin by the intermediate of micelles, increases at the transfer from gastric to intestinal conditions. This expected result appeared only in feeding conditions. The above results allow an estimation of food effects on the *in vivo* dissolution.

Concurrent ingestion of food with fluoroquinolones has been shown not to interfere with their absorption to a clinically significant degree [7]. The dissolution media proved to have discriminatory power, being able to highlight differences in formulation between two non-bioequivalent norfloxacin formulations. (Figure 4).

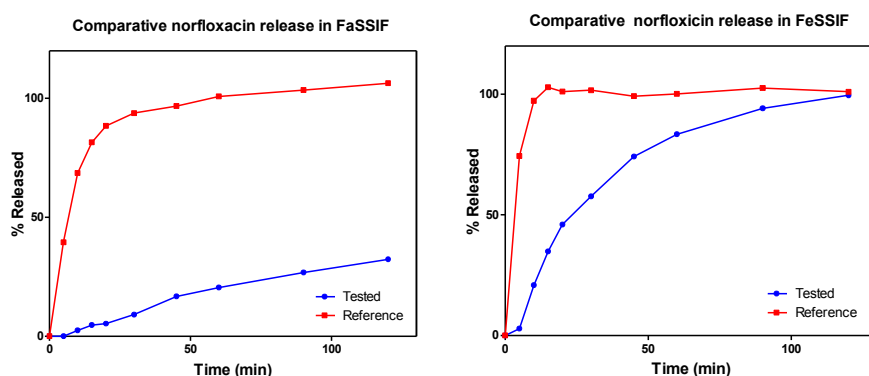


Figure 4

Release profiles for two norfloxacin formulations in simulated intestinal media

Modelling of *in vitro* release kinetics in media simulating intestinal content.

A. Higuchi Model

Since, frequently, the dissolution of the first 60% quantity of substance follows a diffusion mechanism, modelling of norfloxacin release kinetics in biorelevant simulated intestinal media (FaSSIF and FeSSIF) was attempted by comparing goodness of fit for different models describing diffusion [12,15]. The release in SGF and compendial acetate buffer was very rapid, a complete dissolution being achieved within 30 minutes, making modelling not applicable.

Most simple and most frequently diffusion based models are square root laws. The first square root law, Higuchi model, was an application of the Fick's laws to the release in a limit layer at the surface of a

pharmaceutical matrix (i.e. ointment, tablet) toward an external solvent which acts as a perfect sink under pseudo steady-state conditions [5]. Since the assumptions of the model are valid only in the first part of the release process, the application of this law is recommended only for the first 60% of the release curve [9]. In the evaluation of release profiles from ointments and insoluble matrices, the Higuchi law is expressed as a square root function:

$$m = \sqrt{DS(2c_0 - S)t} = \alpha t^{1/2}$$

where D is the diffusion coefficient, c_0 is the initial drug concentration in the matrix and S the solubility of the drug.

The fitting of experimental data appears to be very successful ($r^2 = 0.989$) in the case of release in FaSSIF (Figure 5)

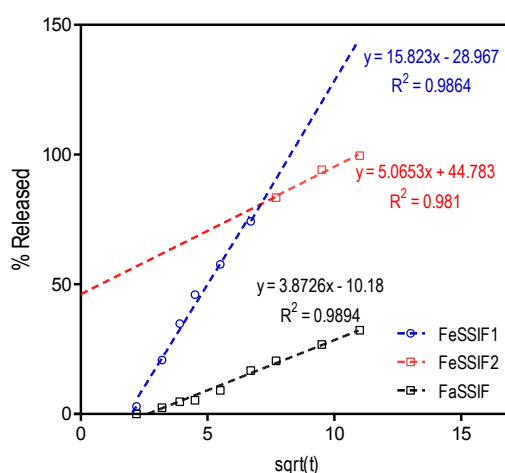


Figure 5
Higuchi modelling of norfloxacin release kinetics

The result was predictable, since the release was slow, no more than 35 % being dissolved within two hours.

For the initial release in FeSSIF (0-45 min), the dependence on the square root of time was clearly linear (Figure 5, FeSSIF1 curve), suggesting a pure diffusion mechanism. A clear change in the diffusion course occurred at a point around 75 %, the last three sampling points, corresponding to the release within the 60-120 min interval, being again connected linearly with the square root of time. (Figure 5, FeSSIF2 curve). Since the application of the square root law is frequently extended toward 70 % of the released drug, a successful fitting of experimental data is to be concluded.

It is to note that the second phase linear dependence in FeSSIF is approximately parallel with the line corresponding to the release in FaSSIF.

B. The Power-Law (Peppas) Model.

The release profiles from a range of formulations were analyzed using a power law equation proposed by Peppas, which was derived by considering both the effects of diffusion and erosion on drug release kinetics from colloidal systems [11].

$$r = \frac{m(t)}{m_{\infty}} = \alpha t^{\beta} \quad \text{or,} \quad \text{transformed in a linear dependence,}$$

$$\ln r = \ln \alpha + \beta \ln t$$

The model proved not to be appropriate, nor for entire domain, nor for 60% release, though a biphasic regression could reduce the residual sum of squares. (Figure 6)

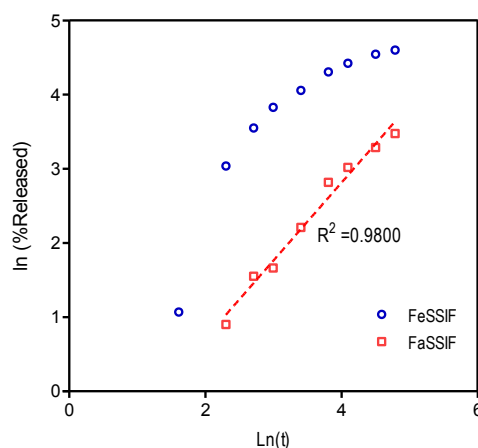


Figure 6

Peppas modelling of norfloxacin release kinetics

C. Noyes Whitney model construction starting from a diffusion equation

Fickian's first law is:

$$\frac{1}{A} \frac{dm}{dt} = J = -D \frac{\partial c}{\partial x}$$

where m is the transferred mass, A area, D diffusion coefficient and c concentration, as concerns the flux of substances J across virtual interfaces in homogenous solutions.

A limit or stationary layer of thickness, h , appears in the receptor solution at the frontier with the pharmaceutical formulation, which is not

affected by the convection currents in the fluid. It is usually accepted the fact that the concentration in the immediate neighborhoods of the pharmaceutical formulation concentration of active substance equals its maximum value c_h (noted also frequently by S) which is determined by its solubility.

Consequently the following approximation can be made:

$$\frac{\partial c}{\partial x} = \frac{c_h - c_s}{h}$$

and Fickian's equation is transformed into

$$\frac{1}{A} \frac{dm}{dt} = -D \frac{(c_h - c_s)}{h} \text{ or } \frac{dm}{dt} = -\frac{AD}{h} (c_h - c_s)$$

In fact, this was experimentally established by Noyes and Whitney over a century ago [10]. This differential equation can easily be solved with initial conditions being expressed as $c_h(t=0) = 0$ with the implicit solution:

$$-\ln\left(1 - \frac{c_h}{c_s}\right) = kt$$

or:

$$-\ln\left(1 - \frac{m(t)}{m_\infty}\right) = -\ln(1 - r) = k't$$

The same two-phase behaviour was suggested after fitting experimental data by means of Noyes-Whiney model (Figure 7).

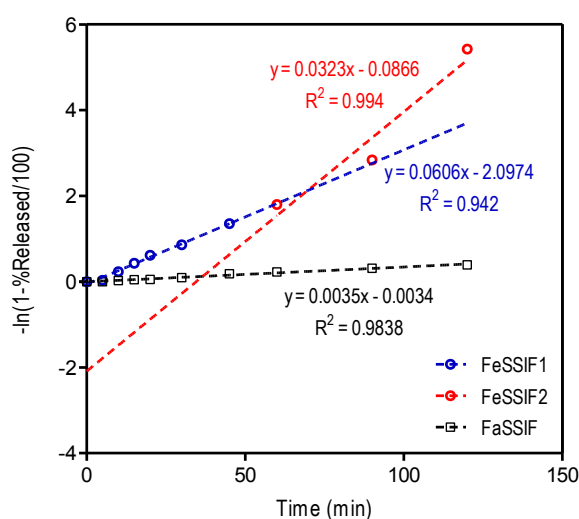


Figure 7

Noyes-Whiney modelling of norfloxacin release kinetics

In this case both one-phase and two-phase regressions are possible. One-phase regression represents a good fitting of all points excepting the last measurement. Two-phase regression can predict the last point, but the application of the F test for the comparison of residual sum of squares, indicated no significant difference between the performances of the two models.

D. Weibull Model

The 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 , or transformed to a linear dependence: $\ln(-\ln(1-r)) = \ln \alpha + \beta \ln t$ [8]. Weibull model proved to be applicable in describing almost all dissolution curves.

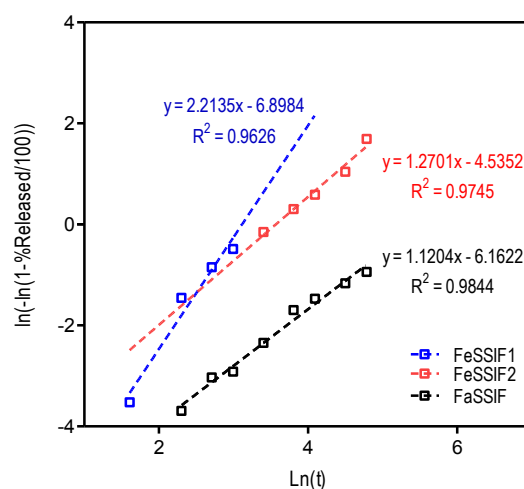


Figure 8
Weibull modelling of norfloxacin release kinetics

Once again the result is unexpected. A one-phase regression represents a poor fitting, while a two-phase linear regression proved to be very successful (Figure 8).

In fact, all of the above analysis suggested a two-phase evolution of the release phenomena. This change in behaviour could be thought as a consequence of solubility changes of norfloxacin during the release as well as its including in micelles.

Conclusions

The release kinetics of norfloxacin was strongly dependent on the dissolution medium as well as on formulation.

Great differences appeared between dissolution in simulated intestinal fluid in fed and fasted conditions probably connected with the large variability of solubility of norfloxacin in the proximity of the isoelectric point, where the behaviour is critical: small pH variations could lead to significant alterations in solubility.

Dissolution in both SGF and compendial acetate buffer (pH 4.0) was very rapid and complete.

In simulated fasted intestinal fluid conditions (pH 6.8), the released quantity increased slowly, approximately linear, but reached only 33 % within two hours. Though bile salts were expected to increase the solubility of norfloxacin by the intermediate of micelles, this result appeared to be significant only in feed conditions.

The Higuchi law, the most simple diffusion based model, described well the released amount for the first 70 % release. A two phase linear regression allowed an extension of the model to 100 % of the released amount.

In the case of Noyes-Whitney and Weibull models, the fitting after linearization could also be applied only as piecewise linear regression. A statistically significant improvement was obtained by using a two-phase instead of one-phase linear regression. The first phase corresponds to what is generally considered a pure diffusion mechanism. In the second phase, the result indicated a transition to a different, more complex mechanism.

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