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RESEARCH PAPER

A Semi-mechanistic Modeling Strategy to Link *In Vitro* and *In Vivo* Drug Release for Modified Release Formulations

Martin Bergstrand • Erik Söderlind • Ulf G Eriksson • Werner Weitschies • Mats O Karlsson

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ABSTRACT

Purpose To develop a semi-mechanistic model linking *in vitro* to *in vivo* drug release.

Methods A nonlinear mixed-effects model describing the *in vitro* drug release for 6 hydrophilic matrix based modified release formulations across different experimental conditions (pH, rotation speed and ionic strength) was developed. It was applied to *in vivo* observations of drug release and tablet gastro intestinal (GI) position assessed with magnetic marker monitoring (MMM). By combining the MMM observations with literature information on pH and ionic strength along the GI tract, the mechanical stress in different parts of the GI tract could be estimated in units equivalent to rotation speed in the *in vitro* USP 2 apparatus.

Results The mechanical stress in the upper and lower stomach was estimated to 94 and 134 rpm, respectively. For the small intestine and colon the estimates of mechanical stress was 93 and 38 rpm. Predictions of *in vivo* drug release including between subject/tablet variability was made for other newly developed formulations based on the drug release model and a model describing tablet GI transit.

Conclusion The paper outlines a modeling approach for predicting *in vivo* behavior from standard *in vitro* experiments and support formulation development and quality control.

KEY WORDS IVIVC · magnetic marker monitoring · modified release · NONMEM

INTRODUCTION

In vitro dissolution testing of solid oral dosage forms is an essential tool in the development of new modified release (MR) formulations (1). One of the important aspects of *in vitro* dissolution testing is to provide predictions of *in vivo* performance of the tested drug product (2,3). There are well established dissolution testing methods for quality control and for the optimization of dosage forms (4). Predictability has sometimes been poor for the case of hydrophilic matrix based MR dosage forms, in particular when they are administered together with a meal (5). Efforts to improve the predictability have primarily focused on the use of bio-relevant dissolution media that more resemble the physiological situation with respect to parameters like pH, buffer capacity, surface tension, viscosity, solubilisation power and enzymatic activity (6,7). Experimental settings for adjusting the pH and other experimental parameters over time to more resemble typical gastro-intestinal (GI) transit have also been proposed (8,9) as well as new dissolution apparatuses that are

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M. Bergstrand (✉) · M. O. Karlsson
Department of Pharmaceutical Biosciences, Uppsala University
P.O. Box 591, SE-751 24 Uppsala, Sweden
e-mail: martin.bergstrand@farmbio.uu.se

E. Söderlind
Pharmaceutical Development, AstraZeneca R&D
Mölndal, Sweden

U. G. Eriksson
AstraZeneca R&D
Mölndal, Sweden

W. Weitschies
Institute of Pharmacy, University of Greifswald
Greifswald, Germany

designed to produce a type of mechanic stress to the formulation more similar to that along the GI tract (10,11). A possibility that could be utilized much more is *in silico* models to link results from standard static *in vitro* dissolution experiments to *in vivo* predictions. In combination with information about GI transit patterns and physiological conditions along the GI tract this approach could provide predictions of both the typical *in vivo* drug release and the expected between subject/tablet variability. There are already several published examples of where *in silico* models have been successfully applied for prospective predictions based on *in vitro* dissolutions data (12–16). There is also several existing commercial software possible to utilize for these purposes (17–19). *In silico* models has however likely not reached its full potential either with regards to implementation nor usage. One source of good information that rarely has been utilized enough is information from advance clinical studies with imaging techniques such as gamma scintigraphy or Magnetic Marker Monitoring.

Magnetic Marker Monitoring (MMM) offers a unique opportunity to simultaneously study the *in vivo* drug release and transit of a solid oral dosage form through the GI tract (20). The technique is based on the determination of the magnetic dipole moment generated by magnetically labeled dosage forms. With the MMM technique the disintegration properties of the solid dosage form can be monitored during its passage through the GI tract by means of the decrease of magnetic moment. For dosage forms where the drug release rate is determined by the erosion of the dosage form, the magnetic moment can be linked to the drug release. In these cases a relationship between decrease in magnetic signal and drug release characterized in *in vitro* experiments can be used to obtain actual *in vivo* drug release profiles (21).

Results from *in vitro* dissolution experiments with MR formulations are typically illustrated graphically with mean fraction dissolved *vs*. time and summarized with descriptive statistics of time to a certain percentage dissolved (*e.g.* $T_{80\%}$) (3). This type of summarization of the results gives no possibility for interpolation/extrapolation to other conditions than those studied. Furthermore, in the development of a new MR formulation several candidate formulations are typically investigated in *in vitro* experiments. Each candidate formulation is typically characterized under different experimental conditions (*e.g.* pH and mechanical stress). Several of the investigated formulations are often closely related with respect to composition with only a single parameter altered between the formulations, *i.e.* the formulations are more similar than different.

In this manuscript we suggest to apply an *in silico* model to 6 closely related candidate formulations across varying experimental conditions. This aims to characterize; (1) the entire drug release profile for all experiments, (2) the effect of experimental conditions (*e.g.* pH) on drug release rate with continuous functions, (3) systematic differences between the different formulations. The model developed based on the *in vitro* experiments was in a second step applied to *in vivo* drug release data from a MMM study (D1250C00018) to estimate the link between *in vitro* and *in vivo* conditions. This represents the first steps in order to perform prospective predictions of the plasma concentration *vs*. time profiles for newly developed formulations. For this to be possible the drug release model will have to be combined with a pharmacokinetic model describing regional absorption properties similar to the one previously described by the authors (22).

AZD0837 is a novel oral direct thrombin inhibitor in clinical development for the prevention of stroke in arterial fibrillation patients (23). It is a prodrug that is bioconverted via an intermediate to its active form, AR-H067637. Development of a suitable extended release (ER) formulation was undertaken to support a once daily dosing (24). Six different Hydroxypropyl Methylcellulose (HPMC) candidate ER formulations for AZD0837 with erosion controlled drug release rates were developed and subsequently used as an example to investigate the potential benefits with application of nonlinear mixed-effects modeling approach, to *in vitro in vivo* correlation (IVIVC) for drug release.

MATERIALS AND METHODS

Substance and Formulations

AZD0837 is a base with pH dependent solubility that decreases with increasing pH. Thus, the solubility at pH 7.5, approximately 0.2 mg/mL, determines the solubility classification of AZD0837 as defined by the Biopharmaceutical Classification System and is not sufficient for a high solubility classification at all doses greater than 50 mg. A formal determination of permeability for BCS classification has not been conducted, but permeability data using a Caco-2 model suggest moderate to high permeability.

The investigational ER formulations in the current study consist to the major part of HPMC and drug substance. The drug release rate from the ER formulations was controlled by using different grades of HPMC and drug substance content.

In Vitro Experiments

In vitro drug release for 6 different investigational HPMC ER formulations was assessed under different experimental conditions with USP apparatus 2 equipped with a stationary basket (4). Formulation Z was also investigated with the modified ERWEKA (m-ERWEKA) dissolution tester (21). The USP 2 experiments were carried out at AstraZeneca R&D, Mölndal Sweden and the m-ERWEKA experiments in Institute of Pharmacy, University of Greifswald,

Greifswald, Germany. Table I describes important formulation characteristics that differ between the formulations and what experimental conditions that were investigated for the different formulations. The formulation characteristics taken into account in the model were the nominal dose of AZD0837 (free form), the fraction active pharmaceutical ingredient (API) in the tablet (weight% for the salt form of AZD0837) and the tablet weight. Experimental conditions varied with respect to: rotational speed (rpm), pH and ionic strength (Table I). A comparison of the *in vitro* drug release profiles for the 6 formulations are provided in the online supplementary material (Fig. 7).

Clinical Study

A randomized, 3-way crossover, single-centre, study in which 6 healthy male volunteers underwent three study sessions each with magnetically marked, single doses of AZD0837 ER tablets (formulation Z, see Table I). Treatment I: tablet administered in fasting state. Treatment II: tablet administered immediately (within 5 min) before intake of a high-fat, high-caloric breakfast. Treatment III: tablet administered 30 min after start of intake of a high-fat, high caloric breakfast. The GI transit and *in vivo* drug release of the magnetically labeled ER tablets were monitored with a biomagnetic measurement device and plasma samples were frequently sampled to characterize the plasma concentration of AZD0837. For this publication only the GI position and drug release information have been considered.

The study (D1250C00019) was conducted at CPU Berlin SocraTec R&D GmbH (Berlin, Germany) under the sponsorship of AstraZeneca R&D (Mölndal, Sweden). All patients signed a written informed consent form. The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and was approved by the ethics committee at Ethik-Kommission, Landesärztekammer Thuringia.

Magnetic Marker Monitoring

Magnetic Marker Monitoring (MMM) is a non-invasive tool for the investigation of the GI transit of ingested dosage forms that are marked as magnetic dipoles. For magnetic labeling, each tablet contained 5 mg of black iron oxide (E172), a color pigment that is commonly used as a colorant for food and orally applied dosage forms. In order to create a magnetic dipole moment, the tablets were magnetized prior to ingestion using a static magnetic field, resulting in a dipole moment of about 30–60 μAm^2. This magnetization was done at the Clinical Pharmacology Unit of SocraTec R&D GmbH in Berlin, Germany. During the *in vivo* investigations the magnetic field that was generated by the magnetized tablet was determined using an extremely sensitive biomagnetic measurement device, multichannel SQUID sensors (Physikalisch-Technische Bundesanstalt Berlin). Assessment of the magnetic signal for localization and characterization of the erosion of the labeled tablets was planned to last 10 min with rests of 20 min duration in between up until 14 h after tablet administration. End of detection was defined when the magnetic moment decreased to a value below 15% of the initial value. In case that a decrease below 15% of the initial value could not be observed until 14 h post dose one further measurement was performed 24 h post dose.

A mathematical relationship between the decrease in magnetical signal and amount of released drug substance was derived based on *in vitro* characterization of the labeled formulation (21). Using this correlation function and the individually measured magnetic moments the theoretical amount of drug remaining to be released at each time point could be calculated.

The position of the labeled tablet was characterized based on previously published principles (25). For the analysis the tablet position was categorized into 7 distinct GI regions: *Proximal stomach*, *distal stomach*, *small intestine* (duodenum, jejunum, early ileum and terminal ileum), *ascending colon* (incl. cecum and hepatic flexture), *transverse colon* (incl. splenic flexture), *descending colon*, *sigmoid colon* (incl. rectum). In some cases, the location could not be differentiated between the proximal and distal stomach and how this was handled is described in the model building section.

Model Building

Software

Data analysis was performed with a nonlinear mixed-effects approach as implemented in the NONMEM software version 7.1.2 (26), run on a Linux cluster with a Red Hat 9 operating system using OpenMosix and a G77 Fortran compiler. First-order conditional estimation method (FOCE) with interaction and the ADVAN6 (general nonlinear kinetics) subroutine was applied for parameter estimation. The so called M3 method was applied to account for observations below the lower limit of quantification (LLOQ) in the parameter estimation (27–29). All NONMEM control files can be provided upon request.

The PsN toolkit version 3.2.7 (30,31) was used in conjunction with NONMEM for automation and post processing purposes. The Xpose 4.3.0 (32,33) package in R (34) was used for graphical diagnostics.

Table I Specification of the Investigated Formulations and the Experimental Conditions that these were Investigated Under

	Formulation characteristics			Investigated experimental conditions			
Formulation	Dose (mg)	API[a] (%)	Weight (mg)	pH	Rotation speed (rpm)	Ionicstrength (mol/l)	# Exp.[b]
A	100	37	353	6.8	50	0.1	6
B	100	32	409	1, 4.5, 6.8, 7.4	50, 100	0.05, 0.1	21
C	100	31	423	6.8	50	0.1	6
Q	200	70	380	1, 6.8	50	0.1	9
X	150	55	360	1, 2, 3, 5, 6, 6.8	10, 50, 100	0.1, 0,2, 0,3	56
Z[d]	200	55	490	1, 1.2, 3, 4.5, 6.8	20[c], 25[c], 30[c], 50	0.05, 0.075, 0.1, 0,2, 0,3	28 (15)

[a] Fraction active pharmacological ingredient (weight percentage for salt form of AZD0837)

[b] Number of experiments in total. Number of experiments with m-ERWEKA set-up within brackets

[c] Rotational speed with m-ERWEKA set-up not equivalent to USP 2 (conversion factor estimated)

[d] Formulation used in clinical MMM study

In Vitro Drug Release

For eroding hydrophilic matrix ER tablets, the dissolution tests measure the composite of drug release and drug dissolution. Under the investigated conditions for the substance at hand, the dissolution of released substance was very fast in comparison to the drug release. Hence, the measurements could be considered describing only drug release.

Formulation X was considered as a reference formulation and the release rate for the other formulations was characterized in relation to that. Different principal model structures were considered for describing the drug release. Zero-order, first-order and combinations of several first and/or zero-order release processes were considered but did not result in a satisfactory fit to the data. A hypothesis was formed around that the rate of drug release was dependent on the surface area of the formulation. An attempt was made to create a model that could describe the drug release as a function of the dynamically changing surface area. The weight of the tablet raised to the power of 2/3 (γ in Eq. 1) was used as an approximation of the surface area of the tablet. This approximation assumes that the substance is evenly distributed in the formulations and that the tablet erodes in a symmetrical fashion (*i.e.* maintain its original shape during the disintegration). Equation 1 features the differential equation that describes how the amount of non-released substance in the tablet ($A1$) decreases with time, dependent on a release rate constant (R), the initial *Tablet weight* (mg), the amount (mg) of AZD0837 in the tablet (*Dose*) and the power factor (γ).

$$\frac{dA1}{dt} = -R \cdot \left(\frac{Dose}{Tablet\ weight}\right) \cdot \left(\frac{Tablet\ weight \cdot A1}{Dose}\right)^{\gamma} \quad (1)$$

$A1$ was initially equal to *Dose* with the dose being the estimated drug content with log-normal between tablet variability (close to nominal dose). The hypothesized model resulted in significantly better fit to the data than any of the previously mentioned parameterizations. By estimating the power factor, γ, rather than fixing it to the theoretical value of 2/3 an even better fit to the data was achieved. This model was also compared to the often used Weibull model (35). The Weibull model resulted in a significantly poorer fit to the data both when applied to individual experiments and when simultaneously analyzing the entire dataset. Suggested more mechanistic models (36) like the Hopfenberg model (37) or the Baker-Lonsdale model were (38) not investigated since they could not easily be applied to time varying predictors of the drug release rate (*e.g.* changes in pH over time).

The effect of experimental conditions was investigated as covariate relationships on the typical release rate $R_{typical}$ (formulation X, rotation speed = 50 rpm, ionic strength = 0.1 mol/l, pH 6.8), see Eq. 2. The relative differences in release rate for formulations other than the reference formulation (X) were also estimated (Cov_{Form}) and the between tablet variability (BTV) in release rate was described with an exponential random effect (η_R).

$$R = R_{typical} \cdot Cov_{Ion} \cdot Cov_{RPM} \cdot Cov_{pH} \cdot (1 + Cov_{Form}) \cdot e^{\eta_R} \quad (2)$$

The effect of ionic strength (E_{Ion}) and rotational speed in the USP 2 apparatus (E_{RPM}) were satisfactory described by linear functions (Eqs.3 and 4). A conversion factor ($mERW$) was estimated to translate measurements of rotational speed (rpm) from the m-ERWEKA experiments to the measurements in USP 2 setup.

$$Cov_{Ion} = 1 + E_{Ion} \cdot (Ionic\ strength - 0.1) \quad (3)$$

$$Cov_{RPM} = 1 + E_{RPM} \cdot ((RPM \cdot mERW) - 50) \quad (4)$$

Initial data exploration suggested that pH in the low range (1–4) affected the release rate but that no apparent effect could be seen with pH>4. This relationship to pH was successfully described with the estimation of a linear slope parameter (E_{pH}) and a breakpoint at the pH where no further effect could be seen ($BreakPoint_{pH}$) according to Eq. 5. Prior information also revealed that the effect of pH on drug release was driven by pH dependent solubility of the active ingredient AZD0837 since the disintegration of the HPMC hydrophilic matrix previously was shown to be relatively insensitive to pH (39). An interaction term between fraction of active ingredient in the formulation (API) and pH was therefore considered in the model. A linear effect of API (normalized to the API of formulation X, 0.55) on the pH covariate relationship was estimated (E_{API}). The statistical significance of the covariate relationships included in the final model was tested with a final backward elimination step where the included covariate relationships were taken away one by one (see Table V in the online supplementary material). All included covariate relationships was highly significant with p-values less than 0.005.

$$Cov_{pH} = 1 + \frac{E_{API} \cdot (API - 0.55) \cdot E_{pH} \cdot \left(pH - BreakPoint_{pH}\right)}{1 + e^{7^{*}\left(pH - BreakPoint_{pH}\right)}} \tag{5}$$

In the final model the dose amount was set to the nominal dose for the experiments where far from complete drug release was achieved before the end of the experiment (<80% of nominal dose released). This was the case for experiments with high ionic strength (>0.1 and/or with low rotation speed, 10 rpm). This was done to avoid a suspected over fit to these data by estimation of a relative drug content far from the nominal dose. One experiment with formulation X resulted in release of 126% of the nominal dose (1 out of three replicates for pH 3, 100 rpm, 0.1 mol/L). An experimental error was suspected in this case and a separate model fit with this experiment excluded was performed to investigate model sensitivity to this suspected outlier. The sensitivity to the suspected outlier was very small. The only noticeable change to parameter estimates upon exclusion was a decrease in variability for the relative dose amount (4.4% to 3.8%), as expected.

The final *in vitro* drug release model was diagnosed with standard goodness-of-fit (GoF) plots and the covariate relationships were assessed by assessment of η_R *versus* the covariates. Eta and Epsilon shrinkage was calculated in order to ensure validity of the diagnostics (40,41). A dissolution experiment (two replicates) with varying experimental conditions over time was available for formulation X. The experimental conditions were: pH 1, 50 rpm (0–2 h), pH 6.8, 50 rpm (2–6 h) and pH 6.8, 10 rpm (6–60 h). The Ionic strength was 0.1 mol/l throughout the entire experiment. Data from this experiment were not utilized for model building but were instead used as an external validation dataset for the final model. A non-parametric 95% confidence interval from the median drug release was calculated based on 500 simulations with the final model and compared to the observed median.

In Vivo Drug Release

The model developed based on the *in vitro* dissolution experiments was applied to the *in vivo* drug release data derived with MMM. The drug release data was complemented with information about the tablet GI position and prior information on pH in the different GI regions. The prior information about typical and between subject variability in pH along the GI tract (Fig. 1) was gathered from the literature (42,43). Literature information about the ionic strength along the GI tract was scarce but the available information pointed towards 0.1 mol/l in the stomach and colon but slightly higher 0.14 mol/l in the small intestine (44).

With the pH and Ionic strength set to the literature value according to the observed GI position and food intake, the only unknown factor in comparison to the *in vitro* experiments was the mechanic stress (*i.e.* rotational speed) in the different GI regions. The mechanic stress in the different GI regions was hence estimated with the final *in vitro* model. All fixed effects of the *in vitro* model were fixed at the final estimates. Random effects were estimated for R and the *relative dose amount*. Random effects were also included for pH in the different GI regions. The pH variance was fixed to literature values describing between subject variability in pH (42,43). The mechanic stress was estimated (RPM) based on the covariate relationship for rotation speed in the *in vitro* experiment and the estimates were hence of a unit equivalent to rpm in the USP 2 apparatus.

In a few cases, no certain distinction in tablet location could be made between proximal and distal stomach. An average of parameters (pH and estimated RPM) for the two positions was then assumed. Preliminary estimates of mechanic stress in the different colon regions indicated similar estimates and relatively high uncertainty due to sparseness of observations for the later colon regions. For this reason the final model only characterized the mechanic stress for colon in total. Since the literature assumption about higher ionic strength (0.14 mol/l) in the small intestine was judged to be uncertain an alternative model fit was performed with the final model and small intestine ionic strength assumed to be 0.1 mol/l. This as expected only affected the estimate of mechanical stress in the small intestine.

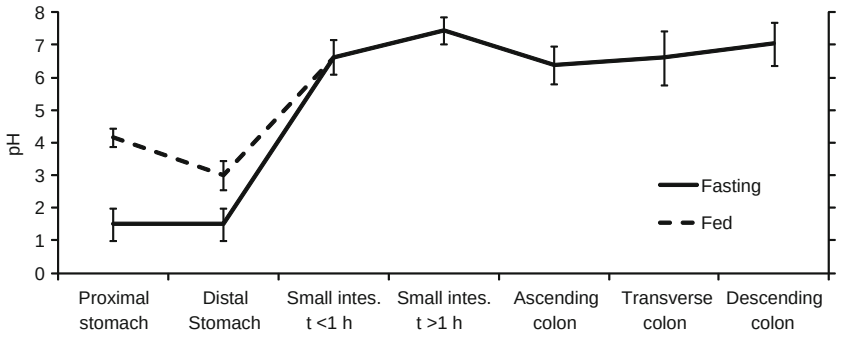

Fig. 1 Literature information on pH throughout the GI tract under fed and fasting conditions (42, 43). Fed (*dashed line*) and fasting (*solid line*) conditions differ for pH in the proximal and distal stomach. Lines represent mean and the error bars standard deviation.

The final model was diagnosed with standard GoF plots and Visual Predictive Checks (VPC) stratified for each treatment (fasting, food-tablet and tablet-food). Due to the low number of subjects the VPCs focused on the median drug release profile and fraction of observation below LLOQ (29). A non stratified prediction and variance corrected VPC (pvcVPC) was used to also assess the accuracy of the predicted between tablet/subject variability. The pvcVPC plot is normalized based on the typical model prediction and the mean predicted within subject variability (45). The normalization facilitates pooling of data from the different treatment cohorts and diagnosis of the between tablet/subject variability not explained by independent variables included in the model (tablet GI position and time).

Tablet GI Transit Model

A model describing tablet transition throughout the GI tract was developed according to a previously described principle (22). The model was a compartmental, differential equation based, Markov chain model describing the probability for transiting between the characterized GI regions (*proximal stomach*, *distal stomach*, *small intestine*, *ascending colon*, *transverse colon*, *descending colon*, *sigmoid colon*). The model developed for this study differed from the previously described model for the felodipine extended release formulation (22) in that no distinction was made between proximal and distal small intestine (not characterized when collecting the data) and that information was present to describe transit between the ascending, transverse and descending colon. A chain of compartments was used to achieve a prolonged retention time in the small intestine. A sufficient number of transit compartments was found by stepwise increasing the number of transit compartments until there was only a marginal improvement in the NONMEM OFV value (<0.5) with additional compartments. In the final model four transit compartments were implemented. All other GI locations were found to be sufficiently described by only one compartment. Parameters were estimated in the form of mean transit times (MTT).

Potential effect of concomitant food intake was evaluated for all parameters in the model. The treatment where the tablet was given prior to food was handled by estimation of a change point time (CHT) when the subjects went from a typical fasting state to typical fed state. This idea came from visual inspection of the GI transit profiles that revealed some individuals with a fast gastric emptying similar to that associated with fasting administration and some with stomach residence time similar to that for fed administration. This approach was compared to considering the treatment similar to fed administration or as a separate food effect on stomach MTTs (see Table VI in online supplementary material).

Simulations

Simulations were performed with the final models for tablet GI transit and *in vivo* drug release to illustrate the expected drug release profiles for the different formulations under fed and fasting conditions. Tablet GI transit profiles were first simulated based on the Markov model. The simulated tablet GI position was sequentially used as a covariate for simulations with the drug release model. Results from 500 simulations of studies with 100 subjects were summarized with the median and a 90% prediction interval (5^{th} and 95^{th} percentile). These metrics were plotted *versus* time after dose intake. In the same plots the proportion of tablets past gastric emptying was also depicted *versus* time after dose.

RESULTS

In Vitro Drug Release

The final *in vitro* model provided a good description of all experiments, across the six formulations and different experimental conditions (Fig. 2). An acceptable, but slightly

worse than average, fit was seen for experiments performed with high ionic strength (0.3 mol/l). For these experiments the drug release rate (R) seemed to decrease with time. This was judged to be of low clinical interest since the anticipated physiological ionic strength was in the range between (0.1–0.14 mol/l) and hence not investigated further.

Final parameter estimates for the *in vitro* drug release model is presented in Table II. The final established covariate relationships for pH, rotational speed and ionic strength are illustrated in Fig. 3. The measure of mechanical stress (rpm) from the m-ERWEKA experiments was estimated to be related to rpm in the USP 2 apparatus via a conversion factor 3.3 ($rpm_{USP2} = 3.3 \cdot rpm_{m\text{-}ERWEKA}$). No indication of any other difference between the drug release profiles between the two different experimental setups could be detected. The best fit to the data was achieved with the power factor (γ) estimated to 0.56 (RSE 2.1). Compared to the originally assumed theoretical value of 2/3 (when the tablet erodes in a symmetrical fashion) the estimate of 0.56 corresponds to a slightly more constant (zero-order) drug release. The estimated relative drug content was very close to the nominal dose (99%), the variability in drug content between tablets was estimated to 4.4%. The possible influence of one suspected outlier is mentioned in the method section.

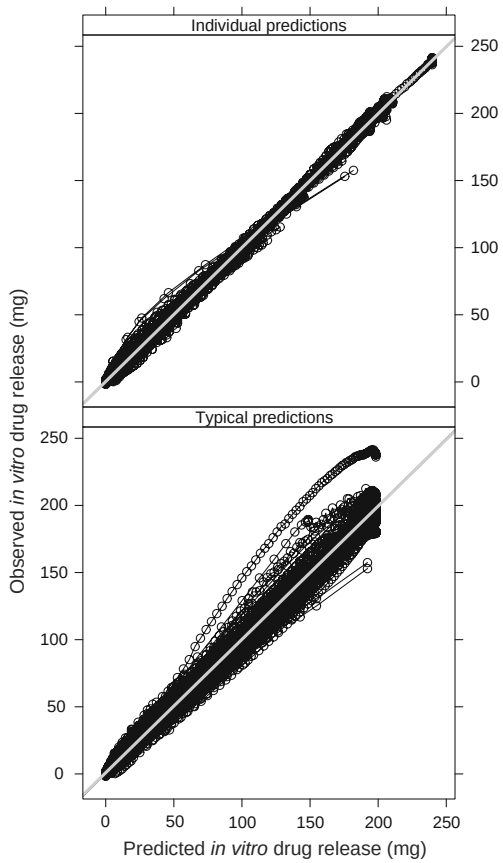

Fig. 2 Observations *vs.* predictions for *in vitro* drug release experiments. Observations *vs.* population prediction (no between tablet variability) at the bottom and observations *vs.* individual experiment prediction on the top. The observations (*black circles*) within a single experiment are connected with black lines, a linear regression line is presented in gray color. Epsilon shrinkage (2%) and eta shrinkage (<9%).

Most of the differences between the different formulations were explained by the structural parts of the model. The remaining difference between the formulations was characterized in relation to release rate for the reference formulation X. Formulation Z was not significantly different from formulation X and the other formulations ranged between having 33% slower to 16% faster drug release rate.

Data from an experiment with formulation X where the experimental conditions were changed over time was used as a validation dataset for the *in vitro* model. A model predicted 95% confidence interval for the median drug release profile is compared to observations from the experiment in Fig. 4. The figure demonstrates good predictive behavior of the model without any obvious deviations.

In Vivo Drug Release

The modeling of the *in vivo* drug release data was based on the model developed for *in vitro* drug release and prior information on pH and ionic strength along the GI tract. The mechanic stress in the different GI regions was estimated in a unit equivalent to rotational speed in the USP 2 *in vitro* apparatus. Parameter estimates are presented in Table III and the assumed pH along the GI tract is presented in Fig. 1.

The mechanic stress was estimated to be highest in the distal stomach (134 rpm) and lowest in colon (38 rpm). The estimate of mechanic stress in the small intestine (93 rpm) was dependent on the assumption about ionic strength. Due to the uncertainty of this assumption (0.14 mol/l) an alternative hypothesis of 0.1 mol/l ionic strength was also investigated. This resulted in an estimate of 79 rpm for the small intestine. No significant effect of concomitant food intake could be detected apart from that explained by differences in stomach pH and longer stomach residence time. Satisfactory predictability was demonstrated both for the typical (median) drug release profile under the different investigated conditions and for the overall predicted variability in drug release (Fig. 5).

Tablet GI Transit

Parameter estimates for the tablet GI transit model are presented in Table IV. Significant food effect was established for the MTT from proximal to distal stomach and

Table II Parameter Estimates for *in vitro* Drug Release Model. Typical Estimates, Between Tablet Variability (BTV) and Associated Relative Standard Errors (RSE)

Parameter (Unit)	Estimate (RSE,%)	% BTV (RSE,%)
Typical release rate, R (h^{-1})	1.03 (7.0)	11 (6.4)
Relative drug content, (% of nominal dose)	99 (0.5)	4.4 (16.8)
Gamma factor, γ	0.56 (2.1)	
E_{RPM} (rpm^{-1})	0.014 (6.7)	
mERWEKA factor[a] (%)	3.30 (11.6)	
E_{Ion} (mol/L^{-1})	−3.2 (2.4)	
E_{pH} ($pH\ unit^{-1}$)	−0.67 (4.7)	
$BreakPoint_{pH}$ (*pH unit*)	3.5 (2.0)	
E_{API} ($\%^{-1}$)	2.6 (8.5)	
Form A relative release rate, R (%)[b]	+16 (30)	
Form B relative release rate, R (%)[b]	−12 (25)	
Form C relative release rate, R (%)[b]	−33 (4.8)	
Form Q relative release rate, R (%)[b]	−14 (26)	
Form Z relative release rate, R (%)[b]	±0	
RUV[c] *for USP 2 (mg)*	3.2 (7.4)	
RUV[c] *for mERWEKA (mg)*	2.1 (9.0)	

[a] Correction factor for mERWEKA rotational speed compared to USP 2

[b] Relative difference in typical release rate (R) compared to formulation X

[c] Residual Unexplained Variability (RUV)

from distal stomach to small intestine. Together these effects result in more than 11 times longer mean residence time in stomach for tablets administered in the fed state. The approach with estimating change point time (CHT) for the case when the tablet was administered just previous to start of food intake resulted in better fit to the data (OFV) than the other investigated approaches. The estimate suggests that if the tablet still remains in the stomach approximately 25 min after the start of breakfast intake it will continue to behave as if it was administered in a fed state. From the distal stomach the tablet can either transit into small intestine or back to the proximal stomach. The parameter estimates corresponds to a 15 min mean residence time in the distal stomach under fasting conditions and 127 min under fed conditions. The probability that the tablet will transit into small intestine is 94% under fasting conditions and 52% under fed conditions. The uncertainty is relatively high for all parameter estimates due to the low number of individuals studied but the estimates are largely in accordance with other published data regarding GI transit of single solid dosage forms (20,22,46,47).

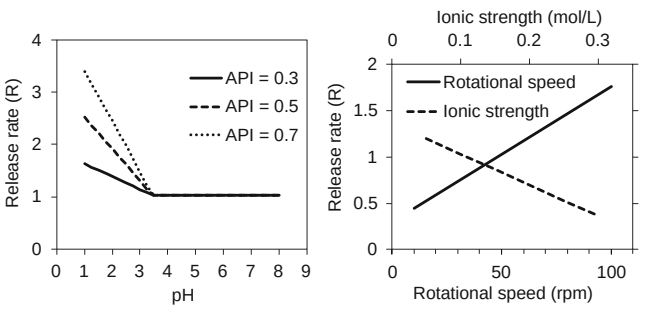

Fig. 3 Covariate relationships for typical release rate (R). *Left*: Relationship between pH and R for three API fractions. *Right*: R as a function of ionic strength (*dashed line*, upper x-axis) and rotational speed (rpm) in the USP 2 apparatus (*solid line*, lower x-axis).

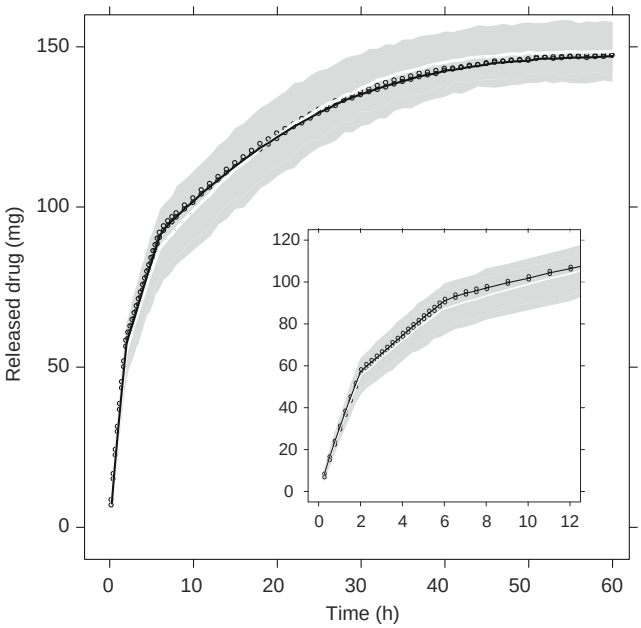

Fig. 4 External validation of the *in vitro* model by prediction of experiment with time varying conditions for formulation X The experimental conditions were: pH 1, 50 rpm (0–2 h), pH 6.8, 50 rpm (2–6 h), pH 6.8, 10 RPM (from 6 h). The Ionic strength was 0.1 mol/L throughout the entire experiment. The solid black line represents the median of the observations (*open circles*) in the two experiments. The model prediction is represented by a solid white line for the median prediction and a gray field for a non-parametric 95% confidence interval.

Table III Parameter Estimates for *in vivo* Drug Release. Typical Estimates, Between Tablet Variability (BTV) and Associated Relative Standard Errors (RSE)

Parameter (Unit)	Estimate (RSE,%)	% BTV (RSE,%)
Typical release rate, R (h^{-1})	1.03[a]	18 (28)
Relative drug content, (% of nominal dose)	103 (1.0)	4.0 (34)
$RPM_{Proximal\ Stomach}$ *(rpm)*	94 (9.6)	
$RPM_{Distal\ Stomach}$ *(rpm)*	134 (4.8)	
$RPM_{Small\ intestine}$ *(rpm)*	93[b] (6.8)	
RPM_{Colon} *(rpm)*	38 (17)	
RUV (mg)	6.3 (8.5)	

[a] Parameter not estimated in model fit to *in vivo* drug release data and hence no RSE

[b] Estimate with ionic strength for small intestine set to 0.14 mol/l. Estimated to 79(7.5) rpm with ionic strength assumed to be 0.1 mol/l

DISCUSSION

The *in vitro* drug release model demonstrates the potential value of simultaneous modeling of several candidate formulations of a similar type. This approach can reduce the number experiments needed to be performed and improve the possibilities for optimizing experimental design. The model structure suggested in this paper is thought to be applicable to formulations with primarily erosion controlled drug release but could likely also be extended to include elements of diffusion. The power parameter (γ) will for low values (<0.2) approximate an almost constant drug release (zero-order) and for the values close to 1 it will be the same as a first-order release rate. A surface area dependent drug release will be in between the extreme cases of a first and zero-order release rate and be represented by a $\gamma = 0.667$ under the assumption of symmetrical erosion. The estimated value for $\gamma = 0.56$ hence indicates that the drug release rate for the investigated formulations are slightly more constant than what would be the case for the theoretical value of 2/3. This could be the consequence of a non symmetrical erosion of the formulation. The lower value of gamma corresponds to a gradual transformation into a shape that has a larger surface area in relation to the volume.

The differences between the formulations not explained by structural parameters of the model (*e.g.* tablet size and API) ranged between 33% slower to 16% faster typical drug release rate. The majority of these differences could be attributed to density of the different formulations (data on file). This further supports the hypothesis that the drug release is related to the surface area of the formulation since the density is what relates the tablet weight to the formulation volume.

Experiments with altered experimental conditions, as the one used as an external validation of the *in vitro* model (Fig. 4), are often suggested (8,9) to simulate a typical passage through the GI tract. Instead the effect of different

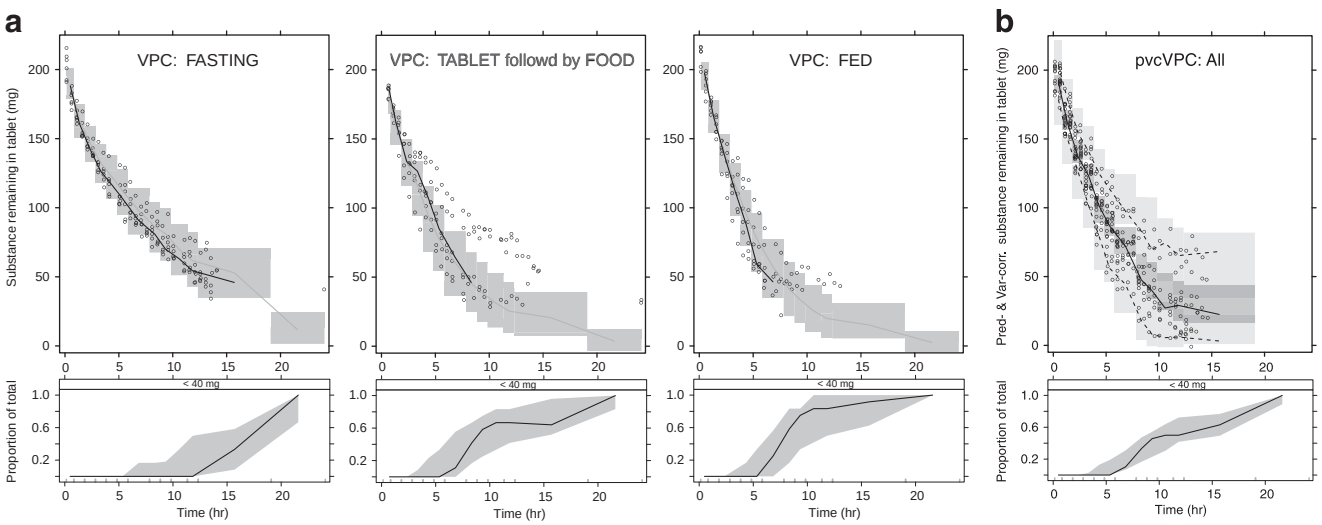

Fig. 5 Model diagnostics for *in vivo* drug release. (**a**) Upper panel: observed median for remaining drug substance in tablet (*black line*) and corresponding model predicted median (*gray line*) and 95% confidence interval (*gray field*) for administration in fasting state and before/after food intake. Lower panel: Observed fraction of observations indicating less than 40 mg remaining in tablet (*black line*) and corresponding model predicted 95% CI (*gray field*). (**b**) Upper panel: pvcVPC with 90% variability interval for all subjects independent of fed or fasting administration (observations and predictions <40 mg censored). Observed median (*black line*) and corresponding model predicted 95% confidence interval (*dark gray field*), the 5th and the 95th observed percentile (*dashed black line*) and corresponding model based 95% confidence intervals (*light gray field*). Lower panel: Observed fraction of observations indicating less than 40 mg remaining in tablet (*black line*) and corresponding model predicted 95% CI (*gray field*).

Table IV Parameter Estimates for Tablet GI Transit

Mean transit times (min)	Estimate (RSE,%)
Prox. Stomach ->Distal Stomach (fasting)	12 (22)
Prox. Stomach ->Distal Stomach (fed)	81 (23)
Distal Stomach ->Proximal Stomach	265 (44)
Distal Stomach ->Small intestine (fasting)	16 (44)
Distal Stomach ->Small intestine (fed)	245 (18)
Small intestine ->Ascending colon	291 (25)
Ascending colon ->Transverse colon	327 (28)
Transverse colon ->Descending colon	185 (64)
Descending colon ->Sigmoidal colon and rectum	240 (17)
CHT (time to fed state for tablet intake followed by food)	33 (27)

conditions can be predicted with the help of *in silico* simulation, as shown in Fig. 4, with a model developed based on static dissolution experiments. The *in silico* approach adds the opportunity to incorporate between subject variability in pH and GI transit profile (Fig. 6). This should result in better predictions of the typical drug release profile and in addition give predictions about the expected variability in drug release.

The results for modeling of the *in vivo* drug release suggest a mechanical stress along the GI tract that corresponds to rotational speed higher than what is typically recommended for *in vitro* predictions (48). The actual investigated rotation speeds is however not of any great importance given that a linear relationship can be established between release rate (R) and rpm in the USP 2 apparatus. The estimates of equivalent rotation speeds in the different GI regions are possibly dependent on the type of formulation that was used since the type of mechanical stress along the GI tract is quite different from the one in a USP 2 apparatus (49). These estimates are therefore primarily thought to be representative for HPMC hydrophilic matrix formulations with erosion controlled drug release. Further investigations based on *in vitro* and *in vivo* drug release data from other types of modified release formulations are necessary in order to assess to what extent these estimates are formulation dependent. The between tablet variability (BTV) for the drug release rate was estimated to be slightly higher for *in vivo* (18%) compared to *in vitro* data (11%). This was considered quite natural taken that ionic strength and mechanic stress are assumed to be identical for all subjects in the application of the model to the *in vivo* data. Variability in relative dose amount was similar between *in vitro* and *in vivo* and within the expected range (~4%).

The estimated GI transit times are largely similar to previous estimates with the same modeling approach (22) and also in agreement with median transit times assessed with other methods (20,46,47). The estimate that stands out as slightly different from that most commonly reported is the small intestinal transit time (SITT). Mean SITT are most often reported to be between 3 and 4 h whereas it in this study was estimated to be close to 5 h. However, other studies have also reported mean SITT longer than 5 h (42). It is further well known that food intake can affect small intestinal emptying into colon. The reports of SITT typically less than 4 h have been suggested to be a consequence of the fact that many clinical study protocols features a meal 4 h after tablet intake (20). The present clinical study had the corresponding meal between 4 and 5 h after dose intake. A meta-analysis of GI transit data across several studies, with different protocols, and types of formulations, would likely result in a better understanding

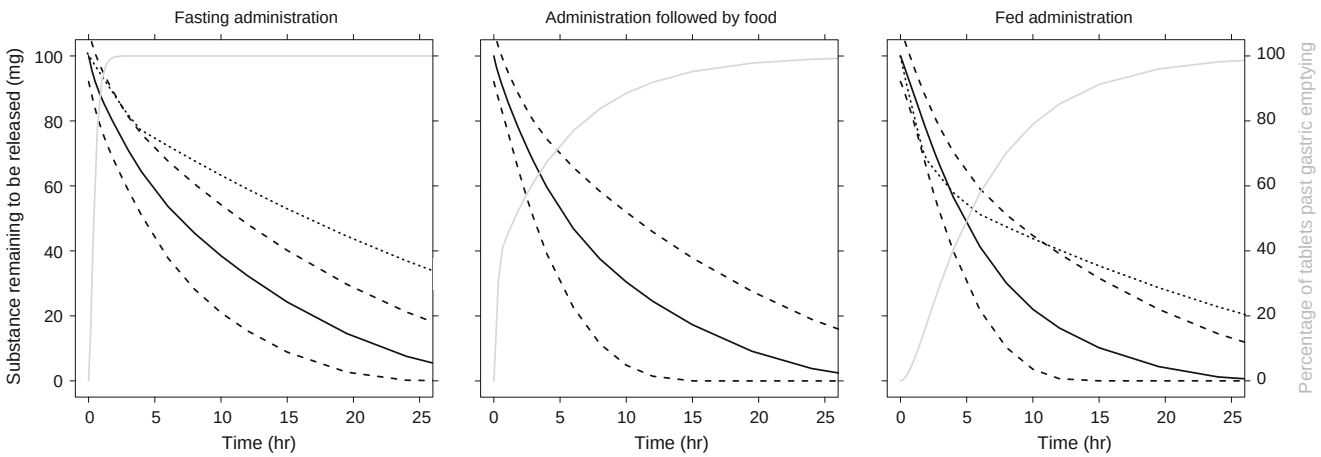

Fig. 6 Simulated *in vivo* drug release predictions for formulation B. Predicted median (*solid black line*) and 90% prediction interval (*dashed black lines*) for substance remaining in the tablet (mg) following administration in fasting state and before/after food intake. As a comparison the drug release profile of a standard *in vitro* experiment to mimic fasting or fed conditions are depicted with a *dotted black line*. The simulated percentage of tablets past gastric emptying is plotted with a *solid gray line*.

of these processes. A well established *in silico* model for tablet GI transit could also serve as a very useful tool for prediction of oral drug absorption.

The *in vivo* predictions for formulation B illustrate the effect of concomitant food intake on the typical drug release profile but also the variability (Fig. 6). The major sources of variability are variability in stomach pH and time to gastric emptying. Food intake affects both of the factors, as it increases stomach pH, decreases the pH variability in the stomach, delays gastric emptying and hence causes larger variability in time to gastric emptying. The full consequence of this can be seen for the case when the tablet is administered prior to food intake. In this case the results become a mix of patterns similar to fed or fasting administration.

CONCLUSION

An *in silico* model structure suitable for estimation of erosion controlled drug release rate was established together with a link between mechanical stress in standard USP 2 apparatus and *in vivo* conditions for HPMC hydrophilic matrix formulations. Simulations of *in vivo* drug release were performed based on a model for tablet GI transit, *in vivo* drug release and literature information about pH variability along the GI tract. The presented modeling approach can be utilized to predict *in vivo* behavior from standard *in vitro* experiments and support formulation development and quality control of MR formulations.

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