Lipid-associated Oral Delivery Systems: A Sensitivity Analysis

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ABSTRACT

Lipids, typically present in food or in lipid-based formulations, are known to impact the absorption of oral drugs in the gastrointestinal (GI) tract, and ultimately drug bio-availability. This is in part due to changes in the physicochemical nature of the intestinal milieu upon lipid ingestion. The mechanism by which lipids impact the intestinal milieu and its effect on drug behavior in the intestine is poorly understood. Hence, available computation models are based primarily on empirical approaches for predicting lipid impact on drug absorption rather than mechanistic ones. Our lab has developed a simple, yet fully mechanistic, computational model that predicts the impact of lipids on drug absorption using carbamazepine as a model drug compound. Specifically, we have studied *in vitro* the mechanism of carbamazepine dissolution, partitioning, volume/area evolution of phases during intestinal lipolysis, and transport across the intestinal epithelium upon co-dosing with a triglyceride at fed concentrations. Equations using transport first principles were developed to describe such processes. Incorporating known drug pharmacokinetic parameters, we could predict a pharmacokinetic (PK) profile. While a validation of this model is still elusive, in this report we conduct a model sensitivity analysis to assess parameters and processes of most relevance to studying and improving upon, for enhanced lipid effect predictions. A sensitivity analysis informs decisions on developing better *in vitro* methods that are more representative of *in vivo* conditions. In addition, it guides decisions on more thorough investigations of the mechanisms behind the most important processes impacting drug absorption. Using the Sobol sensitivity method and Python 3.0 as programming language we concluded that the mass of drug dosed and the drug intestinal permeability are the parameters of greatest impact on the oral PK profile, suggesting that a detailed assessment of the *in vitro/ex vivo/in situ* tools to determine intestinal permeability is largely important. Second-order sensitivity analysis showed large correlations between the drug dose and weight of patient, as well as dose and intestinal permeability, relative to correlations between other parameters.

Introduction

The majority of oral drugs in the market are poorly water soluble, and co-administration with lipids has proven effective in increasing overall absorption, often resulting in several-fold increase in bio-availability. Sometimes this impact of lipids is exploited by developing formulations employing lipids as excipients and sometimes by prescribing drugs in combination with lipid-rich diets. The former approach is not widely used due to a variety of factors, including manufacturing costs, the necessity of specialized equipment, instability and precipitation induced by formulation stabilizing excipients upon administration, etc. The latter is a more desirable approach due to absence of the aforementioned complications. Yet, there is an incomplete mechanistic understanding of and ability to predict food function in the GI tract, particularly due to the composition and structural variability of various food products.

The Advanced Drug Delivery Research Lab at Northeastern University has studied the mechanisms by which lipids in food can impact overall drug absorption, and have developed predictive equations based on such mechanisms. As with many computational models, assumptions and simplifications into ideal conditions are often necessary, thus causing deviations from reality. Sensitivity analysis is a useful tool that provides qualitative information on parameters the model is highly susceptible tool. This knowledge is critical as it guides decisions on more thorough studies on mechanisms of drug-lipid interaction behaviors that matter, i.e. that are of most relevance to overall absorption. Ingestion of lipids triggers a cascade of processes that affect the physical and chemical nature of the gastrointestinal environment, which, in turn, directly affect the behavior of oral drugs in the GI tract. Lipids are digested as soon as they are in contact with gastric and intestinal juices, due to the presence of lipases that break down triglycerides (TG), the most abundant type of lipid in food, into diglycerides (DG), monoglycerides (MG), and fatty acids (FA) as shown below.

$$TG --- > DG --- > MG --- > FA \tag{1}$$

The presence of food-associated lipids in the intestine causes increased secretions of pancreatic juices containing large quantities of enzymes (consisting primarily of lipases and proteases) and bile (consisting of bile salts, phospholipids, and cholesterol). Due to their amphiphilic nature, bile components self-assemble into micelles containing a hydrophobic core and a hydrophilic shell. Bile micelles serve as a niche for partitioning of oral drug compounds, particularly of poorly water soluble drugs. In addition, the final product of TG digestion, i.e. the fatty acid, is also an amphiphilic molecule which partitions into the micelles causing them to swell. An increase in micelle volume is associated with increased drug partitioning into the micellar phase. The altered micellar phase can impact drug dissolution kinetics from its solid formulation into the micelle-rich aqueous lumen. The increased drug concentration in the aqueous lumen serves as a driving force for drug absorption, often resulting in enhanced bioavailability. In Figure 1 we show a mechanistic representation of these concurrent processes that may affect overall drug absorption. Mechanism-based equations have been developed describing each of these processes[1], the simultaneous solution of which provides a drug blood plasma vs. time concentration profile, i.e. a PK profile.

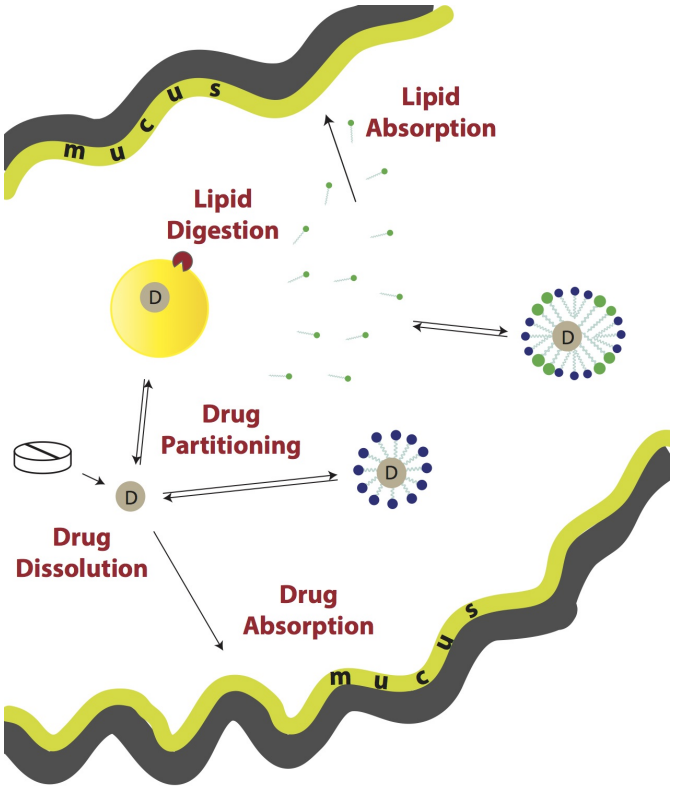

Figure 1. Mechanism of lipid effect on drug adsorption in the intestine[2]

Sensitivity analysis may offer information into which of these processes is most or least sensitive to the area under the curve (AUC) of the PK profile, a critical parameter used for comparisons between PK profiles. For example, if the dissolution process has a high impact on drug absorption, it may be important to study the dissolution process from the moment the drug reaches the stomach to when it leaves the colon, considering that the luminal composition is different in different parts of the GI tract. On the other hand, if the dissolution process is insignificant, then studying the intricacies of each physiological compartment in the GI tract might be unnecessary as it would not enhance the accuracy of the model.

In this report, we employ a previously developed mechanistic model which computes the AUC of the drug plasma concentration vs. time profile following the simultaneous solution of equations describing drug dissolution in the intestine, drug partitioning into the bile micelles, drug partitioning into the lipid droplets, FA production during digestion (allowing computation of volume evolution of the micellar and triglyceride phases), drug absorption, and drug elimination by organs.

Methods

Carbamazepine was used as a model drug and triolein, a long-chain triglyceride, was used as a model food-based lipid. *In vitro* experiments have been conducted to determine input parameters for the model. While it is important to describe the *in vitro* methods and the analytical tools used to determine the parameters used in our model, it is beyond the scope of this report. Below we show the list of equations employed and the physical meaning of each of the parameters.

Systems-Based Modeling Approach

The dissolution of solid drug in the micelle-rich aqueous intestinal environment is governed by the concentration gradient between drug solubility in buffer and micelles to bulk concentration in buffer and micelles, respectively, as shown in Equation 2.

$$dC_{aq}/dt = S/(V*h)*[D_S(C^{eq}_{buffer} - C_{buffer}) + D_m(C^{eq}_{micelles} - C_{micelles})] \tag{2}$$

where C_{aq} is the drug concentration in the micelle-rich aqueous environment, S is the total surface area of the solid drug particles, V is the volume of the intestinal lumen, h is the thickness of the boundary layer at the solid-water interface, D_S and D_m are the diffusivities of the free drug and micelle-bound drug, C_{buffer} and $C_{micelles}$ are the drug concentration in the buffer and micelle phases, respectively, and C^{eq}_{buffer} and $C^{eq}_{micelles}$ are the drug solubilities in the buffer and micellar phases, respectively.

The lipolysis of TG (shown in the form of oil droplets in Figure 1 is given by the following Equation 3.

$$dC_{FA}/dt = K_{dig}A_{oil}/V - K_{inh}*C_{FA} \tag{3}$$

where C_{FA} is the concentration of fatty acid produced due to lipolysis, K_dig and K_inh are the digestion and inhibition rate constants, and A_oil is the total surface area of the oil droplets.
Partitioning into micelles is assumed to be instantaneous and constant Equation 4.

$$K_{m/w} = C_{micelles}/C_{buffer} \tag{4}$$

where $K_{m/w}$ is the micelle-water partitioning coefficient. Partitioning into oil is a slower interfacial process and a function of the droplet surface area.

$$dC_{oil}/dt = -A_{oil}*P_{rel}/V_{oil}*(C^{eq}_{oil} - C_{oil}) \tag{5}$$

where C_{oil} is the drug concentration in the oil (triglyceride) droplets, A_{oil} is the interfacial surface area of the oil-water interface, V_{oil} is the volume of the triglyceride phase, C_{oil} is the drug concentration in the oil phase, and C^{eq}_{oil} is the drug solubility in the oil phase.

Drug absorption for Carbamazepine is known to be passive purely by diffusion as shown in Equation 6 below.

$$dC_{blood}/dt = -A_{intestine}*P_{GI}/V*C_{aq} - k_{el}*C_{blood} \tag{6}$$

where C_{blood} is the drug concentration in plasma, $A_{intestine}$ is the absorbable surface area of the small intestine, P_{GI} is the drug permeability at the intestinal mucosa, k_{el} and is the elimination (by the organs) rate constant.

Sensitivity Analysis

To perform our sensitivity analysis we changed food parameters, species intestinal parameters, and drug parameters. The inputs we chose to use in our sensitivity analysis are shown in Table 1. We chose these parameters to vary for various reasons. The weight of person and starting mass of drug are variables that are certain to change depending on the patient. Intestinal permeability and solubility in ingested oil were originally gotten from literature, but added to the sensitivity analysis to determine the effect they have on drug adsorption. The other variables were added to the sensitivity analysis because they are unknown and expected to vary from patient to patient. Ranges for our sensitivity analysis parameters were determined using our best guesses.

Parameter	Min	Max	Unit	Description
M0	1	500	mg	Starting mass of solid drug
Satw	200	400	μg/mL	Drug solubility in buffer at pH=6.5
SatDiff	1	500	μg/mL	Drug solubility before lypolysis - Satw
r0	1e-3	1e-2	cm	Disintegrated solid drug particle radius
Prel	1e-10	1e-2	cm/min	Relative permeability of drug at oil-water interface
Pgi	1e-5	1e-1	cm/min	Intestinal permeability
C0_oil	1	100	mM	Ingested oil concentration per unit solution volume
Satoil	100	10000	μg/mL	Drug solubility in the type of oil ingested
r0_oil	1e-5	1e-3	cm	Starting radius of oil droplets
Kdig	1e-9	1e-5	mmol/min*cm^2	Lipolysis rate constant
Kinh	1e-2	1	1/min	Inhibition rate constant
Mspecies	50	200	kg	Weight of person

Table 1. Variation of Parameters.

The output of the tested function is the area under the curve of a pharmacokinetic profile, describing Carbamazepine concentration in the bloodstream vs. time. This gives the total amount of drug absorbed. When calculating the area under the curve, the total profile was calculated over a 48 hour time period. Sensitivity analysis determined the input food and intestinal parameters that affect the overall drug substance absorbed.

Using the twelve parameters listed above, a Sobol analysis was conducted to determine the sensitivity of each parameter to the model created. Samples were executed until a sufficiently large number of trials allowed the model to converge. The final Sobol analysis was executed with 20,000 trials that generated 520,000 samples, as calculated by:

$$S = N * (2D + 2) \tag{7}$$

where N is the number of trials chosen and D is the number of model inputs. Computing above this sample size proved not only to be lengthy, but statistically insignificant. The first-order, second-order, and total sensitivities for each parameter were calculated and output to a named set of arrays from the Sobol Python package.

Sobol analysis is a form of variance-based sensitivity analysis that calculates indices using a Quasi Monte Carlo method.[3] Monte Carlo methods for sensitivity analysis assumes that the probability distribution for each parameter is random. Sobol analysis computes the probability distribution using the Sobol Sequence. The Sobol Sequence is a quasi-random low-discrepancy sequence, an image of how this distribution varies from a pseudo-random distribution is shown in Figure 2.

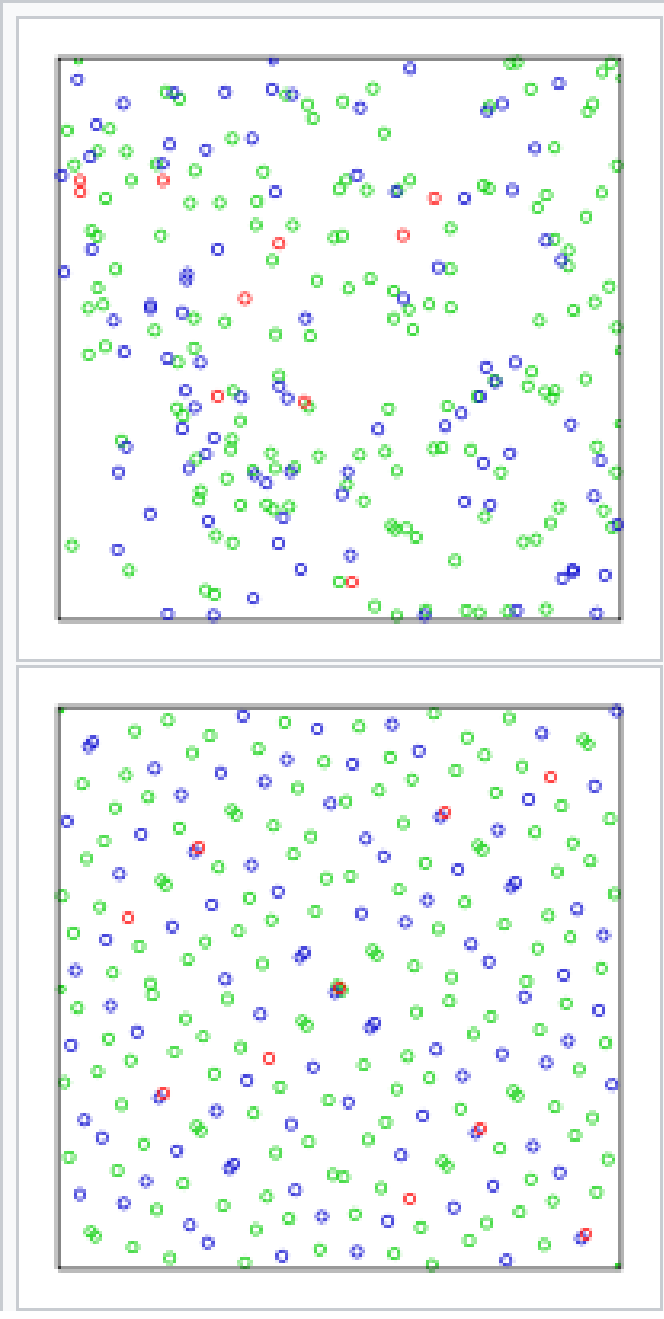

Figure 2. Upper - Pseudo-random sequence, Lower - Sobol sequence[2]

Results

From the Sobol output arrays, the sensitivities of each of the twelve input parameters and their effect on the AUC of the pharmacokinetic profile was analyzed. Three sensitivity indeces are available for each parameter (or set of parameters) from the Sobol analysis: first-order, second-order, and total-effect index. First-order effects (or main effects), give the fractional contribution of variations in **one** variable to the output, as given by:

$$S_i = V_i / Var(Y) \tag{8}$$

where S_i is the sensitivity value (from 0 to 1), V_i is the variance of the output based on the individual parameter, and $Var(Y)$ is the variance of the overall model. This mode of standardization allows for a direct comparison of parameters. The higher the S_i, the more sensitive the parameter is.

Building upon this, the second-order effects give the fractional contribution of variations in **two** variables simultaneously to the output. These contributions continue to an infinite order, the sum of which gives the parameter's total sensitivity index, as given by Equation 9.

$$S_T = \sum_{i=1}^{n} S_i + \sum_{i<j}^{n} S_{ij} + ... + \sum_{i}^{n} S_{i...n} \tag{9}$$

In simple models, a first-order index may be enough to determine a parameter's variability. However, in a model such as this, with 12 varying inputs and likely multiple correlations, high-order sensitivity indices are used to calculate a parameter's total effect on the model's output.

First-order Analysis

Table 2 and Table 3 below both show the first-order and the total sensitivity of each parameter varied. The two tables originate from the same model data, with the only difference being the number of trials executed. It is important to ensure that sufficient trials are run for the model to converge; in other words, enough samples should be taken such that the output data does not significantly change if the sample size is increased. This gives the impression that the output has reached a pseudo-steady state and the model outputs are an accurate representation of the entire population, rather than just a function of the sample size.

The two tables display the trade-off between accurate results and computing capabilities. With a standard processor, Table 2 was able to complete 3,000 trials, or 78,000 samples, in approximately 45 minutes. Similar trials were previously run with smaller sample sizes, leading to believe that no significant changes were occurring once the simulation ran 3,000 times. However, to ensure the highest level of accuracy, a 20,000 trial (520,000 sample) analysis was conducted using Northeastern University's Discovery Cluster. This simulation completed in just over 4 hours on a high-speed compute node. The minor differences between the two tables indicate that the analysis is approaching steady state; the group determined that the 20,000 trial size was sufficient for further analysis.

Parameter	M0	Satw	SatDiff	r0	Prel	Pgi	C0_oil	Satoil	r0_oil	Kdig	Kinh	Mspecies
First-order	0.186	0.038	0.093	0.021	0.005	0.248	-0.008	-0.022	-0.026	-0.030	-0.011	0.057
Total	0.336	0.069	0.158	0.086	0.062	0.465	0.093	0.082	0.112	0.085	0.113	0.262

Table 2. First-order and total sensitivities - 3,000 Trials.

Parameter	M0	Satw	SatDiff	r0	Prel	Pgi	C0_oil	Satoil	r0_oil	Kdig	Kinh	Mspecies
First-order	0.184	0.043	0.080	0.033	0.011	0.251	-0.009	-0.017	-0.017	-0.027	-0.019	0.073
Total	0.355	0.088	0.172	0.102	0.084	0.491	0.112	0.099	0.126	0.099	0.126	0.283

Table 3. First-order and total sensitivities - 20,000 Trials.

As shown in Table 3, many of the first-order parameter sensitivities are virtually zero; changes in such parameters show very little individual effect on Carbamazepine AUC. All parameters a with 0 ± 0.1 value seemingly have no relevant effect on the pharmacokinetic model. Inversely, both M0 (solid drug mass) and Pgi (Permeability in the gastrointestinal tract) showed the highest first-order and total sensitivity values. This demonstrates that M0 and Pgi have a significant effect on the output of the dissolution model. In theory, this is a sensible argument; dosing of pharmaceutical products is primarily based on the amount of the active drug. Further, the ability of the drug to permeate the intestinal tract plays a large role in determining how much drug actually enters the systemic circulation.

Second-order Analysis

Looking beyond the first-order sensitivity, The difference between the total sensitivity of a parameter and its first-order sensitivity is an indicator that higher order sensitivities may be present in the parameter in question. Figure 3

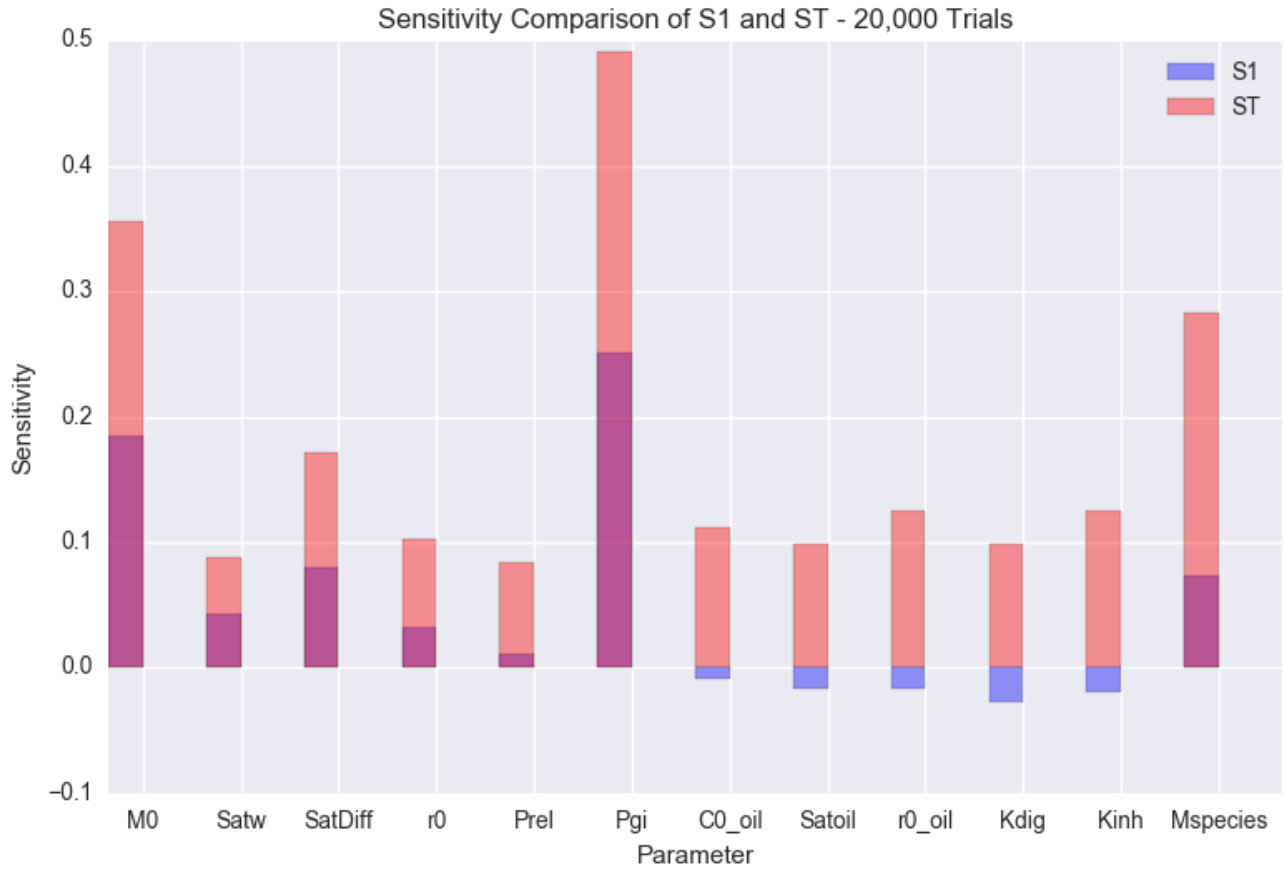

Figure 3. Sensitivity Analysis

Once again, M0 and Pgi are the most susceptible to higher-order interactions. Additionally, Mspecies (human weight) exhibits a much larger total sensitivity in relation to its first order value. Looking in depth at the SALib package, the Sobol analysis has an extended option, which enables the Python script to calculate second-order sensitivities, or interconnections between two variables as they correlate to the overall model. This higher order index gives insight as to the relation (or lack thereof) of certain parameters to each other. The results from this calculation are tabulated in Table 4.

	M0	Satw	SatDiff	r0	Prel	Pgi	C0_oil	Satoil	r0_oil	Kdig	Kinh	Mspecies
M0	-	-0.013	0.010	-0.001	.014	0.082	0.041	0.052	0.066	0.065	0.072	0.097
Satw	-	-	0.035	0.032	0.034	0.057	0.035	0.034	0.037	0.035	0.036	0.042
SatDiff	-	-	-	0.027	0.028	0.060	0.027	0.026	0.032	0.028	0.028	0.040
r0	-	-	-	-	0.031	0.055	0.033	0.033	0.035	0.033	0.034	0.040
Prel	-	-	-	-	-	0.041	0.030	0.030	0.032	0.030	0.031	0.034
Pgi	-	-	-	-	-	-	0.046	0.048	0.052	0.044	0.046	0.076
C0_oil	-	-	-	-	-	-	-	0.035	0.038	0.035	0.037	0.035
Satoil	-	-	-	-	-	-	-	-	0.039	0.039	0.039	0.041
r0_oil	-	-	-	-	-	-	-	-	-	0.038	0.041	0.039
Kdig	-	-	-	-	-	-	-	-	-	-	0.038	0.036
Kinh	-	-	-	-	-	-	-	-	-	-	-	0.029
Mspecies	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Second-order sensitivities - 20,000 Trials.

Referring back to Table 2, few parameters show any statistical significance in the realm of second-order sensitivity. Similar to the first-order scenario, both M0 and Pgi experience elevated levels of S2 sensitivity when compared with other variables. When paired with each other M0 and Pgi experience an S2 sensitivity of 0.082. Other relatively large values of S2 include 0.097 (M0-Mspecies), and .072 (M0-Kinh). Once again, relationships involving M0 and Pgi take into account the largest influence of the PK model; varying the mass of dosed Carbamazepine seems to have a corollary effect on many of the other parameters in the current model. However, at this stage, there is not enough statistical evidence to conclude that any of the chosen parameters have significant second-order sensitivity.

Discussion

A majority of the parameters in the simulated model produced negligible first-order and total sensitivities; the Sobol analysis concluded that varying these parameters had little-to-no-effect on the Carbamazepine AUC. Three parameters, however, emerged as significant inputs and dictated a strong presence in the model. M0 and Pgi had first-order sensitivities that were an order of magnitude higher than any of the ten other tested parameters. Furthermore, their total sensitivities exceeded 0.3, indicating that the two were not only significant when varied alone, but also may have higher-order correlations with other variables. Mspecies also became distinct at this stage, producing a low first-order sensitivity, but boasting the highest proportional total-effect of any of the twelve parameters. Further analysis is required to determine parameters that are distinctly relevant, but we can conclude that solid drug mass, human weight, and intestinal permeability may play a significant role in the absorption and dissolution of Carbamazepine.

The results presented in this report will serve as the foundation for the advancement of future work. Any adjustments of differential equations from the present model and potential future experimentation will utilize the sensitivities of the tested parameters to determine which variables should be more closely monitored. Even more influential is the ability to disregard varying parameters if the sensitivity analysis shows they have little effect on the output of the model. This saves both valuable time and and resources in physical studies and requires exponentially less sampling. Additionally, if there becomes a requirement to analyze supplemental parameters, including controversial literature values or new specifications added to the model, the framework for the baseline code is designed and suitable for adjustment. Rather than forming a complex model from a blank slate, the current Python notebook can be simply fine-tuned to support additional parameter sets.

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Author contributions statement

O.R. conceived the differential equations for the pharmacokinetic model throughout years of extensive research, J.L. and J.L. were primarily responsible for creating a functional sensitivity analysis based on Ms. Rezhdo's hard work. All authors reviewed the manuscript.