IN VITRO DISSOLUTION OF POORLY SOLUBLE DRUGS IN THE PRESENCE OF SURFACE ACTIVE AGENTS - *IN VIVO* PHARMACOKINETICS CORRELATIONS. II. NIMESULIDE

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Abstract

A cross-over two-periods, two sequences clinical study concerned pharmacokinetics of Nimesulide and its active metabolite 4'-hydroxy-Nimesulide after oral administration of 100 mg AULIN® tablets (HELSSIN BIREX) was performed on 24 healthy volunteers. The assay of plasma levels was undertaken using a HPLC validated method. Modelling of data was performed using mono- and bicompartmental models. Application of information and statistical criteria indicated the monocompartmental model was efficient enough in describing time course of concentration of both parent drug and metabolite.

In vitro dissolution of tablets was undertaken using USP apparatus 2 and different experimental conditions, by varying the concentration of the surface active agent Tween 80. Modelling of release data was possible only for the initial kinetics, since a saturation phase appeared as consequence of the poor solubility of nimesulide. Extent of release increased approximately linear with Tween concentration at levels just above critical micellar concentration and strongly linear at much higher concentrations.

In order to establish the most relevant *in vitro* tests, correlation of mean plasma levels with *in vitro* dissolution results was undertaken, by applying Nelson-Wagner method. The experimental set of results was enriched based on models in order to match the *in vitro* and *in vivo* sampling times. Neglecting the concentration term in Nelson Wagner formula, an approximate formula $FRA(t) = [AUC]_0^t / [AUC]_0^\infty$ was derived, which represents rather the „eliminated fraction" than the „absorption fraction". In spite of simplification, the pointwise correlation with *in vitro* released fraction remained practically the same.

Extrapolation of the correlations concerning parent drug to rate of appearance of metabolites in plasma proved that this kinetics also could be well with dissolution.

By comparing the hierarchy of biorelevance of the dissolution tests, higher the Tween 80 concentration, better *in vitro – in vivo* correlation (IVIVC) was obtained. High bioavailability observed *in vivo* and higher concentrations of Tween required for good correlation suggested a possible critical dependence of *in vivo* absorption on physiological surface active agents.

Rezumat

Farmacocinetica Nimesulidului şi a metabolitului său activ 4'-hidroxi-Nimesulid, după administrarea orală de AULIN® tablete (HELSSIN BIREX) conţinând 100mg substanţă activă şi a unui produs testat au fost evaluate printr-un studiu clinic încrucişat cu două secvenţe şi două perioade, pe 24 voluntari sănătoşi. Concentraţiile plasmatice ale celor doi analiţi au fost evaluate printr-o metodă lichid cromatografică validată.

Datele obţinute au fost analizate prin modelare mono şi bicompartimentală. Criteriile informatice şi statistice aplicate au arătat că modelele monocompartimentale descriu suficient de bine datele, atât pentru medicamentul părinte cât şi pentru metabolit.

Cinetica de eliberare *in vitro* din tablete în soluţii de Tween 80 de diferite concentraţii a fost evaluată utilizând aparatul de dizolvare USP2. S-au analizat datele pornind de la modele teoretice. Întrucat s-a ajuns destul de rapid la o fază de saturare, descrierea evoluţiei concentraţiilor în mediul de dizolvare a fost posibilă numai pentru intervalul de la începerea experimentului până la presaturare. Dependenţa cantităţii totale dizolvate în intervalul de timp studiat de concentraţia tensioactivului Tween 80 a fost aproximativ lineară la concentraţii imediat după concentraţia micelară clinică şi puternic lineară la concentraţii mult mai mari.

În scopul stabilirii testului de dizolvare cel mai biorelevant s-au corelat datele de dizolvare *in vitro* cu cele de dizolvare *in vivo*, obţinute prin deconvoluţia curbelor concentraţiilor plasmatice, folosind metoda Nelson-Wagner. Modelele teoretice de dizolvare şi farmacocinetică obţinute au fost utilizate pentru a interpola datele pe curbele teoretice de farmacocinetică şi pentru a obţine evaluări corespunzătoare la timpii de măsurare a dizolvării *in vitro*.

O variantă simplificată a formulei Wagner-Nelson prin neglijarea concentraţiilor plasmatice, care în fapt corelează fracţia dizolvată cu fracţia eliminată a dus la o corelare punctual acceptabilă, foarte asemănătoare celei obţinute prin formula întreagă.

Corelările au fost generalizate şi la rata de apariţie a metabolitului în plasmă. Rezultatele obţinute în cazul metabolitului s-au corelat cu datele pentru medicamentul părinte, rezultat care, ţinând cont şi că dizolvarea a necesitat concentraţii mari de Tween, sugerează o dependenţă critică a dizolvării *in vivo* şi a absorbţiei intestinale de tensioactivi fiziologici.

Keywords: Nimesulide, Tween 80, modelling, *in vitro – in vivo* correlation (IVIVC)

Introduction

The oral dosage form represents the most common route for drug administration into the human body because it leads to a better patient compliance and it is very versatile for what concerns the dosing conditions [1, 2]. Unfortunately, however, this strategy fails when dealing with low bioavailability drugs like those belonging to the widely employed anti-inflammatory class [3].

Bioavailability, defined as the rate and extent to which the active drug is absorbed from a pharmaceutical formulation and becomes available in blood

[4], depends on several factors, usually drug solubility in an aqueous environment and drug permeability through lipophilic membranes playing the role of key parameters [2] and many different techniques being commonly used to improve the bioavailability of poorly water soluble but permeable drugs (Biopharmaceutical Classification System (BCS) class 2 [5]).

Drug bioavailability, in the case of oral administration, is also strongly affected by intestinal permeability. Therefore, drug pharmacokinetics studies are being undertaken for understanding the basic mechanisms ruling the drug transfer through the intestinal epithelium [6].

Nimesulide (4'-nitro-2'-phenoxy methane sulfonanilide) is a weakly acidic (pKa=6.5) non-steroidal anti-inflammatory drug. It differs from other nonsteroidal anti-inflammatory drugs in that its chemical structure contains a sulfonanilide moiety as the acidic group rather than a carboxylic group (Figure 1).

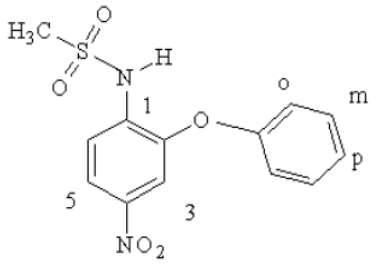


Figure 1
Chemical structure of Nimesulide

Like many non-steroidal anti-inflammatory drugs, Nimesulide is very sparingly soluble in water (≈ 0.01mg/mL) [7]. The poor aqueous solubility and wettability of Nimesulide gives rise to difficulties in realization of pharmaceutical formulations for oral or parenteral delivery and variable bioavailability is the rule [8].

Based on the Biopharmaceutical Classification System (BCS), Nimesulide is considered a BCS 2 drug (poorly soluble and highly permeable) therefore, dissolution is a limiting step for its absorption [9,10].

Solubilization in surfactant solutions above critical micelle concentration (CMC) is the most usual approach to the formulation of poorly soluble drugs [11]. Weakly acidic and basic drugs may be brought into solution by the solubilizing action of surfactants [12]. Parallel to solubilization in surface active agents (SAA) solutions as a formulation improving method, since intestinal media are solutions of physiological SAA, there is a high concern for finding *in vitro* media as close as possible

to *in vivo* release conditions, correlations and models to predict the *in vivo* behaviour.

In this study, *in vivo* pharmacokinetics and *in vitro* release were tested in order to find better models for these processes.

Different concentrations of non-ionic surfactant Polysorbate 80 (Tween 80, CMC 0.012 mM) at pH=7.4 was used to increase the solubility of Nimesulide. Release kinetics were correlated with pharmacokinetics after oral administration in order to establish relevant dissolution tests in Tween 80 solutions.

Materials and Methods

Clinical method

The study protocol was approved by the National Medicine Agency and the Independent Ethics Committee. The study was projected as a single-dose, randomized, two-treatments, two-periods, two-sequences cross-over study under fasting conditions, comparing 200 mg Nimesulide of test and reference products, administered to 24 healthy volunteers. Venous blood samples (approximately 5mL) were collected through a catheter inserted in an antecubital vein, pre-dose (0 hours) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after the drug administration**.**

HPLC conditions

The analyses were carried out using a Waters 600E Multisolvent Delivery System, a Waters 712 WISP autosampler and a Waters 486 Tunable Absorbance Detector (Waters, Milford, MA, USA). The detector was set at 404nm.

The separation was performed on a Microsorb-MV 100-5 C18 (Varian), 150×4.6mm, at 35°C. The mobile phase consisted of a 0.025M potassium phosphate buffer (pH=7.0): acetonitrile mixture (60:40 v/v). The flow-rate was 0.9mL/min, and the injection volume was 100µL. All reagents were HPLC grade.

Processing of plasma samples

The plasma samples were stored in the freezer at -20°C and allowed to thaw at room temperature before processing. To 0.5mL of plasma sample 50µL Internal Standard (10 µg/mL solution of Acenocoumarol in methanol) and 100µL 0.2M phosphate buffer pH=4.5 were added. The samples were vortex-mixed for homogenization, and 3mL methylene chloride were added. The samples were vortexed horizontally for 20 minutes at 120rpm, and 2.5mL of the organic layer was transferred into a conical tube, and

evaporated to dryness under a nitrogen stream, at 45ºC. The dry residue was reconstituted in 300µL of mobile phase.

The entire bioanalytical method was validated according to usual standard procedures in the range 0.1 – 15 µg/mL for Nimesulide and 0.05 – 7.5 µg/mL for its metabolite.

Dissolution methods

The dissolution profiles were studied using USP apparatus 2, in 900mL of dissolution media: USP phosphate buffers of pH 7.4 with 2.5%, 1%, 0.5%, 0.05% Tween 80. Stirring rates were 75 rpm, with a constant temperature bath at 37±0.5^{0}C. Five millilitre samples were drawn at 30, 60, 90, 120, 180, 240, 300 and 360 minutes. Analytical assay method was spectrophotometric, determinations being performed at 274nm. The calibration curve (four standards) was made in phosphate buffer pH=7.4 with 2.5% Tween 80. In all other media, Nimesulide amount exceeded its solubility.

Results and Discussion

Pharmacokinetics

From the 24 volunteers enrolled, only 21 ended the study and 3 were dropped out after first period. Plasma levels curves of Nimesulide and 4'-hydroxy-Nimesulide for volunteers which finalized the study are presented in figure 2:

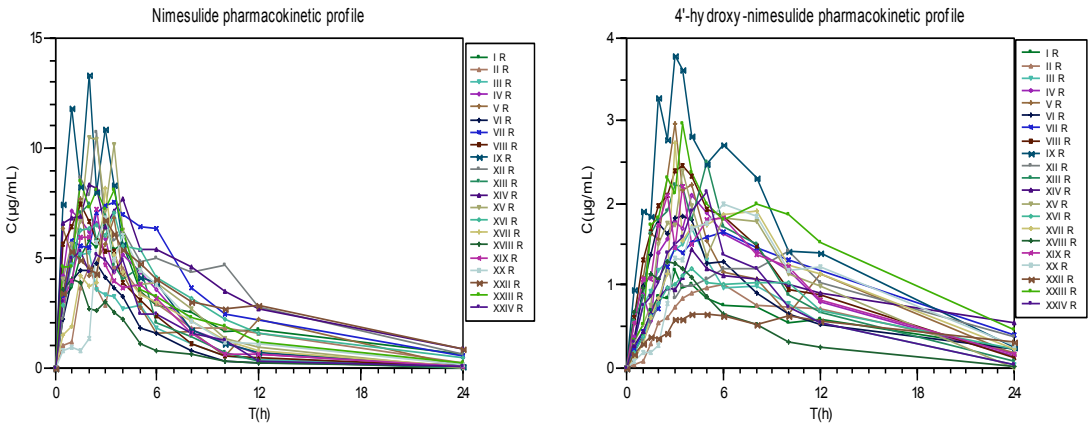


Figure 2
Pharmacokinetic profile for Nimesulide and 4'-hydroxy-Nimesulide

Plasma levels of active metabolite are 2 – 3 times lower than that of parent drug, but not small enough to be neglected. Variability was large enough, but curves were approximately homogenous distributed in space, which justified an analysis of the mean curves (Figure 3).

Since curves were smooth enough it was considered useful to try the fitting the experimental results with solutions of compartmental models in order to permit enrichment plasma level curves with points corresponding to *in vitro* dissolution sampling times.

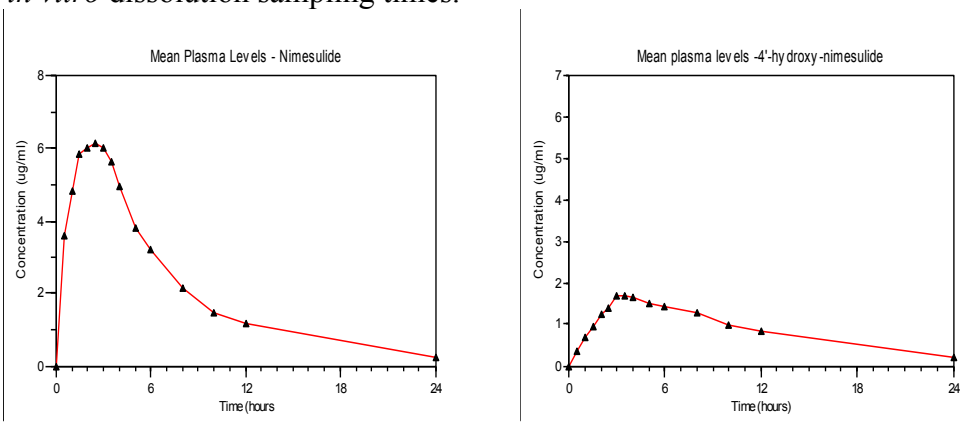


Figure 3
Mean pharmacokinetic profile (n=24 volunteers) for Nimesulide and 4'-hydroxy-Nimesulide

Compartmental analysis

As can be seen in figure 4, in the case of nimesulide both monocompartmental and bicompartmental models fitted well the experimental data. In order to establish the most performant model information and statistical criteria were applied.

Application of statistical and information criteria in comparison of models. The Akaike Information Criterion (AIC) [13,14,15] and the Schwarz Criterion (SC) [16], are somewhat different but both based on the sum of "errors" corrected by a "penalty" function proportional to the number of parameters (p) in the model:

$$AIC = N \ln SS + 2p$$
$$SC = N \ln SS + p \ln N$$

in which SS is the sum of deviations squared with the p-th set of parameters.

The model equation with the smallest AIC and/or SC is a candidate for the "best" representation of the time course plots [17].

F test

A choice between two model equations, with q and respectively $p = q + k$ or parameters, may be based on the F ratio test [16]:

$$F = \frac{SS_q - SS_p}{SS_p} \frac{df_p}{df_q - df_p}$$

where SS is the sum of errors for model and df the number of degrees of freedom.

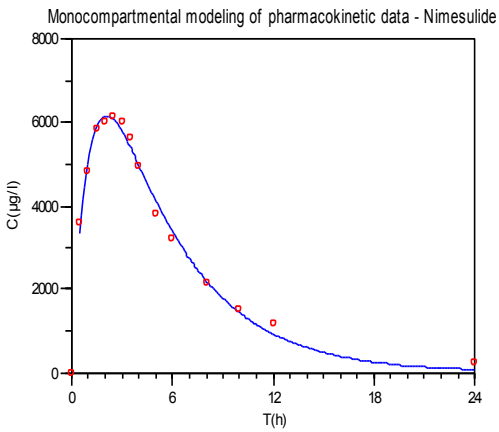
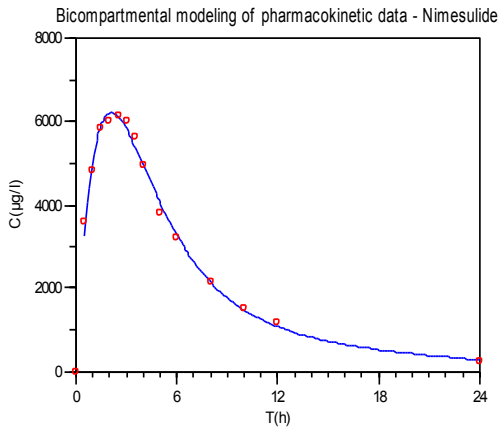
Nimesulide		
AIC	190.49	190.42
SC	188.45	187.01

Figure 4

Monocompartmental and bicompartmental fitting for Nimesulide data

Table I

F-test for comparison of fitting performance for Nimesulide

Model	SS	n	df	F	$F_{2,8,0.95}$
Monocompartmental	528716	14	10	**1.35**	**4.46**
Bicompartmental	395093	14	8		

Both Akaike and Schwartz criteria had smaller values in the case of the monocompartmental model.

Application of F test led to the value 1.35. Since threshold value for 95 % confidence interval is 4.46, the difference in the performance of the models is not significant.

In the case of 4'-hydroxy-Nimesulide metabolite, both monocompartmental and bicompartmental models fitted well the experimental data with the exception of a small interval in the neighbourhood of maximum concentration. Increasing further the number of compartments didn't significantly improve the fitting.

Information criteria voted for bicompartmental model but again, F-test tempered the conclusion, proving the lack of statistical significance for difference. Since as a rule, in such cases the simplest model is preferred, the monocompartmental model was retained. In fact, it's the case of a pseudomonocompartmental behavior, which was reported to be the behavior for many other metabolites [18, 19, 20, 21, 22].

Table II

F-test for comparison of fitting performance for 4'-hydroxy-Nimesulide

Model	SS	n	df	F	$F_{2,7,0.95}$
Monocompartmental	116613	14	10	0.06	4.74
Bicompartmental	115002	14	8		

In vitro dissolution results

The release in 2.5% Tween 80 in 60 minutes (dissolution method of producer) has been very rapid, without "time–lag". However, the release has not been complete, the released quantity being under 92% at the end of the measuring interval.

Since the value did not seemed to be a saturation value, the time interval for measuring release was extended to 6 hours for the rest of Tween solutions.

The release in 1% Tween 80 was instantaneous and the concentration remained constant, without any evidence that it might increase afterwards. Apparently, the release was limitted by solubility, which seemed to be of 68.3μg/mL.

The release in 0.5% Tween 80 was also immediate. It seems that the value of 58.9μg/mL is a saturation value, a little bit less than the saturation value in 1% Tween 80.

In 0.050% Tween 80 apparent saturation value appeared to be 40.5μg/mL.

Comparison of the dissolution profiles

The representation of all dissolution curves (Figure 5) confirmed the "saturation curve" behaviour for release for all concentrations. In these conditions, the study permitted an estimation of the dependence of Nimesulide "solubility" as function of the Tween 80 concentration (Figure 6). For higher concentrations it was obtained a linear dependence.

The result could be explained if we consider that Tween concentrations were both under and above critical micellar concentration.

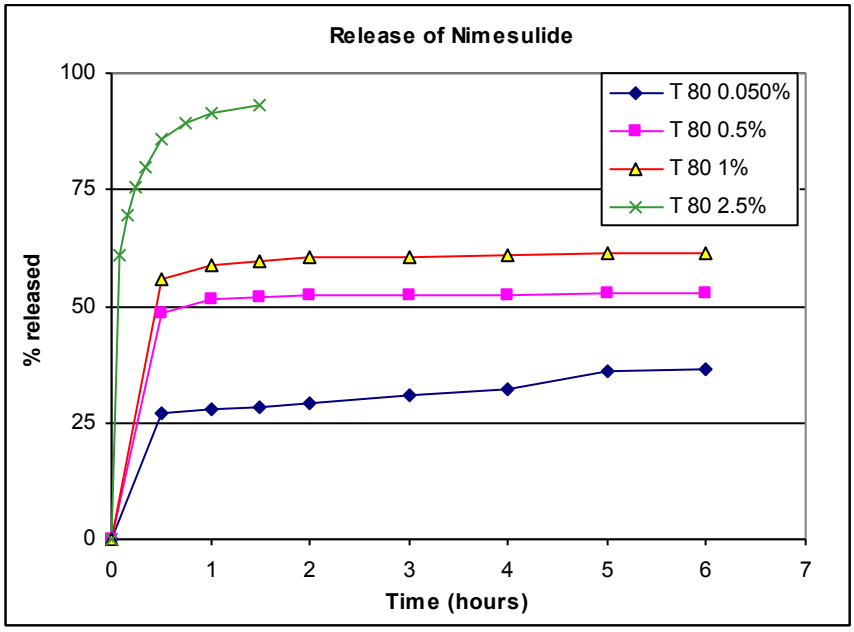


Figure 5
Release profiles in different concentrations of Tween 80 solutions

According to reports of the companies, critical micellar concentration (CMC) for Nimesulude is 0.012 mM [23]. However, effective, more systematic, experimental evaluation has shown that the value depends on the electrolyte concentration present in the solution [24], decreasing from 0.005% (w/v) to 0.003% as concentration of KCl increases. 0.005% w/v (or ~0.01% v/v) are other reported values, correlated to those above, but in other experimental conditions [25].

Consequently, discontinuity or even inversion of a given effect could appear as a critical behaviour around CMC.

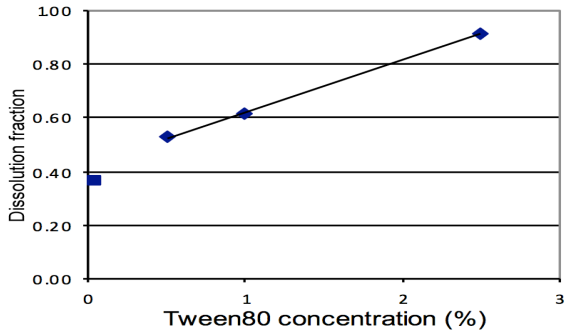


Figure 6
Influence of Tween 80 concentration on Nimesulide solubility

In fact, the surfactant accumulates on the interface of Nimesulide particles – solution and, in that interface, all phenomena and all concentrations significantly differ from those in bulk of solution. Any imagined mechanism concerning the interface phenomena, remains an unverifiable assumption since we have no information about electrical double layers appearing at interfaces.

In the end, we should keep some "abnormal" behaviour, as "normal" in the neighbourhood of CMC.

The release kinetics model

For the 2.5% case, Noayes–Whitney , Higuchi, Peppas and Weibull release models were verified. Both visual inspection and statistical criteria indicated Weibull empirical law as the most adequate mathematical description of dissolution time course (Figure 7).

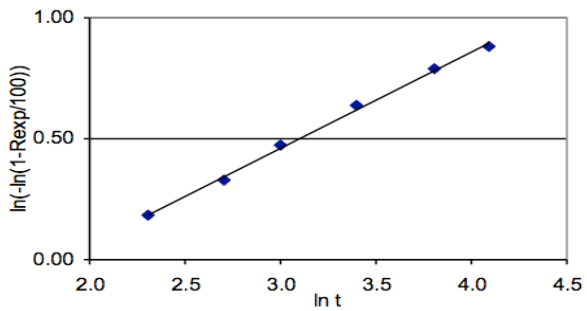


Figure 7
Representation of ln (-ln(1-R/100)) depending on ln*t*

In vitro – in vivo correlations

Correlations were made between dissolution fraction FD and fractions of drug absorbed calculated using Wagner Nelson formula [26]:

$$FRA(t) = \frac{C_p(t) + k[AUC]_0^t}{k_e[AUC]_0^\infty}$$

where:
FRA(t) =Fraction of drug absorbed at time t,
Cp(t) =Plasma drug concentration at time t,
k=Elimination rate constant,
$[AUC]_{0\text{-}t}$=Area under the concentration time curve from time 0 to time t,
$[AUC]_{0\text{-}\infty}$=Area under the concentration time curve from time 0 to infinity.

Neglecting the concentration term, it is possible to use also the approximate formula [18]:

$$FRE(t) = \frac{[AUC]_0^t}{[AUC]_0^\infty}$$

which represents more the „eliminated fraction" (FRE) than the „absorption fraction" (FRA) . The model neglects the quantity of drug in blood face to the eliminated quantity. After the time of maximum concentration the plasma levels decrease and the eliminated quantity increases. Consequently, for the drugs with rapid absoption and small time of maximum concentration the model could be adequate.

Calculation of absorption fraction using Wagner formula and its representation as function of *in vitro* released fraction for different experimental conditions gave the results presented in figure 8.

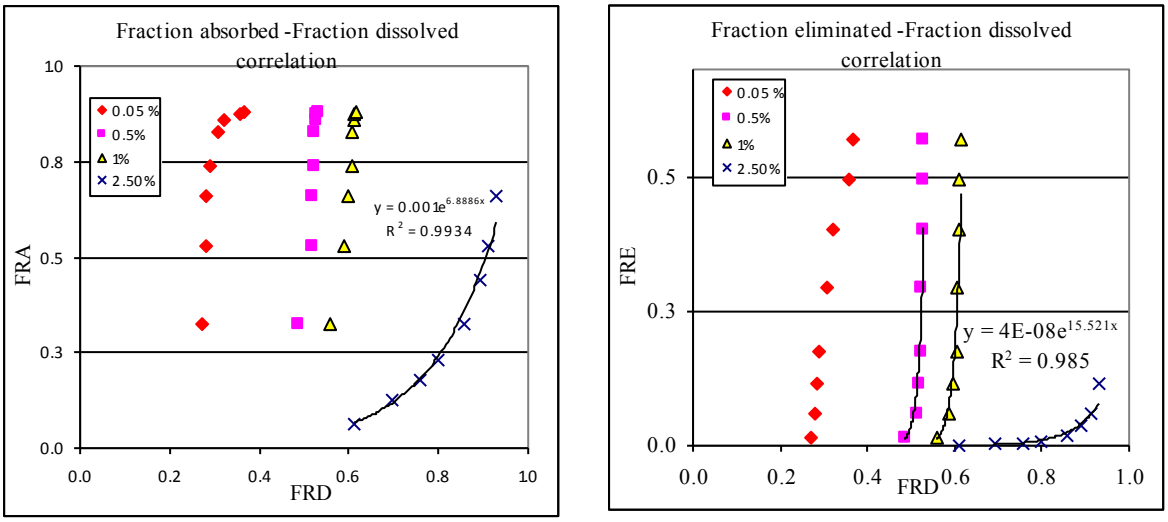


Figure 8
In vitro - in vivo correlations for nimesulide

For concentration 2.5 % it was obtained a good smooth correlation *in vitro – in vivo*, but the *FRA (FRD)* dependence was not linear but rather exponential. Indeed, logarithmic transformation led to a good linear correlation (Figure 9).

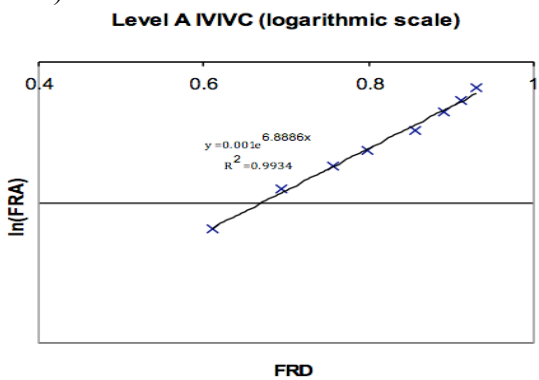


Figure 9
Logarithmic scale IVIVC

For other Tween concentrations the correlation was obscure. Absorption appeared to start at the moment when dissolution reached its saturation value. The situation was similar when replacing FRA with FRE both in the case of 2.5 % Tween and in the case of lower concentrations.

Dissolution – absorption correlations generalized to metabolites

Correlations between dissolution and absorption were used also in a generalized form [27] of correlation between dissolution of parent drug and kinetics of appearance in plasma of its metabolites. The results were similar to those obtained for the parent drug (Figure 10).

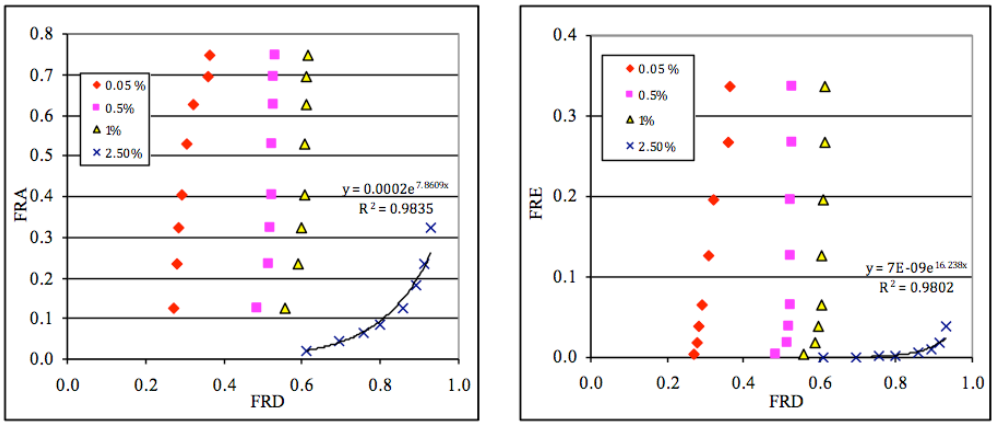


Figure 10
In vitro - in vivo correlations for 4'-hydroxy-Nimesulide

Conclusions

The Nimesulide release from tablets is immediate, the released amount being apparently limited by solubility, a limit which depended on the Tween 80 concentration in the release medium.

The dependence of the released amount on the Tween concentration is not linear, very low Tween 80 concentration showing also a decrease of "solubility", probably connected to a critical micellar concentration at the Nimesulide solution interface.

An almost complete solubilization was obtained at unusual high Tween 80 concentration (2.5 %).

The model followed by the kinetic release is Weibull, but the initial release could be modelled by Higuchi square root law also. *In vivo* plasma levels for both parent drug and metabolite could be well fitted as solutions of monocompartmental pharmacokinetic models.

Most biorelevant dissolution test in media containing Tween 80 appeared to correspond to 2.5% Tween, but even in this case, the correlation *FRA (FRD)* was not linear but rather exponential.

In vitro – in vivo correlations were similar for both classical *FRA (FRD)* and simplified *FRE (FRD)* evaluations.

The *in vitro – in vivo* correlation generalized to metabolite's pharmacokinetics as function of FRD were concordant to correlations associated to parent drug.

Aknownledgements

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