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Sensitivity Analysis of a Pharmacokinetic Model of Vaginal Anti-HIV Microbicide Drug Delivery

Angela M. Jarrett [1,*], Yajing Gao [2], M. Yousuff Hussaini [1], Nicholas G. Cogan [1], David F. Katz [2]

[1] Mathematics, Florida State University, 208 Love Building, 1017 Academic Way, Tallahassee, Florida 32306
[2] Department of Biomedical Engineering, Duke University, 101 Science Drive, Campus Box 90281, Durham, North Carolina 27708

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ABSTRACT

Uncertainties in parameter values in microbicide pharmacokinetics (PK) models confound the models' use in understanding the determinants of drug delivery and in designing and interpreting dosing and sampling in PK studies. A global sensitivity analysis (Sobol' indices) was performed for a compartmental model of the pharmacokinetics of gel delivery of tenofovir to the vaginal mucosa. The model's parameter space was explored to quantify model output sensitivities to parameters characterizing properties for the gel–drug product (volume, drug transport, initial loading) and host environment (thicknesses of the mucosal epithelium and stroma and the role of ambient vaginal fluid in diluting gel). Greatest sensitivities overall were to the initial drug concentration in gel, gel–epithelium partition coefficient for drug, and rate constant for gel dilution by vaginal fluid. Sensitivities for 3 PK measures of drug concentration values were somewhat different than those for the kinetic PK measure. Sensitivities in the stromal compartment (where tenofovir acts against host cells) and a simulated biopsy also depended on thicknesses of epithelium and stroma. This methodology and results here contribute an approach to help interpret uncertainties in measures of vaginal microbicide gel properties and their host environment. In turn, this will inform rational gel design and optimization.

Introduction

Microbicide drugs, that act locally to prevent infection by HIV, are being developed as an alternative to vaccines in the fight to combat HIV/AIDS.[1,2] Recent evidence indicates that diligent, continuous oral administration of microbicide drugs can reduce the likelihood of sexual HIV transmission.[3,4] Alternative microbicide delivery techniques, for example, gels and intravaginal rings, have been in development for some time.[5-8] These diversify the need to administer the drugs, either in on-demand products (e.g., gels, films, dissolving tablets, or suppositories) or in ones that require infrequent administration (e.g., intravaginal rings, subdermal implants, and injections). Gels were the original vaginal dosage form developed for microbicides and have been evaluated for multiple drugs in multiple clinical trials,[9-11] but no gel–drug dosage regimen combination has been proved efficacious in multiple trials. A leading microbicide drug, tenofovir, has been evaluated in 3 phase 3 clinical trials. The first trial demonstrated a significant reduction in sexual transmission of HIV, but the second and third trials did not.[9,12] However, results of the second and third trials were confounded by the very poor adherence of users to the specified gel application regimens.[10] Furthermore, recent post hoc analyses of data for the second trial (Vaginal and Oral Interventions to Control the Epidemic; VOICE) coupled to analysis of the first successful trial (CAPRISA 004), show that if women did apply gel, as instructed, there was a significant reduction in the rate of HIV transmission and that greater adherence to designated administration reduced the rate of transmission.[13] Thus, there remains significant motivation to include gels in the set of dosage forms and products being developed for vaginal microbicide use worldwide. The microbicide field is addressing lessons learned from the gel studies to date, including gaps in the methodologies that are applied in product design and performance evaluation. The hope is to design future products, and their dosage regimens, that foster both pharmacologic success in preventing HIV transmission and behavioral success in willingness to use. The present study is intended to help fill some of those gaps.

Microbicide products function by delivering drugs that prevent HIV virions from infecting host cells through several different mechanisms. Some drugs, termed entry inhibitors, target the processes that enable viral binding and entry into host cells. Vehicles

* *Correspondence to:* Angela M. Jarrett (Telephone: 850-644-2202; Fax: 850-644-4053).
E-mail address: ajarrett@math.fsu.edu (A.M. Jarrett).

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for these drugs (e.g., gels) are intended to deliver some (e.g., cyanovirin, Griffithsin, which target glycoproteins on the viral envelope) to the fluids of the vaginal canal and others (e.g., maraviroc, a CCR5 receptor antagonist) to the host cells in the mucosa, as well. Other antiretroviral drugs act exclusively within the mucosa, on the virion–host cell interaction, by multiple mechanisms including reverse transcriptase inhibition (e.g., tenofovir, dapivirine) and post-transcription integrase inhibition (e.g., raltegravir, cabotegravir). A number of these drugs are already used in oral therapy for previously infected individuals. Delivery of such drugs in sufficient concentrations to target sites at times of exposure to infectious HIV is paramount in the functioning of microbicide products. Such delivery, that is, microbicide pharmacokinetics (PK), has been studied primarily by experiments—for example, studies in which humans or animals are dosed with candidate products, after which tissues and fluids from the lower reproductive tract and blood are collected and evaluated for drug concentrations.[14-17]

There has been a small but increasing amount of theory-based computational work related to microbicides—including deterministic models of vaginal deployment of microbicide gels and films and of drug transport per se as released by gels, films, and intravaginal rings.[18-25] Predictions from recent models of the delivery of the drug tenofovir via a gel to the epithelial and stromal layers of the vaginal mucosa were in good agreement with experimental data in women.[23] The models have parameters characterizing properties of the products: drugs and their delivery vehicles (e.g., diffusion and partition coefficients) and the host environment (e.g., dimensions of the vaginal canal, thicknesses of the epithelial and stromal layers of the vaginal mucosa).

Understanding how the outcomes or predictions of the models depend on variations in values of parameters is of great value when developing, analyzing, and using mathematical modeling in conjunction with experimental studies. For example, this can be used in product design, namely in choosing product properties that optimize drug delivery performance over variations in properties of the host environment. One of the most useful methods for quantifying and describing how an outcome depends on inputs (or parameters) is referred to as sensitivity analysis (SA). This can help us (1) understand how natural biologic variations in product users (i.e., properties of the host environment) affect drug distribution and, thus, help interpret and control for "population variability" in data from experimental PK studies and (2) delineate the roles that different product-based parameters play in drug delivery, informing the design of products in which those parameters are manipulated to optimize such delivery. The goal of the present work was to implement a formal parameter SA to address such questions. We worked with a recent model of tenofovir delivery by a vaginal gel,[23] focusing on parameters that characterize both the gel product and its host environment.

Sensitivity Analysis

SA often has been used to check for robustness of a model, but classifying parameters as sensitive and nonsensitive also helps with 3 additional challenges to model application:

- identifying parameters that most require estimation,
- identifying experimental targets for model application, and
- reducing uncertainty in model results.

Sensitive parameters must be estimated with care due to the fact that small changes in their values lead to large changes in the measured output; a model can only make robust predictions if there is some level of certainty for sensitive parameters' values. Additionally, sensitive parameters can be identified as targets for possible experimental interest because they require the least alterations to have significant impact on the physical system. Furthermore, there may be highly uncertain parameters to which the model is not sensitive and, therefore, have little effect on the predictions—reducing uncertainty in the results. These may reflect biologic processes that need not to be fully explored to understand the behavior of the system to a reasonable degree. There is no clear way to determine which of these 3 aspects of SA are the most important—primarily because "importance" depends on the target audience. Our view is typically that the most useful aspects for biomedical applications are the identification of both sensitive and nonsensitive parameters for the purpose of exposing useful targets and potential dead-ends, respectively.

Another application of SA results is in model reduction. In contrast to classical model reduction, where we use asymptotic arguments to neglect certain terms and reduce the number of state variables, here we refer to the size of the parameter space that must be explored. Model results can depend heavily on particular parameters, but other parameters may be essentially irrelevant to the overall results. Identifying and "freezing" these parameters can reveal simpler models for the same complex biologic system. This reduces the computational demands when dealing with stochastic processes. Also, this reduces the number of "fudge factors" that may need to be introduced.[26,27]

One of the major difficulties that arise when trying to understand the effects of variations in parameters is ranking them in terms of their effects on model predictions. There are many approaches used to quantify such parameter effects—such as differential SA, statistical measures, and different sampling methods.[27,28] Broadly, SA methods can be separated into local SA and global SA methods. Local SA only investigates single parameters at a time, where the impact on the output due to changes in a particular input variable is calculated, whereas the other inputs are kept constant at their given values. Global SA considers variations of all input variables simultaneously, covering the entire parameter space. As a result, global SA methods are less likely fail to identify a significant parameter (i.e., type II errors).[29]

Sobol'[30] sensitivity measures are among the most widely used global SA methods. Their use employs the ANOVA decomposition of the model outputs, and a normalized measure of variance is defined, called the global sensitivity index. There are many methods for quantifying sensitivity, including partial rank correlation coefficient and extended Fourier amplitude sensitivity test,[31] a statistical measure and a variance-based measure, respectively. However, the Sobol' method has several strengths compared with other methods. It is quite flexible and is capable of handling essentially any relationships between inputs and outputs. It is well established in the literature, which means that there are a host of options for algorithms that optimize both efficiency and accuracy of these sensitivity calculations. Finally, the method can provide 3-fold information about the model parameters.

As a variance-based measure, the Sobol' method exploits the recursive construction and orthogonality of the functions given by the ANOVA decomposition in calculating sensitivity indices. These can be obtained for all parameters investigated for every model output.[29,30,32] The importance of the each input can be split into 2 types of effects: main effects and total effects. Total effects are the importance of the input parameter with respect to individual outputs along with any secondary effects from other parameters. Main effects only consist of the effects of the input parameter alone (most other sensitivity methods only give main effects measures). The additional effects, coming from the difference of the total and main effects, can identify parameter interactions that often imply subtle interactions between components of the model that might not be deduced from intuition or heuristics. However, such interactions are premature in the present work, and we have

chosen to focus on total effects as more robust indicators of the pharmacologic significances of the parameters in the model.

The Model

Figure 1 illustrates the model, the predictions of which were shown to be in good agreement with experimental data from 2 human PK trials for the tenofovir gel.[23] The model consists of 3, diffusion-driven, conservation of mass partial differential equations representing the transport of tenofovir diphosphate (TFV) delivered by a gel applied to the vaginal canal,[23] and a mass balance-based ordinary differential equation characterizing volume-averaged drug concentration in the blood. The dependent variables are the concentrations of drug in the gel (C_G), epithelium (C_E), stroma (C_S), and blood (C_B) compartments. The equations are

$$\frac{\partial C_G}{\partial t} = D_G \frac{\partial^2 C_G}{\partial x^2} - k_D C_G \tag{1}$$

$$\frac{\partial C_E}{\partial t} = D_E \frac{\partial^2 C_E}{\partial x^2} \tag{2}$$

$$\frac{\partial C_S}{\partial t} = D_S \frac{\partial^2 C_S}{\partial x^2} - k_B C_S \tag{3}$$

$$V_B \frac{\partial C_B}{\partial t} = \int_0^{h_S} \int_0^{L} k_B C_S \, dx dy - k_L C_B \tag{4}$$

with initial conditions $C_G(x,0) = C_0$, $C_E(x,0) = C_S(x,0) = C_B(x,0) = 0$. The first equation represents drug mass conservation within the gel. There is a loss of the drug due to dilution by vaginal fluids and leakage, and this is modeled as a first-order effect with rate constant k_D. Similarly, in the stroma equation, there is a loss of the drug to the vasculature (there is none in the epithelium) with constant rate k_B. The blood compartment has drug loss due to blood clearance, k_L. The double integral in the equation for the blood, thus, gives the volumetric rate of TFV transport into the blood from the stroma compartment.

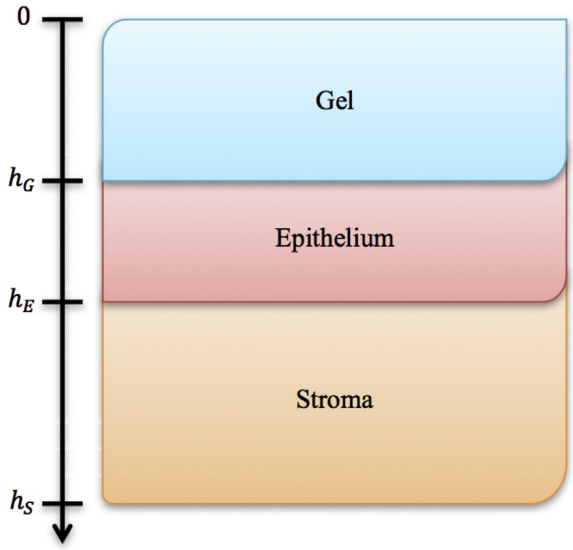
There are 6 boundary conditions applied at the external boundaries and the interfaces between layers. The parameters h_G, h_E, and h_S represent thickness of the gel, epithelium, and stroma, respectively, and Φ_G and Φ_E are the partition coefficients at the gel/epithelium and epithelium/stroma interfaces. See Table 1 for a description of each parameter and its mean value.

To render the model's results suitable for referencing and predicting experimental pharmacokinetic data, the depth-averaged concentrations for each compartment are calculated and the concentrations in the epithelium and stroma compartment are also averaged for an additional output that simulates a biopsy.[23] The curves for these volume-averaged concentrations have characteristic growth and decay behavior which is commonly summarized in pharmacology using measures such as C_{max}, t_{max}, area under the curve (AUC), and C_{24} (see Results). We computed these common PK measures from the model outputs as the focus in our SA. Figure 2 illustrates the curves of volume-averaged concentration versus time using mean values given in Table 1.

Methods

Using the mean values in Table 1, we assume all parameters have uniform distributions with a 50% coefficient of variation. This range is reasonable, we believe, for the majority of the parameters, recognizing that for some it could be smaller or larger. For example, the partition coefficients, Φ_G and Φ_E, are not expected to vary significantly due to physicochemical limitations in both gel design and the tissue at the epithelium/stroma interface. Variabilities for diffusion coefficients and drug loss rate constants are expected to be much greater, reaching differences on the log scale. We standardized the variation within parameters here as a starting point for comparison, and we did not address quantitative values of the variability in all parameters individually (which is a logical follow-up). Sensitivity is presented here in terms of Sobol' indices, which are normalized relative measures of contributions of each parameter to a single-model output measure.

The TFV model equations were solved using the method of lines with MATLAB's ordinary differential equation suite used for the time integration. Then the outputs for sensitivity measures were determined using the average concentrations of each model



$$\frac{\partial C_G(0,t)}{\partial x} = 0$$

$$C_G(h_G,t) = \Phi_G C_E$$

$$\frac{\partial C_G(h_G,t)}{\partial x} = D_E \frac{\partial C_E(h_G,t)}{\partial x}$$

$$C_E(h_G + h_E,t) = \Phi_E C_S$$

$$\frac{\partial C_E(h_G + h_E,t)}{\partial x} = D_S \frac{\partial C_S(h_G + h_E,t)}{\partial x}$$

$$\frac{\partial C_S(h_g + h_E + h_s,t)}{\partial x} = 0$$

Figure 1. Diagram of layers.

Table 1
Parameter Descriptions and Their Mean Values

Symbol	Description	Mean Values
D_G	Diffusion coefficient in gel	6×10^{-6} cm^2/s
D_E	Diffusion coefficient in epithelium	7×10^{-8} cm^2/s
D_S	Diffusion coefficient in stroma	4×10^{-7} cm^2/s
Φ_G	Partition coefficient: gel/epithelium	4/3
Φ_E	Partition coefficient: epithelium/stroma	1
k_D	Death term due to dilution	1.2202 per h
k_B	Loss constant to blood	0.1190 per h
k_L	Time constant of clearance in blood	1.4087 per h
V_G	Gel volume	3.57 mL
C_0	Initial drug concentration	10^7 ng/mL
W	Width of vaginal canal	3.35 cm
L	Length of vaginal canal	13 cm
h_E	Thickness of epithelium	0.02 cm
h_S	Thickness of stroma	0.28 cm
V_B	Blood volume in distribution	75 L

component—calculated using the trapezoidal rule over all space for each individual time point.

- The C_{24} outputs are the volume-average concentration within each compartment at the final time, 24 h (5 total outputs).
- The AUC output values are the time integrals over 24 h of the compartment-averaged concentrations (5 total outputs).
- The maximum average concentrations (C_{max}) were determined by tracking the average concentration for each component over the entire time period (4 total outputs, omitting gel).
- Finally, the time to maximum concentration (t_{max}) was determined concurrently with finding the maximum concentration outputs (4 total outputs, omitting gel).

There are 18 total outputs derived from the model. We used a relatively large number samples to compute the sensitivity indices to ensure that the indices are relatively accurate (48,000 total simulations), where total indices generally converge much faster than first-order indices.

Results

In Figures 3 and 4, we show the total effects for each of the outputs for all the parameters, where the total effects indices represent the combined impact of the variations of each parameter individually together with their variations in combination with all other parameters.

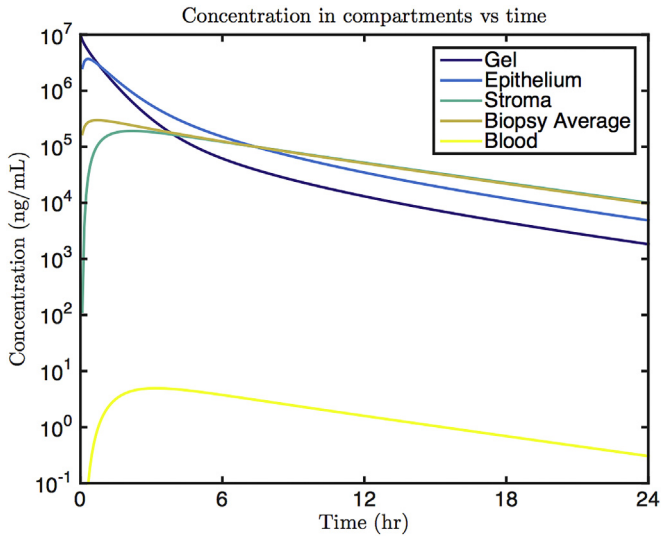


Figure 2. Model outputs for depth-averaged concentrations over 24 h.

Several parameters typically stand out as significant for specific outputs; the threshold for significance with respect to the total index values is chosen subjectively, which is common practice for Sobol' measures. This is a distinction for the Sobol'-based methodology because other global SA methods are less subjective (e.g., partial rank correlation coefficient[33]); however, the methods typically agree with respect to their qualitative rankings. Table 2 indicates which parameters are considered sensitive for each output using a typical threshold value of 0.1 for total effects.

As seen in Table 2, the parameters k_D and C_0 produce sensitivity across all compartments for different types of model outputs and have the greatest overall impact on those outputs. Other parameters give rise to sensitivity for specific compartments: blood—k_L, W, L, and V_B; biopsy and stroma—h_S, whereas others seem to simply correspond to certain types of outputs regardless of compartment such as for t_{max}—D_E and h_E; C_{24}, AUC, and C_{max}—Φ_G; C_{24}—k_B. We also see that there are a handful of parameters to which there is low sensitivity for all outputs—D_G, Φ_E, V_G reducing the uncertainty in the model predictions due to variations in those variables. Further salient implications of results in Table 2 are as follows. First, C_0 does not have a major impact on time-based outputs, t_{max}. Also, D_E generates sensitivity for t_{max} for both the epithelium and stroma but not for the biopsy compartment, which is an average of the epithelium and stroma compartments.

Discussion

In the model here, there are parameters characterizing the gel (C_0, V_G, and D_G), the host environment (W, L, h_E, h_S, and V_B), and the interaction of the drug and host environment (D_E, D_S, Φ_G, Φ_E, k_D, k_L). Values and variabilities in these parameters have multiple origins. Loaded concentration of the drug in the gel (C_0), gel volume (V_G), and diffusion coefficient in the gel (D_G) are controlled in gel design. Vaginal canal dimensions (W, L) and volume of distribution in the blood compartment (V_B) vary with natural physiological variations in body size and mass index. Thickness of the epithelial layer (h_E) varies with phase of the menstrual cycle, whereas stromal thickness (h_S) does not. Drug properties (which are controlled in development) together with those of the mucosal tissue (which are not controlled) govern the D_E, D_S, Φ_G, Φ_G, k_D, and k_L parameters. There are physical and physicochemical interpretations of the sensitivities summarized in Table 2, which we discuss subsequently.

With respect to the gel-associated parameters, the model outputs are not particularly sensitive to the parameters D_G and V_G because drug transport in the gel is much faster than it is in the tissue. As a consequence, the concentration profile in the gel is relatively flat.[23] That is, the rate-limiting step of drug transport occurs in the tissue, so changes in gel parameters for the drug have a negligible effect on the overall transport process. The model is linear with respect to concentration, so the measures C_{24}, AUC, and C_{max} scale directly with load drug concentration in gel, C_0. However, the constants of proportionality vary for the different measures, and thus, the Sobol' indices do as well. This is because the indices are calculated using the variances for each parameter and are then normalized with respect to the variance of the particular output. Although C_0 is a sensitive parameter for the majority of the outputs, it is a precisely known value and consequently reduces uncertainty in the model's predictions. The partition coefficient parameter Φ_G translates the effective concentration up or down the gel—epithelium interface. The magnitude of Φ_G can vary by about a factor of 2 (depending on drug solubility in the gel). In contrast, the partition coefficient between the epithelium and stroma of the tissue Φ_E is not expected to change

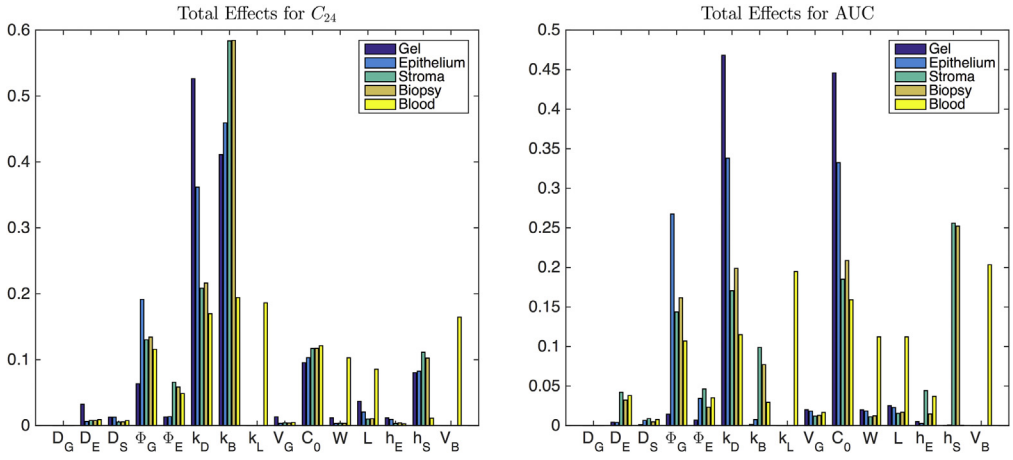


Figure 3. Total effects for C_{24} and AUC outputs.

appreciably with gel design or across individuals. The value of Φ_E is approximately one and causes less sensitivity.

Another gel-related parameter, k_D, the gel dilution coefficient, affects the concentration in the gel over time. It is a first-order approximation for more complicated physical processes, involving gel dilution and also leakage from the vagina. We estimated the overall effects of dilution and leakage by fitting model predictions to experimental PK data.[23] Due to the fact that this rate constant is much higher than those of the other 2 loss terms (k_B, k_L), it has the highest sensitivity. This gel dilution term primarily affects the concentration in the compartments at longer time scales; thus, it is most sensitive for the parameters C_{24} and AUC. The factor k_B formally depends on the collision frequency of drug molecules in the stroma with the vasculature. This kinetic parameter is the most sensitive for the output C_{24} in all compartments because it acts in the long-time scale. It is also sensitive for the C_{max} of the blood compartment because it governs the uptake of drug into the blood stream. The k_L parameter derives from the standard volumetric clearance rate of drug in the blood compartment. Drug transport into the blood is primarily unidirectional, and k_L significantly affects drug concentration in the blood compartment but not the other compartments.

The output t_{max}, the time from the onset of transport to maximum drug concentration in each compartment, is sensitive to D_E, k_D, and h_E (the blood compartment is also sensitive to k_L). Those 3 parameters are, thus, the key factors affecting the time dependence of the drug transport process. D_E and h_E are the key parameters for transport of drug in tissue. The diffusion coefficient in the epithelium is lower than in stroma. Given this distinction, and the fact that the diffusion coefficient in the gel is about 2 logs higher, the rate-limiting step in drug transport is in the epithelium. Notably, at steady state, the epithelium can be approximated by a membrane with transport rate proportional to D_E/h_E.

The diffusion coefficient in the epithelium (D_E) is lower than that of the stroma (D_S). Both diffusion coefficients are effectively volume averages over tenofovir concentrations within and external to cells in both those compartments. They are associated with measurements of drug concentration in biopsies of tissue in experimental PK studies,[16] as referenced in development of this original drug compartmental model.[23] Several factors likely contribute to the lower diffusion coefficient in the epithelium. The vaginal epithelium has a high concentration of cells, whereas the stroma is largely connective tissue with fewer cells and a network of blood vessels (approximately 10% by volume). Thus, tenofovir encounters more obstacles to transport in epithelium than in stroma. Our model here does not include phosphorylation of tenofovir to TFV (TFV-DP) after entering cells in epithelium and stroma, a next step that can now be addressed with the recently published model incorporating TFV-DP model dynamics. The half-life of TFV in cells is of the order of several days,[34,35] much longer than that of tenofovir. The retarding effect of this on drug transport

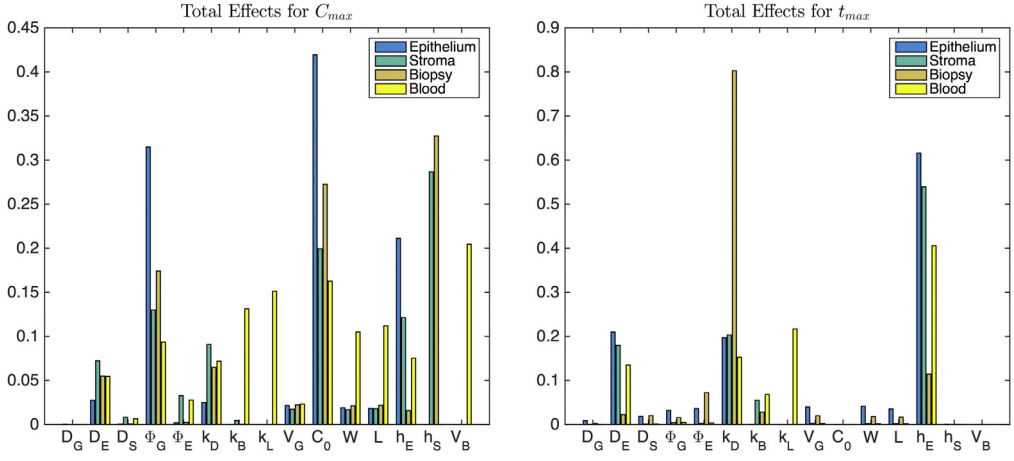


Figure 4. Total effects for C_{max} and t_{max} outputs.

Table 2
Summary of Sobol' Total Effects Indices With Different Sensitivity Thresholds for All Parameters Affecting Each Output in All Compartments: ✓ Indicates SI ≥0.1 and ✓* Indicates SI ≥0.2

	Gel		Epithelium				Stroma				Biopsy				Blood			
	C_{24}	AUC	C_{24}	AUC	C_{max}	t_{max}	C_{24}	AUC	C_{max}	t_{max}	C_{24}	AUC	C_{max}	t_{max}	C_{24}	AUC	C_{max}	t_{max}
D_G																		
D_E						✓*				✓								✓
D_S																		
Φ_G			✓	✓*	✓*		✓	✓	✓		✓	✓	✓		✓	✓		
Φ_E																		
K_D	✓*	✓*	✓*	✓*		✓*	✓*	✓		✓*	✓*	✓*		✓*	✓	✓		✓
K_B	✓*		✓*				✓*	✓			✓*				✓		✓	
K_L															✓	✓	✓	✓*
V_G																		
C_0	✓	✓*	✓	✓*	✓*		✓	✓	✓*		✓	✓*	✓*		✓	✓	✓	
W															✓	✓	✓	
L																✓	✓	
h_E					✓*	✓*			✓	✓*				✓				✓*
h_S							✓	✓*	✓*		✓	✓*	✓*					
V_B															✓	✓*	✓*	

SI, Sobol' Index.

is greater in epithelium than stroma because of the higher concentration of cells there and, thus, also contributes to the reduced diffusion coefficient of tenofovir in epithelium versus stroma.

Due to the fact that D_E is greater than D_S, the rate-limiting drug diffusion step within the tissue is in the epithelial layer. During relatively early times, drug transport is sensitive to D_E. However, by 24 h, the slowing effect of D_E is no longer significant compared with other kinetic factors because most of the drug has transported out of the gel and through the epithelium. This effect also governs the increased sensitivity of C_{max} and t_{max} to D_E at early times versus longer times. Recently, D_E and D_S for tenofovir have been measured directly using confocal Raman spectroscopy.[36,37] This will lead to future reduction of uncertainty in the model.

As noted earlier, epithelial thickness (h_E) varies with the phase of the menstrual cycle (it is thinnest at the time of ovulation in the middle of the cycle). It is a key kinetic parameter for C_{max} and t_{max} in both the epithelium and the stroma but not C_{24} and AUC. Epithelial thickness changes affect the concentration at short times immediately after dosing, but the changes do not significantly alter the concentration at longer times. Additionally, the variance used here might be larger than seen in some population studies, so, with a smaller range, the outputs at longer times would likely be even less sensitive to h_E.

Biopsy punches are commonly used to sample tissue in pharmacokinetic studies of vaginal drug delivery. The harvested tissue is weighed and then homogenized, and drug concentration per unit mass of tissue is measured (e.g., using mass spectrometry). Here the variable h_S depicts not only the histologic thickness of the stroma, but, in the context of a biopsy, it is also the depth to which the tissue is cut. Biopsy outputs C_{24}, AUC, and C_{max} are all sensitive to h_S. Due to slow diffusion in the tissue, most of the drug is concentrated at the top of the tissue, especially the epithelial layer. Deeper biopsy samples lower the average concentration in a biopsy by increasing tissue volume with minimal increase in mass of drug. The sensitivity of spatial-average concentrations in biopsies to thickness, thus, limits their quantitative interpretation. Therefore, measurement of biopsy thickness and total mass of drug would be preferable.

In summary, using the results of Sobol's method for global SA, we have identified multiple parameters of greater and lesser impact on outputs for a model for vaginal mucosal delivery of tenofovir. In doing so, we have considered the biologic and pharmacologic implications of these findings. In particular, the parameters C_0 and Φ_G, the loaded drug concentration in gel and partition coefficient at the gel–mucosal surface (which are controlled in vaginal drug selection and gel design), were found to be good targets for manipulation in optimizing a gel's drug delivery performance. These 2 parameters proved to be sensitive in this analysis, even with tested variances much smaller than their actual pharmacologic ranges. One of the sensitive parameters, k_D (the gel dilution coefficient), merits further investigation with respect to the factors that govern it. In general, sensitivities due to parameters characterizing the host vaginal environment reveal information about the causes of population variability encountered in clinical trials of drug pharmacokinetics. The approach here could inform decisions about stratification of participant populations in PK studies to better understand and account for population-based variability. Our results expand on previous ones emphasizing the need to standardize biopsy thicknesses or, at least, measure those thicknesses after collection.

Finally, the most sensitive parameters produce the greatest amount of variation in the outputs and, thus, elicit the greatest uncertainty in results for predicted PK. Therefore, having good estimates for sensitive parameters decreases the uncertainty and increases the reliability of the model predictions (model robustness). This informs us about which measurements to seek to improve, to use this PK modeling to improve our understanding of the PK process overall. A meaningful follow-up to the work here will be to analyze sensitivities of model outputs while specifying uncertainties in parameters on a per-parameter basis as indicated by specific experimental and computational results.

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