Introduction

Gastrointestinal (GI) fluid volume is a key factor in the dissolution and absorption process of oral drug products. For solid oral dosage forms, the local quantity of fluid can affect disintegration, dissolution, and absorption. Because local GI fluid volume varies significantly due to transit, secretion, and absorption, characterizing the *in vivo* environment is essential to accurately model the oral drug dissolution and absorption process. An accurate model of the oral drug product dissolution and absorption process can facilitate lead drug candidate selection, establish formulation development strategies, and support development of regulatory policies (1).

To predict oral drug absorption, models have incorporated physiological parameters of the GI tract (2, 3). The Compartment Absorption and Transit (CAT) model utilized a small intestine mean residence time to define transit rate (4). The Advanced Compartment Absorption and Transit (ACAT) model utilized mass balance approximations to define each compartment's volume and transit (5). The Advanced Dissolution Absorption and Metabolism (ADAM) utilized volumes reported by water-sensitive magnetic resonance imaging (MRI) to define the volume of each compartment (6).

The Dynamic Fluid Compartment Absorption and Transport (DFCAT) model was developed to characterize the fluid volume and its dynamic changes in the human GI tract from MRI imaging of fluid volume and to evaluate its accuracy of *in vivo* fluid transport based on human GI local concentration of the non-absorbable maker phenol red from a human intubation study. The MRI study quantified the content of water in the stomach and small intestine after dosing healthy human volunteers with 240mL of water (8). The human intubation study measured local GI concentration (stomach, duodenum, proximal jejunum, middle jejunum) of phenol red in healthy human volunteers after dosing 240 mL water with phenol red. These two studies in addition to literature data served to verify and validate the DFCAT model. In future applications, the DFCAT model can be expanded to estimate the *in vivo* drug dissolution process and therefore predict oral drug absorption of oral drug products.

Materials and Methods

A mathematical model, described in detail in the following sections, was derived to capture the essential features of GI fluid transport. The model consists of 62 nonlinear ordinary differential equations (ODE). The stomach is represented by two ODEs (dissolved drug and fluid) with the small intestine represented by 60 ODEs representing a 30-compartment model (each compartment connected in series with a dissolved drug and fluid component). 30 compartments were selected to represent the localized fluid volume as well as capture most cases of small bowel fluid pocket counts observed in the MRI fluid study (7). The conceptualization of a large number of compartments also reflects the long length vs diameter ratio of the small intestine representing the local physiological situation of the small fluid volumes available for dissolution. An illustration of the proposed compartment model and avenues of transport is drawn in **Figure 1**. Matlab 2017a was used for both the simulation of model ODEs and the prediction of rate coefficient parameters. A fixed step ODE solver from the Simulink Package (ODE4) was used in 1s intervals. Calculations were run in parallel model using the parfor method. Visualized data graphics were rendered using Matlab's plot and surf packages. **Table 1** summarizes the parameters obtained and used in the model.

Stomach Compartment Fluid Compartment

The stomach compartment is represented by one ODE for transport of fluid and one ODE for transport of solubilized drug. Fluid transport in the stomach was assumed to be a component of gastric emptying and

net gastric secretion (secretion > absorption). A first order process was used to approximate the typical gastric emptying process (8, 9). Based on the observed trend, secretion on average was assumed to be constant. The result is a fluid transport ODE (**Equation 1**) that is defined by a first order gastric emptying and a zero-order gastric secretion process.

$$\frac{dV_S}{dt} = -\underbrace{k_{qS}V_S}_{\text{Emptying into Intestines}} + \underbrace{k_{sS}}_{\text{Stomach Secretion}} \quad (1)$$

V_S is the volume of fluid in the stomach compartment, k_{qS} is the first order gastric emptying rate constant, and k_{sS} is zero order gastric secretion rate (table 1).

Stomach Compartment Dissolved Drug Compartment

The MRI study quantified fluid as the available free water. However, it is well known that the stomach environment is lined with mucus which can contribute to dissolved drug transport. The model assumed dissolved drug equilibrated instantaneously between the mucosal layer and the fluid. A mucus volume (V_{bS}) was estimated to be a static entity that lines the wall of the stomach but has not yet been fully quantified by clinical measurements. Mucus volume was estimated to be 40mL based on a cylindrical abstraction of the stomach with an average capacity of 0.94L, 10cm diameter, and 1mm thick mucosal layer (10).

Transport of solubilized drug was assumed to follow the gastric emptying process of fluid based on the fraction available in free water. Drug absorption in the stomach is typically assumed to be negligible relative to the small intestine and as such, there is no drug absorption term for the stomach. Drug degradation was also assumed to be minimal. **Equation 2** defines the transport of drug in the stomach compartment.

$$\frac{dM_S}{dt} = -\underbrace{k_{qS}M_S\left(\frac{V_S}{V_S+V_{bS}}\right)}_{\text{Emptying into Intestines}} \quad (2)$$

M_S is the mass of drug in the stomach compartment.

Small Intestine Fluid Compartments

The small intestine was represented by thirty compartments each with one ODE for transport of fluid and one ODE for transport of solubilized drug. Fluid transport in the small intestine was assumed to consist of transit, absorption, and secretion. Small intestine transit behavior was modeled using first order to characterize the forward (anterograde) and reverse (retrograde) transit observed in human physiology (11). Water absorption was characterized by a deuterium-labeled water to approximate a first order absorption process with an absorption rate of 0.0715 min^{-1} (12) (table 1). Net secretion was assumed to occur primarily in the duodenal region from bile and pancreatic secretions. **Equation 3** defines the behavior for a small intestine compartment (the temporal zero order secretion term for the first compartment is not shown).

$$\underbrace{\frac{dV_n}{dt}}_{\text{Net Volume}} = +\underbrace{k_{tF}V_{n-1}}_{\text{Foward In}} - \underbrace{k_{tF}V_n}_{\text{Forward Out}} + \underbrace{k_{tR}V_{n+1}}_{\text{Reverse In}} - \underbrace{k_{tR}V_n}_{\text{Reverse Out}} - \underbrace{k_{aw}V_n}_{\text{Absorption}} \quad (3)$$

The forward and reverse transit rate constants are defined by k_{tF} and k_{tR} respectively. The first order water absorption rate constant is defined as k_{aw}(table 1).

Small Intestine Dissolved Drug Compartments

Transport of dissolved drug in the small intestine was assumed to mimic the mucosal behavior in the stomach compartment as various clinical studies have observed a range of thicknesses of the mucosal layer in the GI tract ranging from 200 to 400 µm (13-15). Based on an average small intestine length of 6.35m, a small intestine diameter assumption of 2.48cm and an average thickness of 300µm, the volume of the mucosal layer in the small intestine was estimated to be 5mL (16, 17). The resulting **Equation 4** describes the behavior of drug with M_n as the mass of the drug presently in n compartments. Absorption is assumed to be first order processes with rate coefficients of k_a.

$$\underbrace{\frac{dM_n}{dt}}_{\text{Net Drug}} = \underbrace{+ k_{tF} M_{n-1}\left(\frac{V_{n-1}}{V_{n-1}+V_b}\right)}_{\text{Forward In}} \underbrace{- k_{tF} M_n\left(\frac{V_n}{V_n+V_b}\right)}_{\text{Forward Out}} \underbrace{+ k_{tR} M_{n+1}\left(\frac{V_{n+1}}{V_{n+1}+V_b}\right)}_{\text{Reverse In}} \underbrace{- k_{tR} M_n\left(\frac{V_n}{V_n+V_b}\right)}_{\text{Reverse Out}} \underbrace{- k_a\left(\left(\frac{M_n}{V_n+V_b}\right) - \left(\frac{M_{central}}{V_d}\right)\right)}_{\text{Absorption}} \quad (4)$$

Model Verification

The DFCAT model was fitted to the small intestine mean residence time (MRT) distribution and observed GI fluid content from an MRI clinical study conducted at the University of Nottingham (7). The study consisted of twelve healthy and fasted individuals. Each individual was administered 240mL and the subsequent GI fluid volumes were measured via MRI at designated intervals over 120 minutes. The variables in the model such as duodenal secretion was adjusted to best fit the average observed fluid over time in the study. Small intestine MRT distribution was obtained from existing models that aggregated clinical data (7, 18). The inclusion of small intestine MRT in the DFCAT model mirrors a major design verification criteria of the original CAT model.

Human Intubation Clinical Study for Model Validation

The DFCAT Model was validated based on the local GI concentration acquired through ahuman clinical intubation study at the University of Michigan using the non-absorbable marker phenol red.

Ethics Statement

The study was approved by University of Michigan IRBMED HUM00085066 and the Food and Drug Administration (RIHSC protocol 14-029D. Study volunteers provided written informed consent. The study was in accordance with study protocol, the International Conference on Harmonization of Good Clinical Practice guidelines, and applicable local regulatory requirements. The ClinicalTrials.gov identifier is NCT02806869.

Materials

USP grade phenol red (phenolsulfonphthalein) was purchased from USP (Rockville, MD, USA) and Avantor Performance Materials (Center Valley, PA, USA). The 0.1 mg/ml phenol red solution was prepared in 250 ml of water. Phenol red was dispensed by the Investigational Drug Service (IDS) at the University of Michigan.

Study Inclusion and Exclusion Criteria

Healthy human volunteers between the ages of 18 and 55 were eligible for the study. Volunteers completed a physical exam and medical history screening by physician to confirm study eligibility. Volunteers all had normal values for vital signs, electrocardiogram, urine drug screen, serum pregnancy test (women only), comprehensive metabolic panel, complete blood count with platelet and differential, and lactate dehydrogenase.

Volunteers were excluded if any of the following applied: inability to consent; mentally incapacitate; prisoners; significant clinical illness within 3 weeks prior to screening; use of concomitant medications

including but not limited to prescription drugs, herbal and dietary supplements, over the counter medications and vitamins within 2 weeks prior to study; received an investigational drug within 60 days prior to study; history of allergy to ibuprofen or other non-steroidal anti-inflammatory drugs (NSAIDS); pregnant or lactating females; history of severe allergic diseases including drug allergies; history of drug addiction or alcohol abuse within 12 months; clinically significant abnormal lab values during screening; any other factor, condition, or disease including but not limited to, cardiovascular, renal, hepatic or gastrointestinal disorders that may, in the opinion of the investigator, jeopardize the safety of the patient or impact the validity of the results.

Study Procedure

Clinical procedures were conducted at either the Michigan Clinical Research Unit or the Medical Procedures Unit of the University of Michigan hospital. Volunteers were instructed to fast 14 hours prior and to avoid consuming water 11 hours prior to dosing. A physical exam was performed to ensure the health of subject prior to GI catheter intubation procedure. Volunteers received a topical anesthetic (1mL of 4% lidocaine before catheter insertion. Lubricating jelly was applied to the GI catheter which was then orally inserted into the GI tract of the volunteer. Catheter placement was confirmed under abdominal fluoroscopy to ensure proper positioning in the GI tract. Upon placement completion, the GI catheter was taped and kept open with saline solution throughout the study duration.

Volunteers were administered a single oral dose of ibuprofen (800 mg tablet) administered with 250 mL of phenol red. This was swallowed by the volunteer and not administered through catheter. GI fluid samples were collected through aspiration of available ports from the catheter. Prior to sample collection, contents from previous aspirations were collected and discarded. This discard volume ranged from 1.7mL to 3.2mL. If air bubbles were observed, at least 30 cc of air/fluid mixture was collected and discarded. GI sample collection times include 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 7 hours post dose. Supernatant was collected after sample centrifugation at 21,000 x g for 5 minutes and stored at -80^{0}C. The GI catheter was removed from the volunteer at 7 hours.

HPLC analysis of Phenol Red in GI Fluid

All samples of phenol red were analyzed with an Agilent 1200 series HPLC system HPLC system (Agilent Technologies, Santa Clara, CA). The HPLC system consisted of Agilent pumps (1100 series), an Agilent autosampler (1200 series), and an Agilent UV-Vis detector (1100 series) controlled by Chemstation® 32 software (version B.01.03). Samples were resolved in Agilent Eclipse XDB-C18 reverse-phase column (3.5 µm, 4.6 × 150 mm) equipped with a guard column for phenol red. The mobile phase consisted of 0.1% TFA/water (Solvent A) and 0.1% TFA/acetonitrile (Solvent B) with the solvent B gradient changing from 0–56% at a rate of 2%/min during a 14-minute run. Standard curves generated for phenol red were utilized for quantitation of integrated area under peaks. The detection wavelength was 430 nm.

Results

Gastric Secretion and Emptying

Average gastric emptying behavior was observed to be first order and net fluxes were attributed to either be emptying or secretion. Temporal stomach fluid content data from the MRI study was fitted using Matlab's fitting toolbox. The equation for fitting is defined in **Equation 5**. The fitted equation is illustrated in **Figure 2**.

$$V(t) = (242) * e^{-k_{qS}*t} + \frac{k_{sS}}{k_{qS}} \quad (5)$$

The initial stomach volume was based on the first observed time point (242mL). The resulting fit found the coefficients for k_{sS} and k_{qS} to be 1.425 mL/min and 0.0699min^{-1} respectively (table 1). The R square for the goodness of fit was 0.993. This is within the range of the typical daily adult gastric secretion of 2-3L or 1.39 to 2.08mL/min. (19)

Small Intestine Transit Rates

To determine the optimal transit rate coefficients, a compartment model was used to simulate the transit of drug through the small intestine based on a 199 min mean residence time (50% exit from small intestine to colon) determined by a previous study (20). Due to having more than one transit variable to solve, a range of forward and reverse rate coefficients were evaluated. A 3D visualization of residual fit as a function of forward and reverse rate coefficients is shown in **Figure 3a**. Figure 4a visualizes the natural relationship between the forward and reverse coefficients which minimizes the predicted transit vs. experimental measurement. The combination of forward and reverse rate transit coefficients with the lowest residual was chosen as the optimized values (0.92 min^{-1} forward and 0.269 min^{-1} reverse) (table 1). The optimized transit rates were then used in the model. The resulting cumulative drug exit in **Figure 3b** closely resembles the small intestine MRT of the original CAT approach.

Small Intestine Secretion Rate

Assuming net absorption throughout the small intestine, the resulting term that allows for the variation necessary to govern total small intestine volume is duodenal secretion. Duodenal secretion was assumed to change over time and the values for duodenal secretion were determined via an optimization algorithm to determine an estimate for duodenal secretion based on the total volumes observed in the simulation. Values for duodenal secretion rate range from 0 to 4.5mL/min (table 1). The final secretion value represents the duodenal secretion necessary to return the system to the basal volume observed in the beginning of the fluid MRI study. Secretion below the pylorus is roughly 4 liters daily (1 liter bile and 3 liter pancreatic) (21) This translates to roughly 2.78 mL/min which is reasonable with the values used in the model.

Average Gastrointestinal Fluid Volume Over Time

Model verification was conducted based on fluid MRI study data that ranged from 0 to 120 minutes. The observed and simulated stomach and small intestine physiological fluid volumes over time are shown in **Figure 5**. The upper and lower small intestine were categorized by the MRI study as the proximal duodenum to proximal jejunum and distal jejunum to distal ileum respectively. This was recognized in model form as small intestine compartments 1-4 and 5-30 respectively based on the approximate distance for each compartment (roughly 20 cm). The observed and simulated stomach and small intestine physiological fluid volumes over time (excluding mucosal volume) are shown in **Figure 4**.

The simulation of stomach volume generally fit within the standard error of the mean (SEM) as the volume decreased after the initial water dose. The use of first order gastric emptying and zero order gastric secretion appear to capture the average transport behavior in the stomach. In comparison, the simulated small intestine results do not follow the experimental profile as well as the stomach volumes. This could be due to the use of an absorption rate constant from another clinical study with a different population, mixing of water with the fluid layer lowering observed fluid volume, and/or large variation in regional absorption. In addition to these possibilities, the observation differences between simulated and experimental fluid volumes in the upper and lower small intestine can also be explained by the natural formation and transport process of fluid pockets in the GI tract.

Model validation with Non-absorbable Phenol Red in the GI

Since phenol red is a non-absorbable maker in the GI tract, the GI local concentration change of phenol red after oral dosing of 100 µg/ml solution is only affected by the GI fluid volume change and transit. Therefore, phenol red GI local concentration is used to validate the DFCAT model. A simulation was conducted for fluid volume and phenol red transit replicating the dosing scenario observed in the phenol red intubation clinical study. The average phenol red concentration was used. The initial dose volume was 274 mL with an average phenol red dose of 23.4mg (85 µg/ml). The simulated volume (excluding mucosal layer), mass, and concentration are shown in **Figure 5**. The blue line corresponds with the first compartment or duodenum.

The design validation results of comparing simulation with experimental results are shown in **Figure 6**. The predicted concentration generally falls within the average and standard error of the mean observed phenol red concentrations. While the behavior cannot be considered ideal in the proximal and mid jejunum as the simulation results in a higher concentration at the early time points, the study volunteers and physiology as well as stochastic variation differ between the two clinical studies. The closeness of the trend was considered well replicated by the simulation.

While the duodenum is the first compartment to peak in terms of total phenol red content, other compartments follow rapidly as it transports down the small intestine, and there is an extensive distribution of phenol red through the small intestine. Within two hours, there is significant phenol red distribution throughout the small intestine. On the other hand, the large initial quantity of fluid ingested means that initial compartments do not experience the highest concentration possible. This occurs in the later compartments where water absorption has contributed significantly to alter the fluid volume in the GI tract.

The frame of an animation of the DFCAT model (supplement 1) is shown in **Figure 7**. The transit of phenol red is rapid, reaching past the middle small intestine by 30 minutes. On an average basis, there is fluid distribution throughout the small intestine with 10mL in the duodenal compartment and just over 5mL in the last ileum compartment. After the initial dosing of water, the average basis fluid volumes do not change rapidly in the small intestine and as such, lead to an overall shape of the mass profile that is similar to the concentration profile over all the small intestine compartments.

Discussion

Recent research efforts have been focused on clarifying the numerous complexities in the oral drug absorption process. The OrBiTo project is such an initiative dedicated to establishing new frameworks and tools for predictive biopharmaceutics regarding oral drug delivery (22). One topic of interest is the local GI fluid volumes within the gut lumen resulting from fluid intake, secretion, and reabsorption. This is a critical factor in the oral absorption process as the change of fluid volume within the gut lumen can have a significant effect on the dissolution of the drug and hence the concentration presented to enzymes and transporters within the enterocyte (2). While MRI studies have provided quantification of GI fluid volumes, the dynamic change of the fluid volume as well as the intermediate process of fluid absorption, secretion, and transit that occurs GI tract remains difficult to characterize.

Understanding the dynamics of GI fluid transport is essential to improvements in predicting *in vivo* dissolution from mechanistic models. Measurement of GI fluid volume through imaging has provided valuable knowledge of GI fluid quantification. However, the data obtained remains as snapshots in time and does not detail the degree of absorption, secretion, and transit that occurs. The DFCAT model was established to mechanistically interpret the dynamic changes and intermediate knowledge using a

methodical approach based on design verification and validation using phenol red concentration in the GI tract.

Design verification was based on the GI fluid quantification via MRI. The MRI method (23) used to obtain the *in-vivo* fluid volume (7) measured only freely mobile water with long transverse relaxation time, hence fluid components with restricted mobility and shorter transverse relaxation times (e.g. water in mucous) were not accounted for. If the mixing of fluid and mucus prevents the fluid from being quantified, it would explain why the total volume of fluid measured was underestimated and that the simulated volume may be closer to reality. This could explain the significant drop when assessing the mass balance of fluid from the stomach into the small intestine. The quantity of mucus in the GI tract has not been well characterized and is not usually considered a major contributor to drug dissolution and oral drug absorption. However, the weight of evidence from this model suggests the mucus layer is present and its volume can affect local GI concentrations given the surface area of the small intestine.

In addition, in the MRI measured fluid volume, a minimum threshold of 0.5 mL per fluid pocket was applied to the quantitation in (7). This was done since the contribution of very small pockets of fluid in the small bowel comprised only 0.5% of the total volume detected in the small bowel whilst their inclusion confused the display and interpretation of data. The 0.5mL is not a lower detection limit, but rather a single MRI image pixel of adequate brightness against the validated calibration (23). Therefore, the total volume of fluid measured by MRI was likely be underestimated.

Other imaging techniques such as Positron Emission Tomography (PET) have obtained an average volume of 313mL which is significantly larger than the MRI derived volume of 105 mL (24, 25). However, this tends to overestimate the total volume as the marker can spread along the small intestine walls. This difference can be explained by the presence of a mucus layer and would suggest that an estimated volume of 150 mL is reasonable to describe the small intestine mucus layer. Design validation of DFCAT was based on the experimentally determined local GI concentrations of phenol red. Despite the variation that was observed between the simulated and experimental fluid profiles, the simulation and experimentally observed phenol red concentrations in the GI tract were similar suggesting the model is representative. It is critical to note that there is a significant variation in the GI local phenol red concentration due to the stochastic nature of the GI tract. The use of a continuous model was to simplify the approach to characterize average tendencies and trends. In this regard, the DFCAT model can explain the change in local GI fluid volumes.
A primary assumption in the DFCAT model is that transport is defined by first order kinetics. First order kinetics was used in the model to define gastric emptying, transit rate in the small intestine, and water absorption. Of these processes, only gastric emptying and water absorption have been experimentally determined to be well approximated by first order kinetics (8, 9, 12). The transit rate in the small intestine has only noted to be faster in the proximal regions and slower in the more distal regions (26). The use of first order kinetics can mathematically approximate this behavior to a certain extent but certainly does not mimic the complete peristaltic effect observed in the small intestine.

The use of verification and validation in establishing the DFCAT model also presents limitations. The model presently is only designed to simulate the fluid volume after intake of water in the fasted state. There are numerous physiochemical aspects in the GI tract such as conductivity, pH, and osmolality that can impact oral drug delivery (26). Changing the drink or simulating a fed state may result in a significantly different profile with different GI secretions. Each change in study conditions would require a new MRI fluid study to quantify the fluid model as well as a new intubation study to obtain the local GI concentrations under fed condition.

The DFCAT as presented is a methodology to model GI fluid transport based on experimentally obtained fluid volume, which is also validated by local phenol red concentration in the GI tract. While the verification and validation do not include drug absorption, the framework can be expanded to predict drug dissolution and oral drug absorption as referenced in the compartmental equations. Since local GI fluid content is critical to drug dissolution and oral absorption, the approach used to construct the DFCAT model can be integrated with existing physiologically based pharmacokinetic (PBPK) models. Along with the fundamental knowledge of the GI tract developed by the OrBiTo project, the integration of local GI fluid and other physiological considerations can integrate in vitro and in silico approaches to improve the oral drug development process.

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