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Research Article

A Case Study of *In Silico* Modelling of Ciprofloxacin Hydrochloride/Metallic Compound Interactions

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Abstract. With the development of physiologically based absorption models, there is an increased scientific and regulatory interest in *in silico* modelling and simulation of drug–drug and drug–food interactions. Clinically significant interactions between ciprofloxacin and metallic compounds are widely documented. In the current study, a previously developed ciprofloxacin-specific *in silico* absorption model was employed in order to simulate ciprofloxacin/metallic compound interaction observed *in vivo*. Commercially available software GastroPlus™ (Simulations Plus Inc., USA) based on the ACAT model was used for gastrointestinal (GI) simulations. The required input parameters, relating to ciprofloxacin hydrochloride physicochemical and pharmacokinetic characteristics, were experimentally determined, taken from the literature or estimated by GastroPlus™. Parameter sensitivity analysis (PSA) was used to assess the importance of selected input parameters (solubility, permeability, stomach and small intestine transit time) in predicting percent drug absorbed. PSA identified solubility and permeability as critical parameters affecting the rate and extent of ciprofloxacin absorption. Using the selected input parameters, it was possible to generate a ciprofloxacin absorption model, without/with metal cation containing preparations co-administration, which matched well the *in vivo* data available. It was found that reduced ciprofloxacin absorption in the presence of aluminium hydroxide, calcium carbonate or multivitamins/zinc was accounted for by reduced drug solubility. The impact of solubility–permeability interplay on ciprofloxacin absorption can be observed in the ciprofloxacin–aluminium interaction, while in ciprofloxacin–calcium and ciprofloxacin–zinc interactions, effect of solubility was more pronounced. The results obtained indicate that *in silico* model developed can be successfully used to complement relevant *in vitro* studies in the simulation of physicochemical ciprofloxacin/metallic compound interactions.

KEY WORDS: absorption profile; drug interaction; GastroPlus; permeability; solubility.

INTRODUCTION

Drug absorption is a complex process that can be affected by numerous physicochemical, pharmaceutical and physiological factors. With the introduction of the Biopharmaceutics Classification System (BCS) and development of physiologically based absorption models, there is an increased scientific and regulatory interest in *in silico* modelling and simulation of drug–drug and drug–food interactions (1–6). According to the BCS concept drug dose, solubility and intestinal permeability are major determinants of drug absorption (1).

Gastrointestinal simulations based on the Advanced Compartmental Absorption and Transit (ACAT) model have become an important *in silico* tool to predict the *in vivo* drug behaviour during drug development and quality approval (6–9). The ACAT model contains nine compartments (stomach, duodenum, jejunum 1, jejunum 2, ileum 1, ileum 2, ileum 3, caecum and ascending colon) to mimic the human gastrointestinal tract. Mass balance equations describe drug transport along the gastrointestinal tract, as well as through membrane. Default physiological parameters under fasted and fed states are population mean values obtained from published data including pH, volume, length, radii and transit time. Drug diffusion coefficient, particle density, particle radius, particle shape factor and experimentally determined solubility are used as input parameters. The dissolution rate constant is calculated by a modified Noyes–Whitney dissolution equation (2). The important feature of ACAT model which contributes to its use in biopharmaceutical drug characterization is that it provides link between formulation performance and drug product pharmacokinetics.

Ciprofloxacin hydrochloride is a BCS class 4 drug that exhibits pH-dependent solubility profile and relatively narrow absorption window in the upper small intestine (10). Reports from *in vivo* studies indicate reduced ciprofloxacin bioavailability when co-administered with a range of metallic ion containing preparations (11–14). The absorption impairment may be significant and potentially lead to the failure of clinical treatment (12,13,15). Formation of nonabsorbable complex has been postulated as the interaction mechanism (11–13), although some authors commented that other physicochemical factors, such as

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solubility, may also play a role (11). Drug solubility and permeability are closely associated indicating that solubility–permeability interplay must be taken into account in order to maximize the overall drug absorption (16).

It has been shown in our previous study (17) that *in silico* simulation can be used together with *in vitro* studies for the biopharmaceutical characterization of the physicochemical ciprofloxacin–iron interaction. This previously developed absorption model was employed in the present study in order to simulate interactions of ciprofloxacin with aluminium, calcium and zinc and elucidate potential interaction mechanism/s.

MATERIALS AND METHODS

In Vivo Data

Literature *in vivo* data related to ciprofloxacin bioavailability studies, without/with metallic compounds co-administered, were used for gastrointestinal simulation model optimization. In the study performed by Polk *et al.* (13), 500-mg ciprofloxacin tablets were administered without/with multivitamins containing zinc in a group of 12 subjects. Multivitamins used in the Polk *et al.* study (13) contained vitamin E; vitamins B_1, B_2, B_3, B_5, B_6 and B_{12}; vitamin C; folic acid; biotin and zinc (23.9 mg) and copper (4 mg). The form of zinc compound was not reported. The authors suggested that, although zinc is probably responsible for the interaction, the possibility that other components of the tablet contribute cannot be excluded (13). The results obtained indicate that ciprofloxacin absorption was reduced by 24% (2–50% range) when given concomitantly with zinc containing multivitamin product. Polk *et al.* (13) reported that area under the curve (AUC) for ciprofloxacin administered with multivitamins containing zinc (i.e. 11.29±2.42) is significantly different from AUCs obtained in the two control studies (i.e. 14.46±2.33 and 15.71±2.84). Frost *et al.* (14) studied the effect of aluminium hydroxide antacid and calcium carbonate antacid on ciprofloxacin bioavailability. Ciprofloxacin (HCl) tablets (750 mg) were administered: (a) alone, (b) with four 850-mg calcium carbonate tablets and (c) with three 600-mg aluminium hydroxide tablets. The relative bioavailability of ciprofloxacin when given with calcium carbonate was approximately 60% (mean AUC value 7.82±3.09) of the control value (i.e. 13.50±4.61). When ciprofloxacin (hydrochloride tablet) was given with aluminium hydroxide, the relative bioavailability was approximately 15% (mean AUC value 1.61±1.44). Pharmacokinetic parameters used in gastrointestinal simulation (rate constants k_{12}, k_{21}, volume of distribution, V_d shown in Table I) were calculated from the *in vivo* data reported following ciprofloxacin i.v. administration (18). Values for V_2 and $t_{1/2}$ were calculated by GastroPlus™ as a result of built-in calculation from the PK parameters obtained from the *in vivo* data (18).

Gastrointestinal Simulation

In silico absorption simulation was performed using the commercially available software GastroPlus™ version 6.0.1004 (Simulations Plus Inc., USA) based on the ACAT model. In *in vivo* studies (13,14), ciprofloxacin was taken concomitantly with metallic cations containing preparations after an overnight fast, so Human Physiology Fasted mode was used for simulation. Model optimization was performed based on the set of input parameters describing drug and dosage form characteristics, which were determined experimentally, taken from the literature or estimated by the software (17). A summary of the input parameters used is given in Table I. GastroPlus™ default value for drug particle density was used. For the diffusion coefficient, GastroPlus™ estimated value was used based on ciprofloxacin molecular weight. The blood–plasma concentration ratio was set to 1 as default GastroPlus™ value. Experimentally determined solubility of ciprofloxacin hydrochloride in water (corresponding to the final pH value 4.04) was used as a reference. P_{eff} value was estimated from data on drug bioavailability assuming that there is no substantial loss by first pass elimination (19) and that fraction dose absorbed (F_a) could be considered equal to drug absolute bioavailability.

Table I. Summary of Ciprofloxacin Input Parameters in GastroPlus

Parameters	Value
Molecular weight	385.8 g/mol
log*P*	1.32[a]
pKa_1	8.62[b]
pKa_2	6.16[b]
Dose	500 mg
Solubility at pH 4.04	42 mg/ml[c]
Diffusion coefficient	0.75×10^5 cm^2/s[d]
Drug particle density	1.2 g/ml[e]
P_{eff} (human jejunal permeability)	1.57×10^{-4} cm/s[f]
Body weight	70 kg
Blood/plasma concentration ratio	1[e]
Unbound percent in plasma	70%[g]
Clearance	35 or 37 l/h[h]
Volume of distribution, V_c	0.56 l/kg[i]
Peripheral volume, V_2	1.347 l/kg[j]
Elimination half-life, $T_{1/2}$ (h)	4.08[j]
Distribution rate constants	
k_{12}	2.3753 l/h[i]
k_{21}	0.98752 l/h[i]

[a] From (20)
[b] From (21)
[c] Experimental value
[d] GastroPlus estimated value based on molecular weight
[e] GastroPlus default values
[f] See text
[g] From (22)
[h] GastroPlus optimized
[i] Calculated by Kinetica program from (18)
[j] GastroPlus calculated (built-in calculation from PK parameters)

The exponential relationship for fraction absorbed *vs.* effective permeability established by Amidon *et al.* (1) was employed:

$$F_a = \left(1 - e^{-1.47P_{eff}}\right) \times 100$$

The absorption scale factors (ASF) in Physiology tab are used to demonstrate the changes in permeability as the drugs travels along the GI tract. ASF scale the effective permeability to account for variations in absorption rate-determining effects (e.g. pH value, the presence of influx and efflux transporters) that differ from one compartment to another (2). The ASF are, generally, calculated automatically based on drug physicochemical characteristics, but can be further adjusted based

on the drug disposition observed in the human *in vivo* studies. ASF values relevant to ciprofloxacin hydrochloride were further optimized to reflect the *in vivo* data indicating rapid drug absorption in the proximal segments of the gastrointestinal tract and narrow absorption window in the upper small intestine (10) as reported previously (17). Consequently, they were scaled to zero below the 'jejunum 2' compartment. The pH value in 'duodenum' compartment was adjusted to 4.04 in accordance with experimental data obtained (pH value of saturated ciprofloxacin hydrochloride solution). The proposed adjustment was justified based on relatively high ciprofloxacin concentration following gastric emptying of a dissolved drug, relatively low fluid volume available (23) and low buffer capacity (24) in the proximal part of intestine. The relevant percent prediction error (PE %) values between the *in vivo* observed and *in silico* predicted pharmacokinetic parameters were calculated as follows (25):

$$\mathrm{PE}(\%) = \frac{\mathrm{PKpredicted} - \mathrm{PKobserved}}{\mathrm{PKobserved}} \times 100$$

Parameter Sensitivity Analysis

Parameter sensitivity analysis (PSA) was used to assess the importance of selected input parameters (solubility, permeability, stomach residence time and intestinal transit time) in predicting the fraction of drug absorbed. During PSA, only one parameter is varied at a time while all other parameters are held at their baseline values. Solubility was varied in the range 0.1–100 mg/ml, while effective drug permeability was varied in the range from 0.79 to 3.14×10^{-4} cm/s, covering one half to 2-fold input value (i.e. according to the default GastroPlus™ settings). Stomach and small intestine transit times were evaluated separately in order to assess the effects of residence time in stomach and small intestine on the percent of drug absorbed. As residence time in the stomach is known to be highly variable, it was varied in the range 0.25–3 h, while the transit time in small intestine has been reported to be relatively constant and physiologically relevant range of 3 to 4 h was employed (26,27).

RESULTS

In Silico Simulation of Ciprofloxacin Absorption

Gastrointestinal simulation for ciprofloxacin (HCl) tablets, based on the input physicochemical and pharmacokinetic data presented in Table I, was performed using the GastroPlus Single Simulation. The predicted ciprofloxacin plasma profiles are presented in Fig. 1a, together with the mean plasma profiles observed *in vivo* after administration of tablets containing 500 mg (13) or 750 mg (14) of ciprofloxacin hydrochloride. The best fit of the actual data observed *in vivo* when ciprofloxacin tablets were given without metallic compounds ('control' study) was obtained with the input solubility of 42 mg/ml, corresponding to the experimentally obtained aqueous solubility of ciprofloxacin hydrochloride. The simulation results indicated fraction of drug absorbed of 80.8% and 81% for the 'control' studies (Fig. 1b).

The predictability of the generated absorption model was measured by the percent prediction error (PE %) between the predicted and *in vivo* observed data. The predicted pharmacokinetic parameters and those observed *in vivo* are presented in Table II. The percent prediction errors obtained were less than 10% for both C_{max} and AUC_{0-t}, indicating good predictability. In addition, regression coefficients for *in silico* predicted 'control' profiles were 0.99 and 0.93, indicating that *in silico* predicted profiles matched well the *in vivo* data reported by Polk *et al.* (13) and Frost *et al.* (14), respectively.

PSA Analysis

The results obtained from PSA analysis are shown in Fig. 2a–d. The outputs indicated that, within the range of values tested, the percent of ciprofloxacin absorbed was sensitive to solubility and permeability, while it was less sensitive to variation in stomach residence time and small intestine transit time. The results obtained show that the percent of ciprofloxacin absorbed is not sensitive to change in stomach residence time in the range of 0.25–3 h. However, the percent of ciprofloxacin absorbed increased slightly with the increase in small intestine transit time. Based on the PSA performed for ciprofloxacin solubility in the range from 0.1 to 100 mg/ml, almost complete absorption (F_a=80%) was achieved with solubility value 42 mg/ml, reflecting ciprofloxacin hydrochloride aqueous solubility. Based on the PSA performed for ciprofloxacin permeability in the range from 0.79 to 3.14×10^{-4} cm/s, it was demonstrated that almost complete absorption is expected if effective permeability is equal to or higher than 1.57×10^{-4} cm/s, indicating that ciprofloxacin absorption is not permeability limited, but could be compromised if less permeable interaction adduct is formed.

Ciprofloxacin/Metallic Ion Interaction

In order to investigate the influence of aluminium, calcium and zinc compounds on ciprofloxacin bioavailability, the simulated C_p–time data were compared with the mean plasma profiles observed *in vivo* after administration of ciprofloxacin tablets with aluminium hydroxide (14), calcium carbonate (14) and multivitamins with zinc (13). Taking into account the expected influence of (a) solubility and (b) permeability as identified above in the PSA analysis and the possible interplay between solubility and permeability, three cases were considered for each ciprofloxacin/metallic cation interaction:

Case 1 In this case, P_{eff} value remained unchanged (P_{eff}=1.57×10^{-4} cm/s), while solubility input value was optimized. It was found that reduced ciprofloxacin absorption observed in the presence of aluminium hydroxide was best described when ciprofloxacin solubility was reduced to 0.07 mg/ml (regression coefficient r^2=0.8). The related *in silico* simulated and *in vivo* observed C_p–time profiles are presented in Fig. 3a. In the case of calcium carbonate, reduced ciprofloxacin absorption was best described when ciprofloxacin solubility was optimized to 1 mg/ml (r^2=0.97). Relevant *in silico* simulated and *in vivo* observed C_p–time profiles are presented in Fig. 4a. Ciprofloxacin absorption when administered with multivitamin containing zinc preparation was best described when ciprofloxacin solubility was

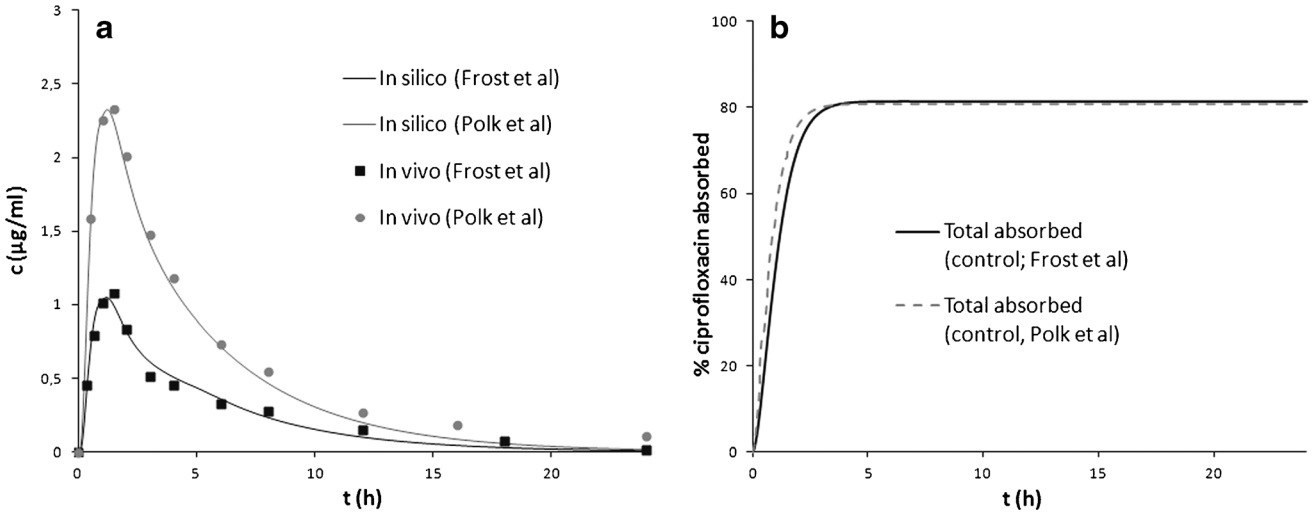

Fig. 1. *In silico* simulated and *in vivo* observed ciprofloxacin plasma C_p−time profiles following oral administration of 500 or 750 mg ciprofloxacin tablet (13,14) (**a**) and predicted absorption profiles (**b**)

optimized to 29 mg/ml (r^2=0.93). Relevant *in silico* simulated and *in vivo* observed C_p−time profiles are presented in Fig. 5a.

Case 2 In the second case, permeability input values have been optimized, while the solubility remained unchanged (i.e. experimentally obtained ciprofloxacin aqueous solubility 42 mg/ml). The results obtained show that reduced ciprofloxacin absorption observed in the presence of aluminium hydroxide or calcium carbonate was best described when permeability was optimized to 0.1×10^{-4} cm/s (r^2=0.74), or 0.8×10^{-4} cm/s (r^2=0.86), respectively. The corresponding *in silico* simulated plasma concentration profiles are presented in Figs. 3a and 4a. Ciprofloxacin absorption in the presence of multivitamin containing zinc preparation was well described *in silico* when permeability input value was optimized to 1.2×10^{-4} cm/s (r^2=0.84). Relevant *in silico* simulated profile is presented in Fig. 5a.

Case 3 In the third case, solubility and permeability input values have been optimized simultaneously. The results obtained indicate that reduced ciprofloxacin absorption in the presence of aluminium hydroxide was best described when solubility and permeability were optimized to 0.3 mg/ml and 0.8×10^{-4} cm/s, respectively (r^2=0.80). The corresponding *in silico* simulated plasma concentration profile is presented in Fig. 3a. In the case of calcium carbonate, reduced ciprofloxacin absorption was best described when ciprofloxacin solubility and permeability were optimized to 2 mg/ml and 1.2×10^{-4} cm/s, respectively (r^2=0.96). Relevant *in silico* simulated profile is presented in Fig. 4a. Ciprofloxacin absorption in the presence of multivitamin containing zinc preparation was well described *in*

Table II. *In Silico* Predicted and *In Vivo* Observed Pharmacokinetic Parameters

Study	C_{max} (µg/ml)			AUC_{0-t} (µgh/ml)			Reference
	Observed	Predicted	PE %	Observed	Predicted	PE %	
Control 1	2.33	2.44	4.70	13.67	13.35	2.30	(13)
Control 2	1.95	2.14	9.74	10.92	11.55	5.70	(14)
Case 1 (optimized solubility)							
Ciprofloxacin/aluminium interaction	0.21	0.21	0	1.20	1.17	2.50	(14)
Ciprofloxacin/calcium interaction	1.08	1.05	2.80	5.88	5.31	9.70	(14)
Ciprofloxacin/multivitamins with zinc interaction	1.67	1.76	5.39	10.20	9.72	4.70	(13)
Case 2 (optimized permeability)							
Ciprofloxacin/aluminium interaction	0.21	0.21	0	1.20	1.63	35.8	(14)
Ciprofloxacin/calcium interaction	1.08	1.18	9.3	5.88	6.5	10.54	(14)
Ciprofloxacin/multivitamins with zinc interaction	1.67	1.59	4.8	10.20	11.53	13.04	(13)
Case 3 (optimized solubility and permeability)							
Ciprofloxacin/aluminium interaction	0.21	0.21	0	1.20	1.19	0.80	(14)
Ciprofloxacin/calcium interaction	1.08	1.05	2.80	5.88	5.47	6.97	(14)
Ciprofloxacin/multivitamins with zinc interaction	1.67	1.75	4.79	10.20	9.53	6.57	(13)

AUC area under the curve, *PE %* percent prediction error values between the *in vivo* observed and *in silico* predicted pharmacokinetic parameters

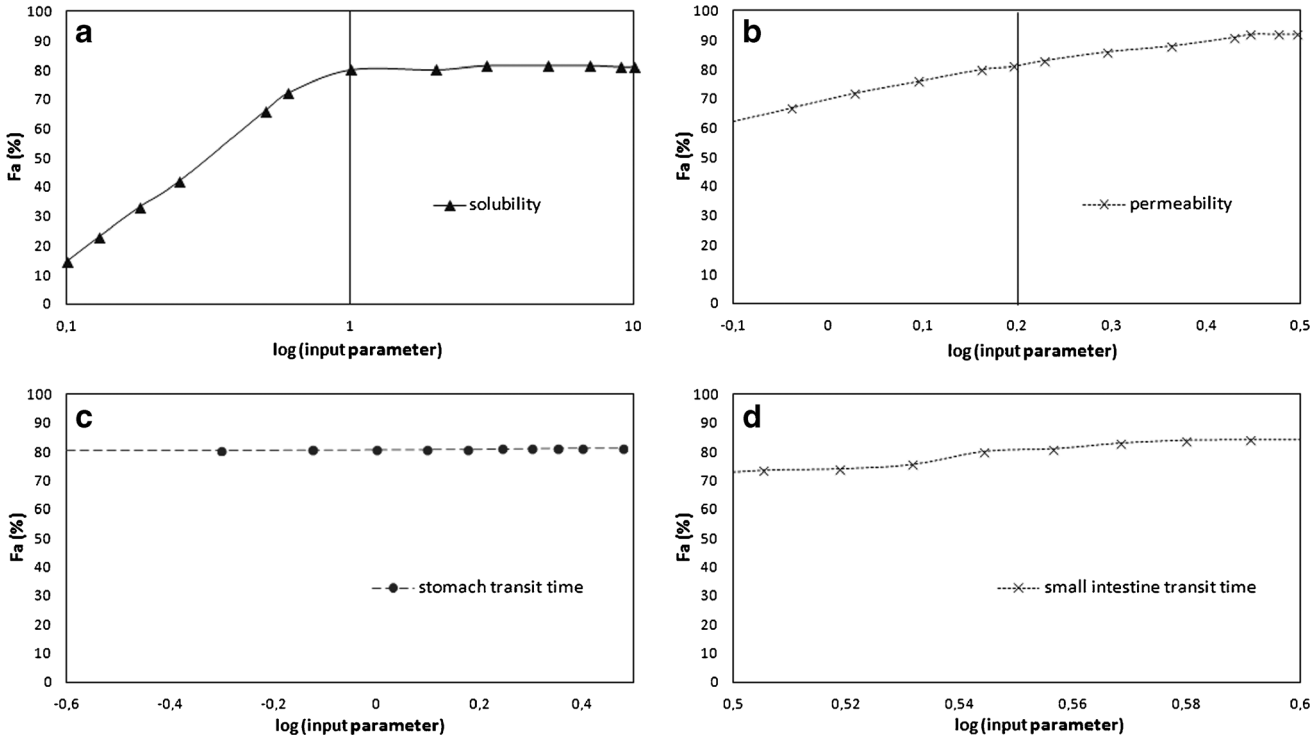

Fig. 2. Parameter sensitivity analysis. The dependence of fraction ciprofloxacin absorbed on different input parameters: **a** solubility, **b** permeability, **c** stomach transit time and **d** small intestine transit time

silico when solubility and permeability input values were optimized to 27 mg/ml and 1.3×10^{-4} cm/s (r^2= 0.95). Relevant *in silico* simulated profile is presented in Fig. 5a.

The predicted pharmacokinetic parameter values (C_{max} and AUC) are given in Table II, together with the data observed in the *in vivo* studies. The calculated percent prediction error values for the *in silico* drug plasma concentration profiles generated based on the optimized solubility values were less than 10% for both C_{max} and AUC (Table II). The calculated percent prediction error values for the *in silico* drug plasma concentration profiles generated based on the optimized permeability values were less than 10% for C_{max} (Table II), while, for AUC, the PE % were 35.8, 10.54 and 13.04 (for aluminium, calcium and multivitamin containing zinc co-administration, respectively). The calculated percent prediction error values for the *in silico* drug plasma concentration profiles generated based on the optimized solubility and permeability values were less than 10% for both C_{max} and AUC (Table II).

According to the percent prediction error values presented in Table II, it would be expected that ciprofloxacin absorption in the presence of metallic cations is more affected

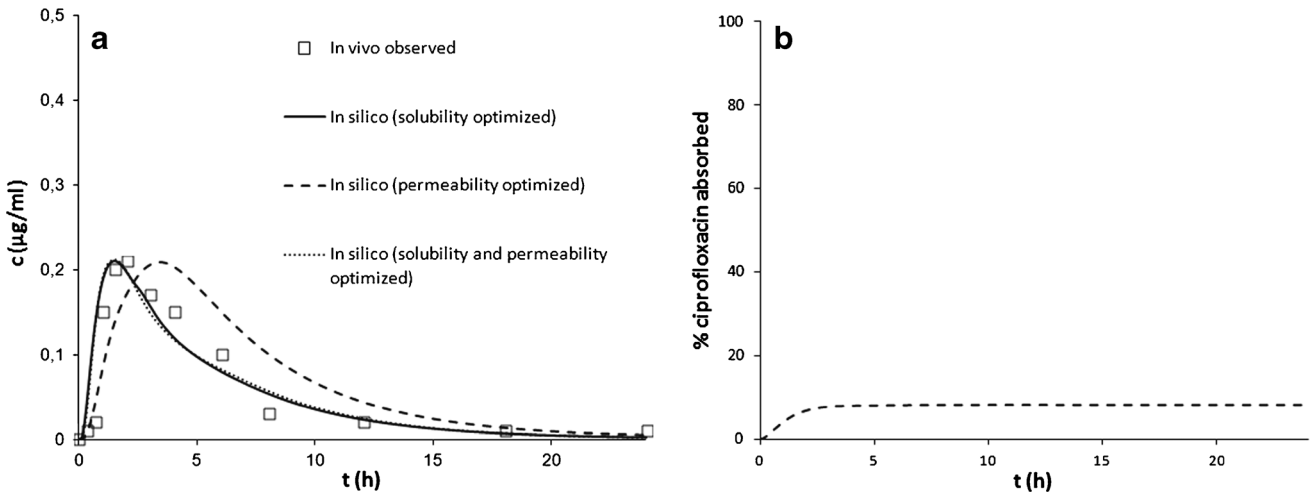

Fig. 3. GastroPlus™ predicted (solubility, permeability or solubility–permeability optimized) and *in vivo* observed mean ciprofloxacin plasma C_p–time profiles with aluminium hydroxide tablets co-administered (**a**) and predicted absorption profile (**b**)

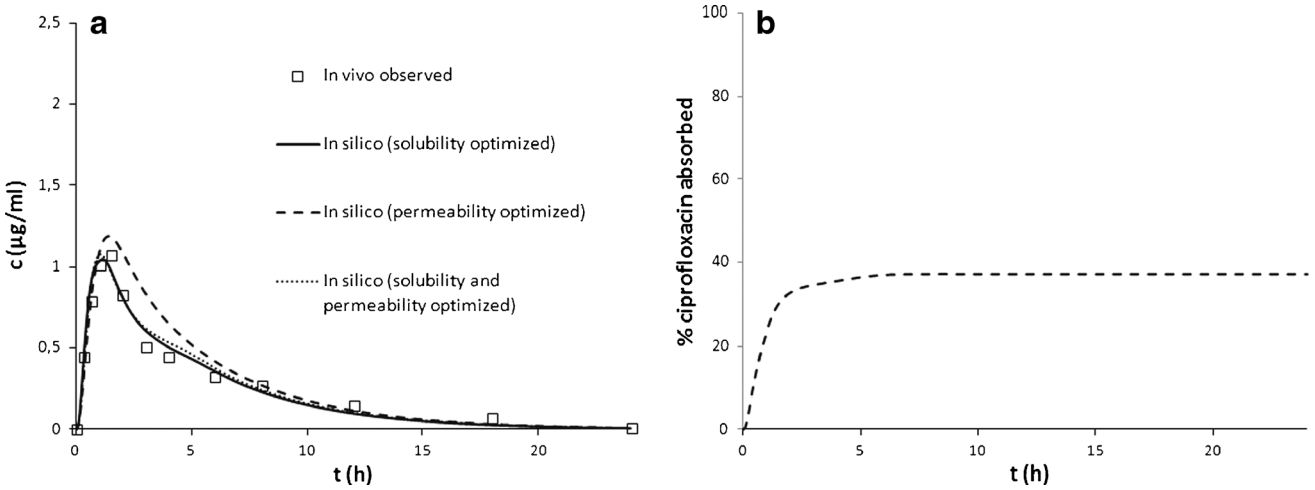

Fig. 4. GastroPlus™ predicted (solubility, permeability or solubility–permeability optimized) and *in vivo* observed mean ciprofloxacin plasma C_p–time profiles with calcium carbonate tablets co-administered (**a**) and predicted absorption profile (**b**)

by solubility than by permeability variation. The impact of solubility–permeability interplay on ciprofloxacin absorption can be observed in the case of ciprofloxacin–aluminium interaction where optimized values for both solubility and permeability were notably lower compared to control input values. In the case of ciprofloxacin–calcium and ciprofloxacin–zinc interactions, effect of solubility was more pronounced and optimized solubility values obtained in case 3 were similar to values obtained in case 1. The simulated ciprofloxacin absorption profiles in the presence of aluminium, calcium and zinc are presented in Figs. 3b, 4b and 5b, respectively.

GastroPlus™ generated regional absorption profile of ciprofloxacin showed that around 81% of the dose administered was absorbed in the proximal parts of GIT. In the presence of metallic compounds, ciprofloxacin absorption was reduced mainly in duodenum and jejunum 1 and 2 (Fig. 6).

DISCUSSION

Gastrointestinal simulation was successfully employed to develop an absorption model for ciprofloxacin immediate release dosage forms. Using the input physicochemical and pharmacokinetic parameters presented in Table I, it was possible to generate the ciprofloxacin absorption profile without metallic cation co-administration that matched well *in vivo* observed data. The predicted fractions of ciprofloxacin absorbed (F_a) for the two 'control' studies were 80.8% and 81%, respectively (Fig. 6). The results obtained are in accordance with the published data stating 60–80% bioavailability of ciprofloxacin after oral administration (28–30).

Literature data related to the effect of metallic cations (aluminium, calcium, zinc) on the solubility of fluoroquinolones are somewhat contradictory. While there are reports indicating increased solubility (31) or no effect (32) in the presence of divalent or trivalent cations, Turel (33) attributed the fluoroquinolone–metallic cation interaction to the decreased fluoroquinolone solubility.

Average absolute percent prediction error (PE %) of 10% or less for C_{max} and AUC indicates good predictability of the *in silico* model developed (25). PE % values for the C_{max} and AUC obtained were lower in the case of gastrointestinal simulation model based on the optimized solubility (Table II). This model gave good prediction of ciprofloxacin

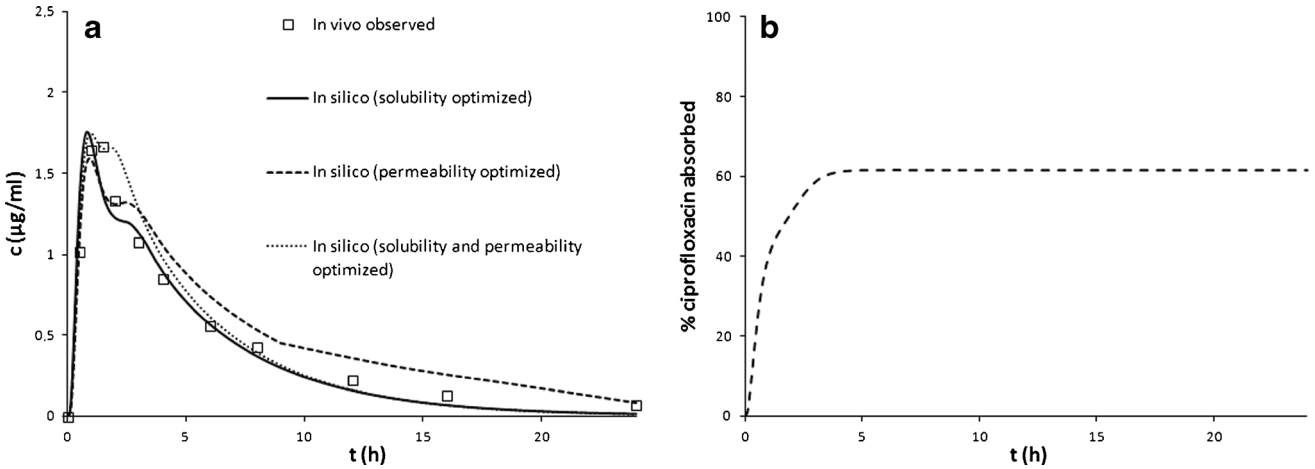

Fig. 5. GastroPlus™ predicted (solubility, permeability or solubility-permeability optimized) and *in vivo* observed mean ciprofloxacin plasma C_p–time profiles with multivitamin preparation containing zinc co-administered (**a**) and predicted absorption profile (**b**)

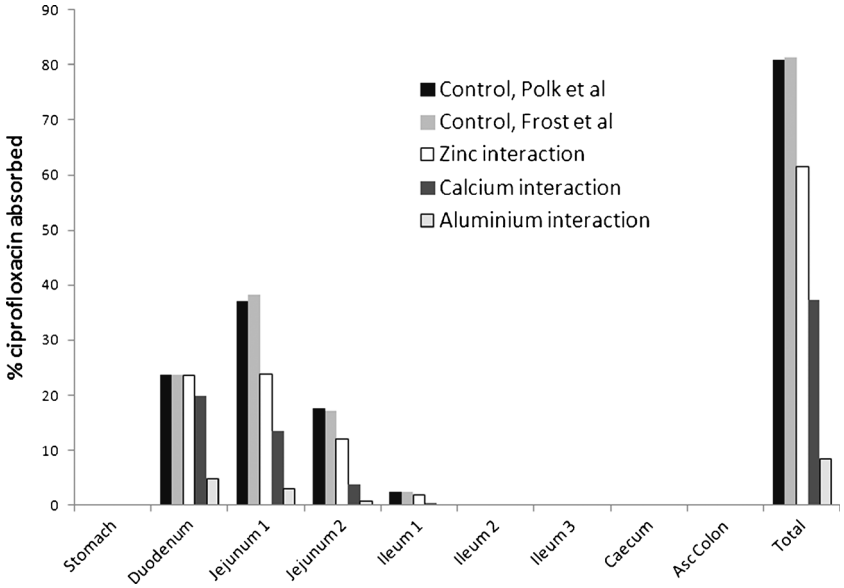

Fig. 6. Regional absorption of ciprofloxacin with/without metallic cations (zinc, calcium, aluminium) co-administration (estimated by GastroPlus™ simulation)

oral absorption with metallic cation containing products (aluminium, calcium, zinc) co-administration. Values of the regression coefficients further confirm good agreement between the *in silico* simulated and *in vivo* observed data.

The absorption model based on optimized permeability value was able to predict well the C_{max} (PE less than 10% in all the simulations), while high degree of deviation from the mean *in vivo* estimated values were observed for AUC (Table II). When interpreting the significance of AUC value prediction, it should be considered that PE % was calculated based on the mean AUC values reported from a particular *in vivo* data set (13,14). Taking into account that reported AUC values after oral administration of ciprofloxacin tablets with multivitamins containing zinc varied between 8.48 and 15.03 μgh/ml (13), the simulated value of 11.53 μgh/ml can be considered as a reasonable estimate. In addition, Frost *et al.* (14) reported that the average area under the ciprofloxacin serum concentration time curve was reduced after concomitant calcium carbonate or aluminium hydroxide product administration, but the effect in individual subjects varied in the case of calcium, while the effect of aluminium hydroxide was similar in all volunteers. According to the values of the regression coefficients obtained in case 2 (varying permeability value), poor correlation was found between *in vivo* observed and *in silico* simulated profiles. The absorption model based on simultaneously optimized solubility and permeability values was able to predict well the C_{max} and AUC values (PE less than 10% in all the simulations) (Table II) indicating the possible impact of solubility–permeability interplay in the drug absorption process.

The regional absorption profiles (Fig. 6) indicate predominant ciprofloxacin absorption in the proximal part of the gastrointestinal tract and are consistent with data reported from the *in vivo* remote control capsule study (10). In addition, the adjusted ASF values in the present model were in accordance with the data on regional drug absorption obtained by remote-controlled drug delivery device (10). Ciprofloxacin is generally absorbed by passive diffusion (34), and it may be assumed that observed absorption behaviour is probably due to high contribution of paracellular permeability in the upper parts of intestine. Such data suggest the existence of a narrow absorption window in the proximal intestine and indicate that potential interactions occurring after drug ingestion may markedly affect its bioavailability. In addition, incomplete drug absorption was observed in *in silico* simulated ciprofloxacin absorption profiles with metallic cations (aluminium, calcium, zinc) co-administered.

PSA indicate that the percent of ciprofloxacin absorbed was not influenced by prolonged residence time in stomach, while it was slightly increased with prolonged transit time in small intestine. It has been reported that ciprofloxacin demonstrates rapid absorption in the proximal part of the intestine (35). Rapid absorption is consistent with the relatively short t_{max} values (in the range of 0.58–1.75 h) and relatively high absorption rate constants, with the values reported being in the range of 1.5–3.6 h^{-1} (28–30). Relatively narrow range in which the intestinal transit time was varied may be also responsible for the negligible effect observed in PSA. According to the *in silico* results obtained, ciprofloxacin absorption in the presence of aluminium hydroxide is more sensitive to solubility than to permeability variation and certain solubility–permeability interplay was evident. Literature data indicate formation of more soluble ciprofloxacin complexes with aluminium (31,36) compared to ciprofloxacin in the range of pH 1–8. It was reported, based on *in vitro* permeability studies, that ciprofloxacin–aluminium complex does not permeate intestinal mucosal membrane (37). Ciprofloxacin tablet dissolution *in vitro* in the presence of aluminium hydroxide has been shown to be retarded (38,39), and also, some authors suggest that adsorption of fluoroquinolones on the aluminium hydroxide particles could significantly decrease the amount of drug available for absorption (39,40). Currently, it is not possible to include all these phenomena in the *in silico* simulation, which emphasize the need for complementary *in vitro* and *in vivo* studies in order to assess the mechanisms involved. In addition, the role

of solubility–permeability interplay in drug absorption should not be neglected.

Žakelj *et al.* (37) reported that ciprofloxacin permeability was not significantly influenced by the presence of Ca^{2+}. According to their results and the results obtained from *in silico* simulations, reduced ciprofloxacin bioavailability, which occurs when it is taken concomitantly with preparations containing calcium, is not necessarily a consequence of diminished intestinal drug permeability. *In silico* optimized ciprofloxacin solubility in the presence of calcium carbonate was found to be 1 mg/ml. We have shown earlier that addition of calcium carbonate elevates pH value of the dissolution media above 6 and affects ciprofloxacin solubility resulting in appearance of notable precipitation (41). Cumulative amount of drug dissolved was reduced by more than 70% (41). *In silico* optimized ciprofloxacin solubility (1 mg/ml) was similar to concentration of ciprofloxacin dissolved in reactive media containing calcium carbonate which was found to be 0.83 mg/ml (41). The simulation results show incomplete drug absorption (Fig. 4b) in the presence of calcium carbonate, indicating that absorption is likely to be solubility-limited at pH values above 6 (i.e. dose number ~2).

In the case of ciprofloxacin–zinc interaction, when zinc compound was administered as the mixture with other minerals and vitamins, it was not possible to distinguish the influence of zinc on ciprofloxacin absorption. The simulation results suggest that drug absorption when co-administered with multivitamins containing zinc was not impaired in 'duodenum', whereas it was substantially decreased in 'jejunum 1 and 2' (Fig. 6). Formation of low soluble ciprofloxacin–zinc complexes was described in the literature (42). *In silico* simulation of ciprofloxacin–zinc interaction identified decreased solubility as the critical factor affecting the rate and the extent of drug absorption, which is consistent with literature data suggesting formation of low soluble ciprofloxacin–zinc complexes.

CONCLUSION

The data presented indicate the potential of 'gastrointestinal simulation technology' to be used for prediction of ciprofloxacin metallic ion interaction. It was possible to use common model for simulation of ciprofloxacin absorption without/with metallic cations co-administration. It should be stressed that, in order to obtain meaningful *in silico* modelling, the necessary input data have to be carefully evaluated if taken from the literature or experimentally verified by performing relevant *in vitro* and *in vivo* studies. Ciprofloxacin absorption in the presence of metallic compounds is mainly impaired in the proximal parts of the intestine, leading to a significant decrease in ciprofloxacin bioavailability and potential failure of therapeutic effect. It was found that reduced solubility of the interaction adduct may be assumed responsible for reduced ciprofloxacin bioavailability in the presence of metallic ions containing preparations. Based on the results of the regional drug absorption study, it can be expected that ciprofloxacin–metallic cation interaction may be circumvented by administering metallic ions containing products 2 h after the ciprofloxacin dose, when majority of drug has been absorbed.

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