Physiologically-based Pharmacokinetics Modeling of Colloidal Nanoparticles

Anh-Dung Le

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PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING
OF COLLOIDAL GOLD NANOPARTICLES

by

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B.S., Biochemistry & Molecular Biology
M.S., Nanoscience & Microsystems

DISSERTATION
Submitted in Partial Fulfillment of the Requirements for the Degree of
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PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF COLLOIDAL GOLD NANOPARTICLES

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ABSTRACT

Physiologically-based Pharmacokinetics (PBPK) modeling—a mechanistic mathematical tool to predict biodistribution to inform drug development, requires knowledge of the physico-chemical properties of the drug in question as well as the anatomy and physiology of the organism. Unlike with molecular drugs, nanoparticle parameters often need to be estimated by fitting models to time-course data, which can result in large uncertainties in parameter estimates due to a high-dimensional parameter space. Our research addresses this challenge by offering a path to parameter insights using global sensitivity analysis. The first chapter is an overview of the PBPK model structure and mathematical framework used in our study of colloidal gold nanoparticles in mice. The second chapter introduces parameter sieving as a method for model reduction. We show that it is possible to obtain the same goodness-of-fit while excluding estimation of insensitive parameters. The final chapter utilizes time-course sensitivities to suggest drug delivery strategies.
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CHAPTER 1:

STREAMLINING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL DESIGN FOR INTRAVENOUS INJECTION DELIVERY OF NANOPARTICLE DRUGS

ABSTRACT

Physiologically-Based Pharmacokinetic (PBPK) modeling for nanoparticles elucidates the nanoparticle drug’s disposition in the body and serves a vital role in drug development and clinical studies. This paper offers a systematic and tutorial-like approach to developing a model structure and writing distribution ordinary differential equations based on asking binary questions involving the physico-chemical nature of the drug in question. Further, by synthesizing existing knowledge, we summarize pertinent aspects in PBPK modeling and create a guide for building model structure and distribution equations, optimizing nanoparticle and non-nanoparticle specific parameters, performing sensitivity analysis and model validation. The purpose of this paper is to facilitate a streamlined model development process for students and practitioners in the field.
BACKGROUND

Nanoparticles have distinct properties that make them unique in their applications in nanomedicine partly due to the high surface area-to-volume ratios which allow for functionalization of drug nanocarriers. For instance, metallic nanoparticles such as iron oxide or gold nanoparticles can be used in medical imaging\textsuperscript{1–3}. This rise in the use of nanotechnology in medicine has crossed paths with pharmacology resulting in the need for understanding of biodistribution (distribution throughout the body) of drugs and their carriers.

There are a number of different nanocarriers that have been used in the development of nano drug delivery systems. Hossen \textit{et al.} reviewed several different nanocarrier drug delivery systems for cancer therapy including colloidal nanoparticles and liposomes\textsuperscript{4}. These nanocarriers have different roles in how they carry the associated drugs throughout the body. For example, colloidal nanoparticles such as gold nanoparticles are a good candidate for a drug carrier because they have good biocompatibility and can be conjugated to different molecular species\textsuperscript{5}. Further, their optical properties as a result of surface plasmon resonance make it possible for them to be used in imaging purposes\textsuperscript{6}. Liposomes can be functionalized to recognize cancer cells while carrying cargos of hydrophilic drugs that can be released upon activation \textsuperscript{4}. Other drugs are turned into their nanocrystalline form to overcome the low solubility in both water and oil\textsuperscript{7}. Such nanocrystal drugs have a dissolution rate constant that must be taken into account during modeling and simulation.
Biodistribution of nanoparticles can be modeled *in silico* using pharmacokinetics modeling. Physiologically-Based Pharmacokinetic (PBPK) modeling takes into account more physiological processes compared to traditional one-compartment pharmacokinetics modeling and is important in the field of pharmacology. The Food and Drug Administration (FDA) regulates PBPK modeling for regimenting drug dosages in order to safely predict efficacious therapeutic indices for drugs. Moreover, PBPK modeling is also important in the field of environmental science and engineering due to the risk of exposure to toxic nanoparticles and the need for rational design of nanoparticles, respectively. PBPK modeling may be further applied in areas relating to diagnostics, for example, predicting the biodistribution of monoclonal antibodies.

PBPK modeling is a powerful tool used in the research and development of drugs partly due to the ability to predict and understand drug behavior. PBPK modeling may be used both, *a priori*, to mechanistically predict drug disposition, as well as, *a posteriori*, to empirically understand drug behaviors by estimating parameters. Empirical data may also be used to validate model predictions (Figure 1.1). PBPK modeling has been used to study the biokinetics of nanoparticle drugs in terms of modeling past experimental data as well as predicting appropriate dosages through simulations. However, one major limitation that has been ascribed to PBPK modeling of nanoparticles is the application of the resulting model for only one type of nanoparticle at a time.
Every new nanoparticle formulation would require reparameterization due to changes in the physico-chemical properties of the particles being used in the model as well as how certain factors such as cell permeability and macrophage uptake rate may be affected. This limitation results in a shortage of models that can keep up with the demands of different types of nanoparticle drugs being developed in the pharmaceutical industry. This paper focuses on providing a unifying framework—to understand how to develop a nanoparticle PBPK model via a systematic approach where major parameters, model compartments and physiological factors are considered.
COMPARTMENTAL MODEL STRUCTURE

PBPK models can consist of many compartments, for example including the venous blood, arterial blood, lungs, heart, muscle, brain, kidneys, liver, gut, spleen, adipose tissue, skin, and bone. Some of these compartments may be grouped together depending on their influence on biodistribution. Venous and arterial blood are sometimes considered as one compartment called the plasma or blood circulation. However, other slightly different combinations of compartments including the lymph nodes and thymus may also be included, for example. Most other PBPK models incorporate a miscellaneous or remainder compartment that may include tissues either insignificant or not sampled to further account for the total amount of mass of the administered dose.

PBPK model design often consists of both arterial blood as well as venous blood compartments in order to account for the lag in drug distribution when an IV dose is administered. On the other hand, in a single blood circulation compartment, any IV dose administered will take on the presumption that the drug will travel directly to all the organs, in other words, having 100% biodistribution much quicker. More accurately, in a dual blood circulation compartment, an IV injection will typically take place on the accessory cephalic vein, which is located...
on the back of the arm, delivering the drug at the site of the vein carrying
deoxygenated blood\textsuperscript{28}. In experiments involving mice for example, the tail vein is
the place of injection into the venous blood.

Figure 1.2 shows the structures of a typical multicompartment PBPK model
currently used across the literature for modeling disposition of nanoparticle
drugs. The multi-compartment model structure containing separate venous and
arterial blood compartments can be slightly modified to describe whether the
model is flow-limited or membrane-limited, or whether or not it contains
phagocytizing sub-compartments, for instance.
Figure 1. 2. Multi-compartment PBPK model structures for different hypothetical scenarios.

(A) non-nanoparticle, flow-limited structure (with specific examples found in \(^{25,31}\)); (B) non-dissolvable, colloidal-nanoparticle, membrane-limited structure; (C) non-dissolvable, colloidal-nanoparticle, membrane-limited structure, with phagocytizing cell sub-
compartments (with specific examples found in 20,32,33); and (D) dissolvable nanoparticle, flow-limited structure (with specific example found in 7).

In all parts in Figure 1.2, the multi-compartment PBPK model structure utilizes both the venous and the arterial blood compartments instead of only one plasma compartment. However, a model structure that utilizes one plasma compartment for colloidal nanoparticles has also been demonstrated to be effective21. Such a model structure would experience latency in the calculated distribution time accounting for blood flowing to the lungs and arterial blood before going to peripheral organs28. In dissolvable nanoparticle, membrane-limited structures, a structure similar to that of Figure 1.2(D) could be used with the presence of capillary compartments similar to that of Figure 1.2(B) and (C).

The organ compartments involved in the hepatic portal circulation in Figure 1.2 are summarized in one compartment for the purpose of illustrating a simplified model structure. However, these different organs have their own designated compartment since they have different parameters such as blood flow, organ volume, permeability coefficients, and partition coefficients. On the other hand, compartments not involved in the pharmacokinetics study will be grouped together in the model structure as the remainder compartment. The total organ volume of the remainder compartment will be the difference between the organism's body weight and the weight of the organs already accounted for, including the venous and arterial blood. The blood flow to the remainder compartment should be similar since they are lowly perfused organs, hence a reason for their lack of attention in pharmacokinetics studies. As far as colloidal
nanoparticles are concerned, their distribution between organs and the blood circulation is not a reflection of the thermodynamic equilibrium between two immiscible liquids\textsuperscript{34}. Therefore, the "partition" coefficient may be the same for similar nanoparticles between the blood and similar tissues.

MATHEMATICAL FRAMEWORK

Building upon traditional pharmacokinetic frameworks, PBPK models for nanoparticles would also incorporate specific physiological processes such as macrophage uptake\textsuperscript{7,21,35,36}. Distribution equations are used to account for the drug’s location within the body after a given amount of time. Depending on whether nanoparticles undergo certain transitional states, the differential equations governing a PBPK model can be affected. For instance, for nanoparticles undergoing dissolution, the distribution equation will need to include a term to account for this drug release process.

The construction of the mathematical distribution equations depends on our knowledge of the physico-chemical nature of the drug. In order to build a model that best reflects the underlying biodistribution processes, the modeler should ask questions pertaining to their understanding of the drug formulation such as:

- whether it is a hydrophilic or hydrophobic substance;
- whether it is a small or large molecule;
• whether the nanoparticles in the formulation will undergo dissolution or not; and
• whether there is macrophage uptake of the nanoparticles or not.

Imposing these questions as a systematic approach to developing a PBPK model can streamline the model structure development process.

Figure 1.3 Flow diagram of the basic decision-making process in constructing a PBPK mathematical framework.
The final mathematical formulation of the differential equation describing the amount of drug in a particular organ compartment depends on the physico-chemical properties of the drug formulation. This process is followed by subsequent steps in the model building process.

The flow diagram in Figure 1.3 can be used to construct a PBPK model structure that is consistent with published models reflecting the physico-chemical characteristics of a drug. For example, in a model for SNX-2112, a poorly soluble molecular anticancer drug that was formulated into dissolvable nanocrystals for evaluating its disposition in rats, used a release constant (\(k_{rel}\)) in the distribution equations, and adopted a flow-limited structure with no phagocytizing cell compartment\(^7\). In the case of colloidal nanoparticles, non-dissolvable nanoparticles are described using membrane-limited model structures with phagocytizing cell subcompartments\(^{32,37}\). Equation 1 begins with the basic components of the differential equation utilizing the law of mass action:

\[
\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right)
\]

Eq. 1

where \(\frac{dM}{dt}\) is the rate of change of the amount of drug in compartment 2 at time \(t\); \(Q\), with units of volume per time, is the blood flow from compartment 1 into compartment 2; \(C_1\) and \(C_2\), with units of amount per volume, are the drug concentrations in compartments 1 and 2, respectively; and \(k_p\), with no units, is the partition coefficient. The partition coefficient is the ratio of a substance in two immiscible or slightly miscible solvents in thermodynamic equilibrium. Note that
the distribution equation for the plasma compartment applies the partition coefficient towards the concentration of the drugs coming from the lungs and not away from the plasma. This is because partition coefficients are applied to concentration of drugs leaving an organ compartment and into a plasma compartment. In other words, there is no partitioning taking place when drugs enter an organ compartment from the plasma. When applying this coefficient to model construction for colloidal nanoparticles, it is not thermodynamic equilibrium between the tissue compartment and the circulation that the coefficient reflects. Therefore, a better name for partition coefficient in such an instance would be distribution coefficient\textsuperscript{34}.

The drug will ultimately be eliminated from the body through either renal excretion where drugs will be excreted from the kidneys into the urine, or biliary excretion where drugs are excreted into the feces by the bile\textsuperscript{38}. The clearance rate can vary depending on the drug. The mass equation ultimately includes the summation of both of these processes. Thus, for compartments involving renal or biliary excretion:

\[
\frac{dM}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) - \frac{dM_{ex}}{dt} \\
\text{Eq. 2}
\]

\[
\frac{dM_{ex}}{dt} = (M_l \times CL_b + M_k \times CL_r) \\
\text{Eq. 3}
\]
where \( \frac{dM_{ex}}{dt} \) in units of amount per time, is the rate of excretion of drugs; \( M_l \) is the amount of nanoparticles in the liver tissue; \( M_k \) is the amount of nanoparticles in the kidney’s capillary blood; \( CL_b \), in units of time\(^{-1}\), is the clearance to feces from the liver tissue; and \( CL_r \), in units of time\(^{-1}\) is the clearance to urine from the kidney’s capillary blood.

Nanoparticle Transitions

In oral administration, whether the drug is subject to quick or slow release, it is only the bioavailability - the proportion of drug that enters the circulation - that has its active effect, which varies from formulation to formulation. It is assumed that once the drug reaches the circulation, its biodistribution does not depend on dissolution kinetics inside the circulation since all of the dissolution has already taken place. Nanoparticle formulations, where cargos of drugs are loaded or where the drug molecules form nanocrystals through self-assembly, for example, require the use of decomposition or dissolution kinetics in formulating a PBPK model--since additional time is needed for the nanoparticle state to decompose or dissolve into the free drug state. Nanoparticle drug delivery systems containing drugs which could later be released in the systemic circulation and in organ compartments, would include a term to describe the transition between the nanoparticle and the dissolved states. This transition may be integrated into PBPK modeling. In this case, release constants are needed in the distribution
equations. These constants may be measured or obtained from the literature via prior in vitro studies.

One physiological phenomenon that can affect nanoparticle states is the formation of what was initially coined as protein coronas, which has been studied since at least 2007\textsuperscript{39}. It is a phenomenon in which a protein adsorption layer is formed around a foreign colloidal nanoparticle\textsuperscript{39–42}. The adsorbed proteins may include fibrinogen, vitronectin, human serum albumin, and cytochrome C\textsuperscript{43}. A mechanistic study has shown surface properties of nanoparticles play an important role in determining interactions with the host’s immune responses even more so than nanoparticle sizes when these particles are initially introduced into the circulation\textsuperscript{44}. The formation of protein coronas involves both hetero- and homo-aggregation, where nanoparticles aggregate with proteins or with themselves, respectively, based on ionic concentration of the environment\textsuperscript{42}. The effect of protein coronas can act as a confounding variable affecting cell-specific targeting and uptake of nanoparticles, for example\textsuperscript{45}. The formation of this corona layer around nanoparticles does beg the question of whether or not this plays into the kinetics of dissolution, and thus, the PBPK model itself. In fact, some efforts do exist in order to explain the kinetics of the protein corona\textsuperscript{43,46}. In light of this knowledge, the pharmacokineticist must decide whether or not to take into account the effects of this protein corona. A global sensitivity analysis may help determine the influence of a parameter, and in this case, the rate of dissolution of nanoparticulate drugs having a protein corona layer. Understanding that the state
of nanoparticulate drugs need to be taken into account will allow the pharmacokineticist to incorporate dissolution equations into the PBPK models. Additionally, nanoparticulate drugs may not always decompose through dissolution as in the case of nanocrystals of SNX-2112\textsuperscript{7}. Rather, they may be released through other means. For example, drugs may be packaged in mesoporous nanoparticles that can released via various triggering mechanisms\textsuperscript{47}. Moreover, nanoparticles may be delivered to the circulation in lipid-based vesicles, via functionalized-gold nanoparticles as carriers, or even in micelles\textsuperscript{48–50}. Thus, understanding of the drug release mechanisms of the different formulations is needed in order to take into account the drug release term in the PBPK model. Understanding the chemico-physical properties of the drug (ie. dissolvable vs. colloidal, membrane-limited vs. diffusion-limited) is crucial in designing the model structure since these properties dictate the inclusion of additional reactions and compartments.

When nanoparticle formulations are used, the release of drugs through dissolution can occur and a release constant is needed in the calculation. However, colloidal nanoparticles used as drug carriers or as contrast agents in magnetic imaging modalities, do not undergo dissolution and therefore, there will not be any inclusion of dissolution reaction in the model. This can further affect whether the particles will take on a diffusion-limited or membrane-limited structure of the model, for instance.
Dissolvable nanoparticles undergo dissolution in the aqueous milieu. Therefore, subsequent to an I.V. administration, a first-order release term with constant $k_{rel}$ must be used to account for this dissolution process in the plasma. An example of the utilization of $k_{rel}$ is demonstrated in Wu et al. where biodistribution of nanocrystals of an anticancer agent SNX-2112 was modeled$^7$. Where nanoparticle dissolution takes place, a term must be used to account for the change in amount of dissolved drug with respect to time. Therefore, the distribution equation describing the nanoparticle amount while considering dissolution in compartments will include $-\left( k_{rel} \times V_t \times C \right)$ and the distribution equation describing the corresponding dissolved drug will include $+\left( k_{rel} \times V_t \times C \right)$ such that:

$$\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{K_p} \right) \pm \left( k_{rel} \times V_t \times C \right)$$

Eq. 4

where $V_t$ is the volume of the compartment where dissolution is taking place and $C$ is the concentration of the nanoparticle drug before dissolution in that compartment (either compartment 1 or 2). Of course, if all nanoparticles dissolve completely in the venous blood compartment (ie. after I.V. administration), then nanoparticle dissolution will no longer apply to subsequent compartments. However, all of that will depend on the $k_{rel}$ constant.
Colloidal Nanoparticles

Colloidal nanoparticles do not dissolve in aqueous milieu and there is no evidence of dissolution in the circulation\textsuperscript{32}. Therefore, the release constant is not included. Thus, crossing the membrane into the intracellular fluid would be a rate-limiting step and the distribution equation will be multiplied by a term describing permeability under the membrane-limited framework as presented previously by Li \textit{et al}\textsuperscript{51}:

\[
\frac{dM}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X} - \frac{dM_{ex}}{dt}
\]

Eq. 5

Where $X$ is the unitless permeability coefficient. Notice that the distribution equation is directly affected by the derived term $\frac{X}{1 + X}$. This term is a result of the nanoparticle residence time in the capillary being small compared to its residence time in tissue compartments. Therefore, the capillary is considered as a quasi-compartment and not taken into account during the derivation process \textsuperscript{52}. The resulting term summarizes permeability between arterial/venous and tissue compartments.
Perfusion (flow)- limited vs. Diffusion (membrane)- limited model

Generally, the PBPK model is either a diffusion-limited (permeability-limited) or a perfusion-limited (flow-limited) model\textsuperscript{53–55}. However, in certain cases, it can be both. Perfusion-limited models are utilized where small lipophilic molecules can partition into tissues rapidly and the rate of blood flow is the limiting rate. Compounds that are large and hydrophilic have a harder time crossing the cell membrane and therefore diffusion-limited (membrane-limited) models would be utilized to model the disposition of those compounds\textsuperscript{18}. On the other hand, hydrophobic or small compounds have an easier time crossing the cell membrane and thus perfusion-limited (or flow-limited) models would be utilized. In the case of gold nanoparticles, the proposed PBPK framework will be a membrane-limited model in order to take into account the rate limiting effects of nanoparticles crossing the cell membranes. Therefore, the model will describe the capillary blood and the tissue compartments separately\textsuperscript{33}.

In diffusion-limited (membrane-limited) models, the rate of biodistribution depends on the permeability of the membrane with respect to the drug. However, in perfusion-limited (flow-limited) models, the rate-limiting step resides in the tissue partitioning of the drug. Identifying where the rate-limiting step will help to determine the paradigm of biodistribution of drugs. Further, the flow and connectivity of the organs will provide an overall picture of the PBPK model where differential equations may be applied to reflect the connections between organ compartments.
Sub-Compartmentalization

Anatomical Sub-Compartmentalization

It is beneficial to consider anatomical sub-compartmentalization in some instances. For example, an intestinal model can anatomically break down the gut into sub-compartments since enterocytes—which are intestinal absorptive cells—can line the walls of the stomach all the way to the colon. This sub-compartmentalization is important because the ultimate possible routes where the drug ends up after an oral administration may not only be excretion through the colon, but also absorption throughout the different anatomical sub-compartments within the gut where the drug can travel into the hepatic portal vein. Sub-compartmentalization details several different other compartments that exist within a main compartment of the PBPK model and thus helps to improve results leading to better model validation. Sub-compartmentalization in PBPK modeling has also been performed in the lungs, dermal, and nasal tissue. In one instance, modeling of pulmonary drug biodistribution where detailed sub-compartments of different regions of the lung including right and left lung, and lower and upper airways have been considered.
Physiological Sub-Compartmentalization

Colloidal nanoparticles do not dissolve in the aqueous milieu and therefore will encounter the body's immune response via macrophage uptake. Macrophage uptake may also be taken into account in the PBPK model with a phagocytizing cells (PC) compartment which will account for clearance of nanoparticle drugs. Currently, macrophage uptake is composed of two basic different steps which include absorption governed by the rate constant $k_{ab}$ and desorption governed by the rate constant $k_{de}$ via the law of mass action\(^{37}\) (shown in Figure 1.4). Absorbed nanoparticles are ultimately released by the macrophage. The resident amount of time that any foreign substance possesses in a particular organ tissue is reflected in the equilibrium between the absorption and desorption rates.

![Figure 1.4 Phagocytizing cells as a physiological sub-compartment within compartments with high concentrations of macrophage.](image)

Figure 1.4 shows the phagocytizing cells being used as a physiological sub-compartment. In these instances, the distribution equation for a compartment with high concentrations of macrophages, such as in the liver where there is high occurrence of Kupffer cells, will need to account for the absorption and desorption processes:
\[
\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X} + \left[ (k_{de} \times M_{2,m}) - (k_{ab} \times V_2 \times C_2) \right]
\]

Eq. 6

Where \( V_2 \) is the volume of organ in compartment 2; \( C_2 \) is the concentration of nanoparticles in organ compartment 2; \( k_{ab} \) is the uptake rate of nanoparticles by phagocytizing cells in organ compartment 2; \( k_{de} \) is the desorption rate of nanoparticles by phagocytizing cells in organ compartment 2; and \( M_{2,m} \) is the amount of nanoparticles captured by phagocytizing cells in organ compartment 2. Organ compartment 2 was used in this example for absorption and desorption because it represents the compartment containing the nanoparticle drugs being calculated.

While some compartmental models currently used in the literature for PBPK modeling do account for macrophage uptake, these models may not account for the existence of confounding variables in the phagocytizing events. For instance, they may not consider the effects of adsorption and internalization which can affect the maximum uptake constant. The importance of considering adsorption and internalization of nanoparticles when it comes to cellular uptake was demonstrated by Yeo and colleagues where differential labeling using electron microscopy revealed three different processes including “attachment”, “in between”, and “internalized” 62. Wilhelm and colleagues have further approached the topic using superparamagnetic iron oxide nanoparticles (SPIONS)'s
interactions with macrophages and built a mathematical framework around their adsorption and internalization kinetics\textsuperscript{63}. The progression of the macrophage uptake process including both adsorption and internalization was validated further by epifluorescence microscopy\textsuperscript{62}. Development of the sub-compartmentalization of macrophage uptake can further be validated with semiquantitative data from the literature\textsuperscript{62}. Much of the literature shows macrophage uptake data that includes consideration for absorption and desorption. For example, Liu et al. discusses a quantitative approach to obtaining absorption (\textit{kin}) and desorption (\textit{kout}) hepatic constants\textsuperscript{64}. The current inclusion of macrophages as a "compartment" within PBPK modeling makes several assumptions including homogeneity of macrophages' uptake rate. Additional consideration regarding what affects adsorption and internalization may be helpful to understanding the overall phagocytizing process. Qie et al have shown that surface modification of nanoparticles can influence phagocytic clearance\textsuperscript{65}.

Parameters

Non-nanoparticle specific parameters

Input parameters are key for PBPK model simulations. Input parameters describe three types of properties: physico-chemical properties; drug-biological properties; anatomical and physiological properties\textsuperscript{66}. Parameters that come from physico-chemical properties are drug-dependent and include the partition coefficient, pH-
dependent partition coefficient, membrane affinity, molecular weight, equilibrium constants, and solubility\textsuperscript{53,66}. Parameters that come from drug-biological properties depend on both drug and organism properties. These parameters include fraction of unbound drugs, Michaelis-Menten constant, dissociation constant, maximum velocity. Since drug-biological properties depend not only on the organism, but also the drugs, partition coefficients and permeability of drugs are also considered as drug-biological properties for they depend on both the organism and the physico-chemical properties of the drug. Parameters deriving from anatomical or physiological properties (organ-specific) include organ volume, surface areas, tissue composition, blood flow rates, and expression levels\textsuperscript{67}. Some of these parameters are used in PBPK modeling to predict the pharmacokinetic disposition for different populations. For example, expression levels can help determine the gene expression for a group of metabolizing enzymes in different organs for different populations\textsuperscript{66,68–73}. Gene expression data may be represented as a normalized relative value with respect to the tissue or organ with the highest expression\textsuperscript{66}. Thus, with different metabolizing enzyme expressions, results for clearance will be reflected in different populations having different levels of expression. Using gene expression data can aid the PBPK model in achieving resolution in the amount of metabolizing enzymes as well as other physiological parameters that aid in determining the dosing regimen\textsuperscript{68,69}.
Nanoparticle-specific parameters

PBPK models built for nanoparticles will also include nanoparticle-specific parameters which may include nanoparticle release constant, maximum uptake rate in phagocytic cells, Hill coefficient, and phagocytic cells release constant (desorption). Tables 1 and 2 show examples of important organ-specific and nanoparticle-specific parameters used in nanoparticle PBPK modeling.

<table>
<thead>
<tr>
<th>Organ specific parameters</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organ volumes based on % of body weight</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>liter</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart</td>
<td>liter</td>
<td>9.5E-5</td>
</tr>
<tr>
<td>Brain</td>
<td>liter</td>
<td>0.00017</td>
</tr>
<tr>
<td>Spleen</td>
<td>liter</td>
<td>0.001</td>
</tr>
<tr>
<td>Kidneys</td>
<td>liter</td>
<td>0.00034</td>
</tr>
<tr>
<td>Liver</td>
<td>liter</td>
<td>0.0013</td>
</tr>
<tr>
<td>Pancreas</td>
<td>liter</td>
<td>0.0013</td>
</tr>
<tr>
<td>Stomach</td>
<td>liter</td>
<td>0.00011</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>liter</td>
<td>0.000228182</td>
</tr>
<tr>
<td>Venous blood</td>
<td>liter</td>
<td>0.000524818</td>
</tr>
<tr>
<td><strong>Blood flow</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>l/min</td>
<td>5.47E-3</td>
</tr>
<tr>
<td>Heart</td>
<td>l/min</td>
<td>2.80E-4</td>
</tr>
<tr>
<td>Brain</td>
<td>l/min</td>
<td>1.30E-4</td>
</tr>
<tr>
<td>Spleen</td>
<td>l/min</td>
<td>9.00E-5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>l/min</td>
<td>1.30E-3</td>
</tr>
<tr>
<td>Liver</td>
<td>l/min</td>
<td>3.50E-4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>l/min</td>
<td>5.20E-5</td>
</tr>
<tr>
<td>Stomach</td>
<td>l/min</td>
<td>1.10E-4</td>
</tr>
<tr>
<td>Portal vein</td>
<td>l/min</td>
<td>1.75E-3</td>
</tr>
</tbody>
</table>

Table 1. Typical organ-specific parameters used in both nanoparticle and non-nanoparticle PBPK modeling.

<sup>a</sup>Can also be calculated by taking the percentage of the weight of a mouse in (g) to give the organ volume in (mL). Other sources in the literature include<sup>28,33,74–76</sup>. However, these values are based on 20 g mouse calculated by PK-Sim® 8 database.

<sup>b</sup>Based on values obtained by PK-Sim® 8 database for mouse.
<table>
<thead>
<tr>
<th>Nanoparticle specific parameters</th>
<th>Descript-ion</th>
<th>Lung-s</th>
<th>Hear-t</th>
<th>Live-er</th>
<th>Kid-ne-y</th>
<th>Spleen</th>
<th>Pancre-a</th>
<th>Bra-in</th>
<th>Stoma-ch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partition  (distribution coefficient)c</td>
<td>0.15</td>
<td>0.15</td>
<td>0.08</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Permeability coefficient between blood and tissued</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.001</td>
<td>0.03</td>
<td>0.00000</td>
<td>0.0</td>
<td>0.00000</td>
</tr>
<tr>
<td></td>
<td>Max uptake rate constant for PCd</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>0.112</td>
<td>0.00</td>
<td>0990</td>
<td>gene-ric</td>
</tr>
<tr>
<td></td>
<td>PC release (desorption) rate constant d</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>gene-ric</td>
<td>gener-i-c</td>
<td>gener-i-c</td>
<td>gene-ric</td>
<td>gene-ric</td>
</tr>
<tr>
<td></td>
<td>Excretion rate constant e</td>
<td>N/A</td>
<td>N/A</td>
<td>1.18</td>
<td>6.56× 10^{-3}</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 1. 2 Colloidal nanoparticle specific parameters taking into account the reticuloendothelial system (macrophage uptake) of nanoparticles.

According to Li and other sources in the literature, arterial and venous blood take up 20% and 80% of the total body blood, respectively \(^{32,33}\).

\(^{c}\)Taken from table in\(^{34}\), which also come from other sources. Source provides data for liver, spleen, kidneys, lungs, brain, and rest of the body. Therefore, any organ compartment not directly provided by source, rest of the body values are used.

\(^{d}\)Values obtained from \(^{77}\). Some assumptions were made since these values were used for rats under different colloidal nanoparticles. Generic values are equal to 16.1±0.306 for absorption and 4.90 \(\times 10^{-19}\)±7.26\(\times 10^{-17}\) for desorption.

\(^{e}\)Values obtained from \(^{77}\).

Some of the parameters, such as liver transporter kinetic data, metabolic enzymes (CYPs), permeability data, and transporter-mediated uptake, can be
determined via *in vitro* experiments and then applied to different stages of absorption, distribution, metabolism, and excretion of the PBPK model to obtain simulated *in vivo* data. More specifically, nanoparticle-related parameters such as macrophage uptake rate and desorption rate constants may be determined *in vitro* and then applied to a nanoparticle PBPK model.

**PARAMETER ESTIMATION**

Predicting biodistribution requires accurate input parameters. While many parameters may be obtained through established literature sources, some cannot be relied upon for predicting biodistribution. When nanoparticle pharmacokinetic disposition is needed for a new formulation, prior parameter estimates and some assumptions may be used. For example, blood flow may be obtained from the literature. Uptake capacity values may be recycled from PK parameters of nanoparticles with similar physical properties. However, one must use some caution in relying on predetermined nano-specific parameters when dealing with nanoparticles even with a slight change in the surface chemistry which can affect their interactions, for example, with macrophages or membranes, and thus can ultimately affect the macrophage uptake rate or permeability coefficients. Therefore, to obtain a good model fit to biodistribution data, a parameter optimization process is needed. For parameter optimization, only a select group of parameters would be required to be optimized. Since organ-specific parameters (Table 1.1) can readily be obtained from databases as well as being
values of minimal variation, these parameters can be exempt from optimization. If we know the range of plausible values for a parameter being optimized, constraints may be added to the parameters being optimized. A variety of local and global optimization techniques can found in the literature and implemented on MATLAB®, for example. Optimizing parameters will not only provide the best-fit model, it also allows the researcher to compare parameters under different nanoparticle formulations. Thus, providing a way to study the effects of different engineered nanoparticles on their corresponding estimated parameters.

SENSITIVITY ANALYSIS (SA)

Sensitivity analysis generates sensitivity indices for each of the parameters to gauge the effects on model output when input parameters are varied. The difference between global and local SAs is that local SA assesses variation in model output based on the changes of one parameter at a time (while all other parameters are held constant), whereas global SA examines not only the overall model response based on variation in all input parameters, but also the variance in model output due to interactions between parameters. SA tests can help to reduce model complexity and elucidate highly sensitive parameters. Conceptually, SA tests for nanoparticle PBPK models should be the same as for non-nanoparticle ones. Nanoparticle-specific parameters such as macrophage uptake absorption and desorption constants may be of interest.
Local SA

Every PBPK model developed that reflects the specific virtual population as well as the nanoparticulate/non-particulate drug system used will have a unique concentration-time curve for any particular compartment based on the input parameters. The degree of influence of a parameter on the concentration-time curve may not be obvious without a sensitivity analysis (SA). Typically, two approaches have been used for local PBPK SA tests. One approach is to multiply or divide each parameter by a predetermined value and observe the concentration output or amount of nanoparticles in each of the compartment with respect to time. Another approach is to measure the change in area-under-the-curve (AUC) after a 1% change in the parameter value. More specifically:

\[
\text{Sensitivity Coefficient} = \frac{dAUC/AUC}{dp/p}
\]

Eq. 7

where AUC is the area under the concentration-time curve in a compartment and dAUC is the change in AUC of that compartment reflecting a 1% change in the parameter \(dp/p = 0.01\). When conducting a sensitivity analysis, the higher the sensitivity coefficient for a parameter, the larger the influence of that parameter on the model output for a particular compartment. However, the equation above only gives the local sensitivity coefficient. A more global and systematic approach to sensitivity analysis is needed to show the influence of a parameter over a set of all possible input parameters. Further, local sensitivity
analysis is only appropriate when interactions between parameters are negligible\textsuperscript{84}.

Global SA

Global sensitivity analysis (GSA) falls into one of two categories which are elementary effect and variance-based GSA methods\textsuperscript{85}. Two GSA methods have been employed in PBPK modeling are the Morris screening (an elementary effect method) and the extended Fourier Amplitude Sensitivity Test (eFAST) (a variance-based method) which can be used to study the effects of input parameters on pharmacokinetic outputs\textsuperscript{85–87}. The Morris test is a qualitative test to identify non-influential input parameters in PBPK modeling, which can be fixed without consequences on output uncertainty. It is a preliminary test typically used as a first step in some PBPK global sensitivity analyses within a GSA workflow giving rough estimations with a limited number of calculations\textsuperscript{84–86}. The Morris method gives 2 measures of sensitivity consisting of $\mu$ which measures a variable’s overall influence and $\sigma$ which approximates the non-linear effects of the variables in the model which are then plotted on a $\sigma$ vs $\mu$ plot\textsuperscript{88}. Using the Morris method (Figure 1.5), simulations that yield low measures of $\mu$ and low measures of $\sigma$ indicate that the input parameter has a negligible effect; simulations that yield high $\mu$ but low $\sigma$ indicate that while the parameter is sensitive, it still has very little interaction with other parameters, or that it has linear effects; and simulations that yield both high $\mu$ and high $\sigma$ indicate parameters that are both sensitive and either interact with other parameters or are non-linear\textsuperscript{89}. 

30
Figure 1.5. Results of the Morris method, a qualitative test, can be visualized on a $\sigma$ versus $\mu$ plot.

Only simulations that yield both high $\sigma$ and $\mu$ indicate parameters are both sensitive and either interact with other parameters or are non-linear.

On the other hand, the eFAST method is a quantitative method for a subset of explanatory selected parameters. These selected parameters may be chosen from the previously discussed Morris method. McNally and colleagues demonstrated the use of Lowry plots to display how much variances may be accounted for in model outputs if all parameters are included up a certain point within the plot as well as discussed the calculation of the upper and lower bounds of the variances. More common GSA methods may also be applied to
nanoparticle PBPK model development to assess outlier or counterintuitive effects of certain parameters on the model. Good practice in GSA application includes applying multiple methods, reiterating choices made, and graphically visualizing results for effective communication of parameter influences.

MODEL VALIDATION

Model validation takes place after key parameters have been determined by either performing parameter estimation or obtained through previous literature findings. This essentially means that validation requires a different set of empirical data in order to validate the model built on previous findings or assumptions.

$R^2$ analysis is typically used to evaluate a model which is based on the deviation from the line of unity between $\log_{10}$ of measured and predicted values. Geometric standard deviations ($\text{GSD}^2$) may also be used to further validate a model. A GSD$^2$ less than 10 will indicate that the accuracies of prediction of individual data points are of maximum one order of magnitude, for instance. Additionally, results of PBPK models may also be validated based on either looking at percent ($\%$) errors or fold errors. Area under the curve (AUC) and maximum concentrations (Cmax) and clearance (CL) are all model outputs that can be used in the validation discussion. Percent error is the measure of the difference in predicted and actual values over the predicted values. These values may be
lowered after optimization of a model. For example, Mavroudis and colleagues showed that in three different formulations of paracetamol, by lowering the gastric emptying time (GET) and dissolution time (DT), and by altering the dissolution shape (DS) parameter, they were able to decrease the percent error of results which were previously obtained by a different group\textsuperscript{91}. Optimization of models is a way to further elucidate our understanding of the impact of our parameters.

Another method of analyzing the validity of models is through the use of average fold-errors which are ratios of predicted over observed values\textsuperscript{92}. This method is typically used in analyzing the validity of predicted clearances (CL) and other model outputs where a 2.0 or less fold-error is preferred\textsuperscript{92,93}. The absolute average fold error (AAFE) is the average of all fold errors for a particular model output\textsuperscript{94}:

$$\textit{Absolute Average Fold Error (AAFE)} = 10\frac{1}{n} \sum_{i=1}^{n} \left| \frac{\log_{10}\text{predicted}}{\log_{10}\text{observed}} \right|$$

where $n$ is the size of the data. The AAFE value may be plotted with its standard deviation. In one example, Zhou and colleagues have analyzed the performance of model in 6 different age groups under 4 different drugs which yielded a 0.5 - 2.0 fold-error\textsuperscript{92}: The closer to the predicted/observed ratio of 1.0 along with variations not extending beyond a predetermined range, in this case, 0.5-2.0, the more confidence we have in the model. Other ways to analyze the model based on fold-errors is to plot the percentage of data points falling within a 2.0 fold-error
or plotting absolute average fold-errors of model outputs by various model approaches. It is important to note the optimization of a model depends on the reiteration of that model based on improved understanding of parameter influences as well as error analysis results. Therefore, effective PBPK model development is a workflow that relies on our understanding of: how to build the model structure mechanistically, the mathematical framework underlying the biodistribution of the nanoparticles, the importance of the contributions of each of the parameters, and how to evaluate the effectiveness of the model through model validation methods.

INTERSPECIES EXTRAPOLATION

While there are a plethora of available in vivo pharmacokinetic data deriving from rodent studies that can be used as an empirical aspect to PBPK model development, there remains translational questions regarding how to further our elucidation of biodistribution investigations and apply that in the clinical setting. Part of the reason for PBPK modeling is to circumvent the need for excessive animal studies, and thereby reduce the resources needed to obtain information on dosing, for example. Mechanistic PBPK modeling has presented more of a first-principles approach to modeling and simulating nanoparticle drug biodistribution. Therefore, its interspecies extrapolation to humans also requires a more mechanistic approach, compared to that of traditional allometric scaling. Allometric scaling only takes into account weight and size factors but not
fundamental biochemical mechanisms, and therefore does not offer much more than “black box” inter- and intra-species extrapolation\textsuperscript{95}. Hall \textit{et al.} propose a multiscale biological system model describing not only the fate of drugs in cells, tissues, organs, whole body, but also intra- and interspecies by scaling: hepatocytes to account for metabolic activity; mass transport area to account for mass transfer of active transport; and remaining physiological and anatomical parameters to account for biodistribution across species\textsuperscript{95}. Lin \textit{et al.} also present interspecies extrapolation by scaling physiological and endocytic parameters while keeping nanoparticle-specific parameters the same\textsuperscript{96}. While it is possible to scale endocytic parameters based on the macrophage’s occurrence within an organ tissue, their specific kinetic parameters may be experimentally determined, albeit, with a few suggestions. For example, primary cell types are preferred over immortalized cell lines, and a time-dependent study to determine when the cells are at maximum uptake rate as well as a concentration-dependent study to determine macrophage uptake kinetics\textsuperscript{96}.

CONCLUSION

This tutorial paper gives an overview of how to build a PBPK model for nanoparticle drugs using a flow diagram-decision making process which requires an understanding of the nanoparticle physico-chemical nature. The main components of a PBPK model comprises of the model structure and the pharmacokinetic mathematical framework. Both the model structure and the
mathematical framework are built based on several initial questions which are: whether the nanoparticle drugs are dissolvable or colloidal; and if so, whether the dissolved nanoparticle drug is a large or molecular hydrophilic drug. Specifically, dissolvable nanoparticles will need a release term to account for the increase in the amount of drug molecules from nanoparticle dissolution. Further, the model for dissolvable nanoparticles will either take on perfusion-limited or membrane-limited structures. Consequently, membrane-limited models do not have a permeability term since blood flow to the organs are the limiting step in the model. Since colloidal nanoparticles do not undergo dissolution, a membrane-limited model is assumed. Within the model structure, anatomical and physiological sub-compartmentalization may be applied, and thus, add further complexity to the model. The rest of the model building process comprises of looking at appropriate general and nanoparticle-specific parameters, an overview of sensitivity analysis as well as model validation. With a more streamlined approach to building a PBPK model, as synthesized in this paper, understanding and working with pharmacokinetic modeling can be enhanced.
CHAPTER 2:

SYSTEMATIC MODEL REDUCTION FOR PHYSIOLOGICALLY-BASED PHARMACOKINETICS (PBPK) MODELS USING SENSITIVITY ANALYSIS

ABSTRACT

Physiologically-Based Pharmacokinetic (PBPK) modeling is an effective predictive tool that can provide insights into colloidal nanoparticle biodistribution. Identifying and estimating physiological parameters that are pertinent to the model and understanding the impact of parameter changes on model output can assist with nanomedicine drug development, for example, in dose regimenting. However, complex systems pharmacological models such as PBPK models contain a high-dimensional parameter space, which can result in large uncertainty in parameter estimates when fitting model outputs to data. In this study, we developed a systematic model reduction method for PBPK models based on global sensitivity analysis. The reduced model is developed by identifying parameters with high sensitivity indices while not estimating the parameters with low sensitivity indices—Sobol indices having a value less than 0.02 (2%). Our results consist of: (1) global sensitivity of the full model using the Sobol method; (2) percent of initial dose versus time biodistribution profiles for the full and reduced models; and (3) parameter estimation with standard error of the means for the full and reduced models. We use Akaike’s Information Criterion
(AIC) to compare the two models. The reduced model’s AIC score is significantly lower than that of the full model, suggesting a successful model reduction effort.

INTRODUCTION

The advantages of manipulating the chemical and physical properties of nanotechnology can be exploited in the field of medicine leading to the advent of nanomedicine \(^{97,98}\). The utilization of these properties include: (1) manipulating nanocarrier type and morphology to enhance surface area-to-volume ratio improving surface recognition \(^{99,100}\); (2) functionalizing nanoparticles and nanocarriers enabling targeted delivery of cargos of drugs directly at tissues of interests \(^{101}\) thereby improving therapeutic index; and (3) taking advantage of the enhanced permeation of nanoparticles in tumor tissues, a phenomenon called the enhanced permeability and retention (EPR) effect, for the purpose of accumulating anticancer drugs at the tumor site \(^{102,103}\). These advances require elucidation of nanomedicines’ pharmacologic properties such as understanding absorption, distribution, metabolism, and excretion (ADME). Nanoparticle biodistribution cannot be inferred from conventional molecular or peptide drugs, for instance, due to the aforementioned properties of nanoparticles adding additional complexity to what happens in the body of the subject \(^{104}\). Physiologically-based pharmacokinetic (PBPK) modeling is an \textit{in silico} method of elucidating the biodistribution of drugs. Granted, PBPK modeling and simulation of non-particulate drugs have been previously accomplished \(^{105–108}\). However,
PBPK modeling of nanoparticles is newer and more challenging, in part, due to the added levels of complexity such as nanoparticle size, functionalization, surface recognition by macrophages, and potential for bio-corona formation. Recently, there has been an increase in PBPK modeling of nanoparticle delivery. For instance, Zhoumeng Lin and colleagues performed a meta-analysis study analyzing 376 datasets of tumor nanomedicines and their delivery efficiencies for more than a decade in order to identify influential factors affecting tumor delivery kinetics. There is, of course, much interest in determining what factors affect delivery of nanoparticle drugs. In this study, we use Takeuchi’s experimental dataset on gold nanoparticles biodistribution in mice which includes 4 different formulations (2 pegylated and 2 non-pegylated). We aim to develop a method to reduce complex systems pharmacologic models by (1) using a global sensitivity analysis mapping the influence of parameters on model outputs; (2) determining and sieving out non-sensitive parameters based on the global sensitivity analysis results; (3) building a reduced model by fixing and not estimating these non-sensitive parameters; and (4) validating the method by comparing Akaike’s Information Criteria (AIC) scores of both the full and reduced models. We consider that the model is successfully reduced, and model parsimony is increased, when the AIC score of the reduced model is lower than that of the full model. This can occur even if the reduced model results in a lower goodness-of-fit due to fewer estimable parameters.

RESULTS AND DISCUSSION
Parameter estimates of the full model

The estimated parameters from the full model show large values for standard error of the means. See the Associated Content Section Figure 2.7. In particular, the estimated value for the partition coefficient for each of the four formulations had errors of at least 2 times larger than the corresponding mean (Figure 2.1a). For the 20nm bare gold nanoparticle formulation, the standard error was almost 3 times larger than the mean. This large error associated with the estimated parameters prevents us from drawing insights regarding physiological changes as a result of changes in the nanoparticle formulation. However, other noticeable differences in parameter estimates, albeit containing large errors, may still help us to elucidate physiological insights due to surface modification. For example, the permeability coefficients for the lungs show markedly different values between the pegylated and non-pegylated formulations (see the Associated Content Section Figure 2.7). The increase in value for the permeability coefficient for the lungs in the full model explains the sharp decrease in nanoparticle concentration in the non-pegylated formulation as seen in the blood compartment (Figures 6c and 6d in the Associated Content).

Nevertheless, even when we look at some of the parameter estimates in the full model for all four formulations, the estimate-to-error ratios are still large where the errors of a parameter in one formulation overlap with the value of the same parameter of another formulation. One way to decrease errors in parameter estimates is to decrease the number of parameters being estimated. However, some parameters are highly sensitive such that a slight change in the input
parameter significantly alters the model output. On the other hand, some parameters are relatively insensitive, and any change in their value has minimal impact on model output. For example, we note that initial input parameters of uptake capacities, $M_{2,\text{cap}}$, on the denominator in the macrophage absorption term have values on the order of $10^8$ to $10^9$ micrograms per liter, making the ratio of $\frac{M_{2,m}}{M_{2,\text{cap}}}$ small enough whereby changing this uptake capacity parameter by several orders of magnitude does not impact the model output. Additionally, this ratio of $\frac{M_{2,m}}{M_{2,\text{cap}}}$ after parameter estimation in the full model is much smaller compared to when $M_{2,\text{cap}}$ initial parameter values were used and therefore further confirms the uptake capacities’ insensitivity. Sensitivity analysis can aid in the determination of which parameters to not estimate.
Global sensitivity analysis (GSA) using the Sobol method

1st order Sobol indices

Figure 2. 1. Heat map of 1st order Sobol indices for the full model.

Vertical labels represent model outputs which are concentrations of nanoparticles in respective compartments as well as in phagocytic cells in 4 different compartments. Horizontal labels represent input parameters for: permeability coefficients for lungs (luX), heart (hX), brain (bX), kidneys (kX), spleen (spX), lI (liver), pancreas (pX), stomach (stX), and remainder (rX); maximum absorption rates for kidneys (K.a), liver (Li.a), lungs (Lu.a), and spleen (Sp.a); uptake capacities for kidneys (K.mt), liver (Li.mt), lungs (Lu.mt), and spleen (Sp.mt); excretion forward rate constants for "kidneys à urine" ([K-U].f) and "liver à feces" ([Li-F].f); and partition coefficients.

Based on the first order Sobol indices for the full model, seven parameters have little to no influence on model output variance and are considered insensitive (1st order Sobol indices < 0.02 for all model variables). These parameters are the 4
uptake capacity values (kidneys, liver, lungs, and spleen) as well as the partition coefficients for the liver, spleen and remainder compartments. All other parameters are also considered insensitive in some cases, albeit not across all model outputs. The permeability coefficients for the brain, heart, pancreas, stomach, and remainder are insensitive with respect to all but one compartment corresponding to their respective organ. Other parameters such as the maximum absorption constant for kidneys, liver, lungs, and spleen are insensitive in all but the kidneys, liver, lungs, and spleen compartments, respectively. The forward excretion rates for the urine and feces are also insensitive in all compartments except in the urine and feces.

Parameters such as the permeability coefficient for the kidneys, spleen, and liver are sensitive in several compartments. This may be attributed to these organs being involved in the hepatic portal system where blood travels back to the liver via the portal vein instead of the venous blood compartment. Even in these cases, the parameters are straightforwardly sensitive with respect to the compartment they are connected to. However, the partition coefficient is highly sensitive for the lungs (1st order Sobol index of 0.4 and 1 for the lungs PC cells and lungs, respectively). Interestingly, the permeability coefficient for the lungs is sensitive in all compartments except in the lungs and its respective PC cell sub-compartment, which is the opposite of how the other parameters behaved.

Parameter estimates of the reduced model
The reduced model is obtained by fixing the seven insensitive parameters at their average values from the literature and then estimating the remaining parameters. The parameter estimates shown in Figure 2.1b represent the reduced model partition coefficients and their respective error bars. Notably, the estimated partition coefficients for the bare gold nanoparticles of the reduced model are approximately 10 fold that of the full model. Both the full and reduced model estimates for bare nanoparticles have partition coefficient values greater than that of pegylated nanoparticles. The rest of the parameter estimates and their error plots are shown in the Associated Content section. Generally speaking, the standard errors of parameter estimates are smaller for the reduced model. In particular, the permeability coefficients for the lungs, spleen, liver, pancreas, remainder; maximum absorption constant for the PC cells in the lungs; the urine excretion rate constant; and partition coefficient show reduced errors—or an increase in estimate-to-error ratios. While these parameters show markedly different estimate-to-error ratios between the full and reduced models, actual estimates, even in the reduced model, do not seem to be dependable for intra- or inter-species extrapolation (extrapolating from rodents to humans where assuming appropriate scaling) purposes due to their relatively high standard errors.
Comparison of temporal dynamics of both models

Starting with the blood compartments, both the full and reduced models predict biodistribution for all 4 formulations well—regarding smooth and sharp nanoparticle concentration curve declines for the pegylated and non-pegylated formulations, respectively (See Figure 2.3 and Associated Content Figure 2.6). While the results of all 4 formulations have a steep decline in the initial dose, the pegylated formulations (10nm PEG2000 and 20nm PEG5000) circulate in the blood compartment longer than their non-pegylated counterparts (20nm bare and
50nm bare). One explanation attributing to the fact that the pegylated formulations (10nm PEG2000 and 20nm PEG5000) circulate in the blood compartment longer their non-pegylated counterparts may be due to polyethylene glycol’s biocompatibility, which enables the nanoparticles to exist in a stealth mode thereby avoiding opsonization in the reticuloendothelial system. It has been demonstrated that PEGylation of PEG2000 could prolong the blood circulation time of engineered micellar nanoparticles. Additionally, PEGylation of PLGA nanoparticles results in substantial blood residence time. Residence times in the lungs are very short for all 4 formulations ($t_{1/2} < 0.5$ hr). While we allowed provisions for phagocytizing cell compartments in the lungs in our model structure, simulations show insignificant amounts of nanoparticles in the phagocytizing cells compartment. If phagocytizing cells had a significant impact on drug uptake in the lungs in mice, then experimental data would have indicated a much longer residence time. The fact that the lung compartments in all four datasets did not contain many nanoparticles relative to other organs may be attributed to these alveolar phagocytizing cells not uptaking nanoparticles efficiently. On the other hand, these alveolar phagocytizing cells would have a more significant role in uptaking nanoparticles in inhaled delivery as demonstrated using cerium oxide nanoparticles in rats by Li and colleagues. Therefore, IV injection may not be a good way to deliver nanoparticle drugs to the lungs due its low residence time as well as insignificant macrophage uptake. For both models, predictions for the heart, brain, pancreas, and stomach did not perform well (Figures 3 and 6). These organs are lowly perfused organs (due to
blood flow and low nanoparticle concentrations) which peak at <0.5 %ID for all four formulations. These peaks are low and are approximately 100x less compared to the liver, for example, a more highly perfused organ. We note that the simulated results for the pancreas in the full model show a sharp rise in gold nanoparticles with a rapid fall whereas there is a gradual increase and plateauing in the reduced model. For the heart, brain, and stomach, the sharp rise and fall shape occurs in all 4 formulations in the full model but only in the pegylated formulations in the reduced model. For the reduced model, concentrations of these 3 compartments show a gradual increase and eventual plateauing effect. However, the percent of initial dose (%ID) for maximum absorption for any of these 4 compartments range from only 0.02 to 0.2% for any given formulation. Further, Takeuchi’s experimental results’ biodistribution’s standard deviation are large for all 4 formulations. Therefore, we may not be able to depend on Takeuchi and colleagues’ empirical data for these compartments for inference of the pharmacokinetics (PK). However, simulated results do give us an idea of the pharmacokinetic behaviors for these lowly perfused compartments. Simulations are consistent with in vivo data for the spleen compartments for both pegylated formulations in Figures 4 and 5. Macrophage uptake is modeled into the spleen compartments for the models in this study. It is noted, however, that since uptake capacities were determined to be insensitive parameters using the Sobol method, these parameters were not estimated but held fixed in the reduced model. In the reduced model, macrophage uptake predictions in the spleen compartments coincided with the total amount in all but the 10nm
PEG5000 formulation. For these instances where the macrophage uptake measurement and total predicted amount aligns, we can suggest that any amount of nanoparticle that goes into the spleen is quickly taken up by phagocytizing cells. In the case of 10nmPEG 5000 in the spleen where predictions show less macrophage uptake, it is uncertain whether the higher molecular weight polyethylene glycol surface plays a role in increasing the nanoparticle’s stealthiness. Since uptake capacity is held fixed in the reduced model, macrophage uptake predictions are only controlled by the maximum uptake rate.

Regarding the predicted results for both the full and reduced models in the kidneys and their associated PC cell compartments, the kidney compartments’ maximum absorption peaks are approximately 1% ID or less for both models. Predicted results for the kidneys demonstrate examples in which nanoparticles have reached their uptake capacity swiftly. Macrophage uptake that has reached saturation levels will plateau below the total amount. Since nanoparticle desorption and cell apoptosis are not considered in the model, any amount captured by macrophages is assumed to remain in that tissue for the duration of the 48-hour simulated event. The rewards for fine-tuning parameters so that the predicted results fit better in these lowly perfused organs (ie. heart, brain, pancreas, stomach, and kidneys) are not high compared to highly perfused organs (ie. liver) where the maximum absorptions are in the double digit %IDs. Therefore, this prompts the question of whether or not it would be beneficial to fit to data in lowly perfused compartments.
Predictions for the liver fit well with *in vivo* data for the pegylated nanoparticles, which show an initial swift increase but slowdown in accumulation. For the non-pegylated nanoparticles in the liver, the simulations do not follow the trend of the *in vivo* data. The rise in nanoparticle concentration in both 10nmPEG2000 and 10nmPEG5000 formulations are met with fluctuations throughout the time course in experimental results. Unlike other peripheral organs, the liver not only receives incoming nanoparticles from the arteries, but also from the spleen and the pancreas via the hepatic portal vein. Thus, there should be an expectation of increased nanoparticle concentration due to the combined effects of receiving blood from the arteries as well as from the portal vein. However, if non-pegylated formulations are more prone to opsonization leading to higher macrophage uptake, then that would explain the observed fluctuations in the *in vivo* results. Additionally, our model does not consider the possible occurrence of a biocorona formation which may increase nanoparticle size and agglomeration. The algorithm we used to fit the data would estimate the permeability coefficient to address this. However, this is presented with the limitation that permeability coefficients are the same for entering and exiting each organ compartment.
Figure 2.3. Reduced Model output results for (a) 10nm PEG200; (b) 10nm PEG5000; (c) 20nm bare; and (d) 50nm bare gold nanoparticles.
Figure 2.3b.
Figure 2.3c.
Figure 2.3d.
Original data points were extracted using ImageJ. Solid lines represent predicted data. Dashed lines for all non-excretion plots represent predicted amounts of nanoparticles in the PC cells compartments. Dots represent observed data. For the excretion plots, solid line represents predicted values for nanoparticles in the feces and dashed lines represent predicted values for nanoparticles in the urine.

Assessment of the goodness-of-fit of both models

Akaike information criterion (AIC) scores were used to evaluate and compare the effectiveness between the full and reduced models. AIC scores take into account both the goodness-of-fit (in terms of likelihood of the data given the model) and the number of parameters. While maximizing the likelihood function fulfills the purpose of yielding parameter estimations, it is possible to over-fit a model. The full model does not restrict the optimization algorithm to only fine-tune the sensitive parameters. As a result, insensitive parameters are excessively tuned resulting in far from acceptable physiological values. Thus, model evaluation using AIC scores rewards models with a better fit while penalizing ones with a larger set of parameters.

\[
AIC = 2k - 2 \ln(L) ;
\]

Eq. 1

where k is the number of estimated parameters; and \( \ln(L) \) is the loglikelihood of the model \(^{119}\).

Initial model evaluation shows that the reduced model has a lower AIC score (see Table 2.1). We found that the reduced model had a higher loglikelihood
value than that of the full model which suggests that the full model’s parameter estimation did not arrive at the global minimum. With more parameters to estimate, the full model should have achieved a better fit. However, this full model’s lower loglikelihood could be attributed to the algorithm reaching some stopping criterion before it found a maximum greater than that of the reduced model. If the calculated log-likelihood of the reduced model is lower than that of the full model, then in order to assert that the reduced model is a more parsimonious model, the reduced model needs to have a smaller AIC score. However, since our reduced model already has a better goodness-of-fit, and we know that the solution is nested within the full model by way of utilizing the same parameter boundary constraints, we can assert that the reduced model is a more parsimonious model based on the fact that less parameters (16) are being estimated.

Nevertheless, to ensure that the reduced model is nested within the full model, we updated the full model with the estimated parameters from the reduced model using the same upper and lower parameter boundaries. Our results show that the loglikelihood of both models were the same confirming our assertion that global optimization solutions for the reduced model is contained within the full model’s solutions.

AIC scores are typically presented by the difference between a model’s score and the lowest model’s score ($\Delta AIC$), therefore giving the lowest $\Delta AIC = 0$. It is possible to show that there is an even bigger AIC score gap between the full and reduced model by looking at the corrected AIC scores (AICc). With smaller
datasets, the AIC will select models with more parameters that overfit the observed values. Even though our reduced model already has a smaller AIC score, we can further show an even greater preference for the reduced model using 120:

\[
AICc = AIC + \frac{2k^2 + 2k}{n - k - 1};
\]

Eq. 2

where \( n \) is the sample size (number of data points). Notice that as \( n \) approaches infinity, AICc approaches AIC. Table 2.1 summarizes our model evaluation scores. In both AIC and AICc analysis, the reduced model is the selected one. However, notice \( \Delta AICc \) is greater than \( \Delta AIC \) by almost 2 fold indicating that the sample size (\( n \)) was not large enough to have made the corrected term negligible.

<table>
<thead>
<tr>
<th></th>
<th>FULL MODEL with starting parameters from literature</th>
<th>FULL MODEL with starting parameters from reduced model</th>
<th>REDUCED MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC (( \Delta AIC ))</td>
<td>2099 (41)</td>
<td>2072 (14)</td>
<td>2058 (0)</td>
</tr>
<tr>
<td>AICc (( \Delta AIC ))</td>
<td>2114 (50)</td>
<td>2087 (23)</td>
<td>2064 (0)</td>
</tr>
<tr>
<td># of parameters (k)</td>
<td>23</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>LogLikelihood</td>
<td>-1027</td>
<td>-1013</td>
<td>-1013</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>9 x 11 = 99</td>
<td>9 x 11 = 99</td>
<td>9 x 11 = 99</td>
</tr>
</tbody>
</table>

Table 2.1 Model evaluation values showing AIC, AICc, # of parameters, loglikelihood, and sample size of the full and reduced models. Values were rounded to the nearest integer.
Both models have the same initial constraints for parameters. Sample size \( (n) = 99 \) due to the 11 compartments being estimated, each with 9 time points. \( \Delta AIC \) represents the difference between AIC score for the model of interest and the model with the lowest AIC score.

Note: the initial loglikelihood value for the full model was lower than that of the reduced model. This is opposite of what was expected due to the full model having more estimable parameters. Since the parameter space of the reduced model is nested within the full model, we used estimated parameter values from the reduced model in the full model and confirmed that the full and reduced models have the same loglikelihood.

CONCLUSION

In complex systems pharmacology models, the challenge is not only to understand the underlying pharmacokinetic behaviors of nanoparticles, but also to estimate parameters involved in these models in order to allow analyses that would lead to the elucidation of these behaviors. Thus, less complex models are preferred and gaining popularity in order to reduce parameter estimation issues. One issue arising from the parameter estimation of complex models is the possibility of multiple local optima which make it difficult to find a global optimum causing large uncertainty in the estimated parameters. Even if estimated parameters between different formulations have stark contrasts, their standard error of the means overlap each other making it difficult to draw conclusions when analyzing changes in parameter values. Therefore, no strong assertions for relationships could be formed in these instances.

From a PK perspective, it is difficult to ascertain physiological events by looking at experimental data alone—especially when dealing with lowly perfused organs. Therefore, one might say that one of the hopes of simulated results is the
elucidation of PK activities in these organs. However, as stated earlier, high uncertainties of estimated parameters do not give us the confidence needed for comparison and analysis. Research questions such as what happens to the permeability coefficients for the liver between pegylated and non-pegylated, and between 25 and 50nm nanoparticles, cannot be answered with large uncertainties in parameter estimates. Therefore, mere use of PBPK modeling in order to predict biodistribution is insufficient to elucidate PK insights such as the role of PEGylation on physiological processes regarding macrophage uptake, for example.
This motivated us to attempt to reduce the model by circumventing the need to estimate insensitive parameters. Our effort to lower the standard errors of parameter estimates was successful. Also, parameter estimate-to-error ratios generally increased in the reduced model indicating a general trend of reduced uncertainty in the estimation process. Therefore, our approach in the model reduction of PBPK modeling for intravenous colloidal gold nanoparticles in mice is a feasible approach when it comes to decreasing the AIC score and increasing parameter estimate-to-error ratios. From our studies, we have shown that model reduction through global sensitivity analysis is useful, for example, due to fact that simulated results for the full and reduced models would result in different PK inferences in lowly perfused organs as well as PC cells.
Our investigation has used global sensitivity analysis as a means of sieving out insensitive parameters in order to reduce the model, which is validated by an improved AIC score. While we hope to use this approach in future PBPK model
reduction studies, it is noted that there are still some challenges even when we observe smaller uncertainties in parameter estimations in the reduced model. High-dimensional differential equations systems in PBPK modeling with large parameter spaces (even in the reduced model the number of estimated parameters (16) is still high) may require additional reduction processes. In order to further reduce the model, however, a more mechanistic approach to holding parameters constant or nullifying the need to fit simulation results for certain compartments could be applied. This could eventually lead to elucidation of the effects of changing nanoparticle formulations, for example, through PEGylation, on macrophage uptake. Additionally, during sensitivity analysis, we considered the overall Sobol indices peak to obtain a bird’s eye view of the non-sensitive parameters. However, there may still be valuable information, especially when applied to dose regimenting, if we examined the changes in sensitivities temporally. For example, what happens to Sobol indices for each of the parameters at a certain time point compared to another time point? The answer to this question might prove to be useful in enabling rational design of drugs as well as drug administering strategies. In the meantime, our model reduction method for PBPK modeling of colloidal gold nanoparticles applied in this paper can provide systems pharmacologists and toxicologists a way to improve model parsimony, systematically.
METHODS

Empirical data

The empirical data source came from Takeuchi and colleagues consisting of 4 datasets with 2 from bare gold nanoparticles and 2 from pegylated gold nanoparticles. PEGylation refers to the coating of nanoparticles with polyethylene glycol, a biocompatible polymer known to elicit less response from the reticuloendothelial system (RES). Table 2.5 from the Associated Content Section lists measured nanoparticle diameters for each of the 4 formulations adapted from the Takeuchi study. While the original empirical data were presented as concentration [\( \mu g \frac{\text{nanoparticle}}{g \text{ tissue}} \)] versus time [hr] biodistribution plots, the simulation, overlayed with the original empirical data have been converted to percent of initial dose [%ID] in the y-axis, in order to easily compare to the relative amounts of nanoparticles in each of the organ compartments.

While Takeuchi disclosed biodistribution plots showing amounts of nanoparticles in the blood as well as the solution volume used per body weight, the publication did not disclose the actual injected dosage of nanoparticle concentration or weight. The y-intercept of our non-linear curve fit for the blood compartments produced different results for the 4 different formulations. The highest value predicted was used to prevent underestimating. Further, since the initial decay for the ‘20nm bare’ and ‘50nm bare’ formulation concentration-time curves were
much sharper than their pegylated counterparts and the first recorded time was at 30 minutes—which is relatively short compared to the 48-hour time range, it is possible that a decision to use predicted initial dosages from either one of these 2 concentration-time curves would result in an underestimation.

Initial parameters

Organ-specific parameters such as anatomical organ volumes and physiological parameters such as blood flows were obtained from the PK-Sim® 8 database, an open source software within the Open Systems Pharmacology Suite 122. Nanoparticle-specific parameters such as partition coefficients, permeability coefficients, maximum uptake rates, and uptake capacities were obtained from the literature 32,77 (see Table 2.3). The organ volumes were calculated by PK-Sim® 8 by using predetermined values for percent of body weight of a typical 20g mouse.
<table>
<thead>
<tr>
<th>Organ specific parameters</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ volumes based on % of body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>liter</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart</td>
<td>liter</td>
<td>9.5E-5</td>
</tr>
<tr>
<td>Brain</td>
<td>liter</td>
<td>0.00017</td>
</tr>
<tr>
<td>Spleen</td>
<td>liter</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kidneys</td>
<td>liter</td>
<td>0.00034</td>
</tr>
<tr>
<td>Liver</td>
<td>liter</td>
<td>0.0013</td>
</tr>
<tr>
<td>Pancreas</td>
<td>liter</td>
<td>0.00013</td>
</tr>
<tr>
<td>Stomach</td>
<td>liter</td>
<td>0.00011</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>liter</td>
<td>0.000228182</td>
</tr>
<tr>
<td>Venous blood</td>
<td>liter</td>
<td>0.000524818</td>
</tr>
<tr>
<td>Blood flow&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>l/min</td>
<td>5.47E-3</td>
</tr>
<tr>
<td>Heart</td>
<td>l/min</td>
<td>2.80E-4</td>
</tr>
<tr>
<td>Brain</td>
<td>l/min</td>
<td>1.30E-4</td>
</tr>
<tr>
<td>Spleen</td>
<td>l/min</td>
<td>9.00E-5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>l/min</td>
<td>1.30E-3</td>
</tr>
<tr>
<td>Liver</td>
<td>l/min</td>
<td>3.50E-4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>l/min</td>
<td>5.20E-5</td>
</tr>
<tr>
<td>Stomach</td>
<td>l/min</td>
<td>1.10E-4</td>
</tr>
<tr>
<td>Portal vein</td>
<td>l/min</td>
<td>1.75E-3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Can also be calculated by taking the percentage of the weight of a mouse in (g) to give the organ volume in (mL). Other sources in the literature include<sup>28,33,74–76</sup>.  
<sup>b</sup>Based on values obtained by PK-Sim® 8 database for mouse.
Table 2. 3. Nanoparticle specific parameters

<table>
<thead>
<tr>
<th>Nanoparticle specific parameter units</th>
<th>Description</th>
<th>Lungs</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Pancreas</th>
<th>Brain</th>
<th>Stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partition (distribution coefficient)</td>
<td>0.1</td>
<td>0.15</td>
<td>0.0</td>
<td>0.15</td>
<td>0.15</td>
<td>0.1</td>
<td>0.15</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Permeability coefficient between blood and tissue</td>
<td>0.009</td>
<td>0.001</td>
<td>0.03</td>
<td>0.0000</td>
<td>0.0</td>
<td>0.000</td>
<td>0.001</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Max uptake rate constant for PC</td>
<td>2.009</td>
<td>0.11</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
</tr>
<tr>
<td>PC release (desorption) rate constant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excretion rate constant</td>
<td>N/A</td>
<td>N/A</td>
<td>1.1</td>
<td>6.56×10⁻³</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Uptake capacity</td>
<td>1.4</td>
<td>N/A</td>
<td>7.0</td>
<td>9.524</td>
<td>7.97</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

According to Li and other sources in the literature, arterial and venous blood take up 20% and 80% of the total body blood, respectively.\(^2\)\(^3\)\(^4\)\(^5\)

\(^a\)Taken from table in\(^3\), which also come from other sources. Source provides data for liver, spleen, kidneys, lungs, brain, and rest of the body. Therefore, any organ compartment not directly provided by source, rest of the body values are used.

\(^b\)Values obtained from\(^7\). Some assumptions were made since these values were used for rats under different colloidal nanoparticles. Generic values are equal to 16.1±0.306
for absorption and $4.90 \times 10^{-19} \pm 7.26 \times 10^{-17}$ for desorption.

*Values obtained from 77.

1Original values reported in micrograms of nanoparticles per gram of tissue 77.

Model Description

Setup

Model structure and simulation were built in Matlab® academic version R2020a using the corresponding SimBiology application.

Table 2. 4. Parameter estimation specifications.

<table>
<thead>
<tr>
<th></th>
<th>Full model</th>
<th>Reduced model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total compartments</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Blood compartment</td>
<td>venous and arterial</td>
<td>venous and arterial</td>
</tr>
<tr>
<td>Total parameters estimated</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>Partition coefficients</td>
<td>general, liver, spleen, remainder (4)</td>
<td>general (1)</td>
</tr>
<tr>
<td>Permeability coefficients</td>
<td>1 for every organ (9)</td>
<td>1 for every organ (9)</td>
</tr>
<tr>
<td>Max uptake</td>
<td>Lu, Ki, Sp, Li (4)</td>
<td>Lu, Ki, Sp, Li (4)</td>
</tr>
<tr>
<td>Uptake capacity</td>
<td>4</td>
<td>0 (did not estimate but included)</td>
</tr>
<tr>
<td>Estimation method (global solver)</td>
<td>Scatter search</td>
<td>Scatter search</td>
</tr>
<tr>
<td>local solver</td>
<td>lsqnonlin</td>
<td>lsqnonlin</td>
</tr>
</tbody>
</table>

In the parameter estimation for both models, the same constraint limits, optimization methods, ode local solvers, step tolerance, function tolerance, and optimality tolerance were used. The lower parameter estimation constraints were
set at 0. For permeability coefficients, the upper constraints were set at 1 representing maximum permeation. For other parameters, the upper constraints were set at $1 \times 10^6$ fold higher than initial values. Step tolerance was set at $1.00 \times 10^{-8}$, function tolerance at $1.00 \times 10^{-8}$, and optimality tolerance at $1 \times 10^{-6}$. 
Figure 2.4. Multicompartment PBPK model structure for colloidal gold nanoparticles in mice.
Blue arrows represent direction of blood flow to and from the venous compartment. Red arrows represent direction of blood flow to and from the arterial compartment. Black arrows flow between capillary and tissue compartments. Arrows going to phagocytizing cell (PC) compartments are assumed irreversible.

The multicompartment PBPK model in Figure 2.4 was designed to include both the venous and arterial compartments. While some other models include only one blood compartment accounting for both the venous and arterial compartments [11], [12], this makes the assumption that the latent time associated with nanoparticles traveling from the venous compartment to the lungs and then to the arterial compartment is brief enough to not have a negative effect on the simulated results on the lungs. Our preliminary attempt showed that the predicted values did not fit well with the observed values in the lung compartment when both the arterial and venous compartments were combined. This is attributed to the fact that we no longer treated the lungs as the first organs to receive the nanoparticles after intravenous injection. Therefore, we used a 2-blood compartment model structure instead. Phagocytic cells (PC) compartments were in placed in the lungs, kidneys, spleen, and liver. These sub-compartments will contribute to the simulated accumulation of nanoparticles in these organs.

While it is possible to place PCs in all of the compartments, these 4 organs have the greatest number of endocytic cells. We did not account for monocytes circulating in the blood with the assumption that blood flow would have caused nanoparticles to travel to a tissue compartment before these phagocytic white blood cells would engulf any noticeable amount of nanoparticles. While all the compartment sizes could be obtained from existing database or literature, feces
and urine compartments act as a “sink” rather than tissues directly contributing to the volume of distribution. Therefore, their sizes were arbitrarily assumed to be 0.1 mL putting them in the same order of magnitude as most of the other organs. Ultimately, all nanoparticle concentrations in biodistribution plots were presented as %ID, in part, to circumvent the need to show nanoparticle concentrations in compartments in which their sizes were arbitrarily assigned.

The permeability coefficients with respect to each of the compartments are assumed to be the same for nanoparticles both entering and exiting a tissue compartment. In other words, even if there were differences in the membranes at both terminals, the effects on nanoparticle transport are assumed to be negligible by virtue of the same tissue medium at the interface. This assumption may further be investigated in studies where the effects of biocorona formation may possibly be great enough to influence the outcome of biodistribution, even at intra-compartment.

Equations

Derivation of the distribution equations containing nanoparticle distribution, permeability, and phagocytizing cell components have been demonstrated by Li et al. 52. A list of all the ordinary differential equations used in this model may be obtained from the supplementary material. Briefly, a distribution equation used in a typical compartment without macrophage uptake is as follows:
\[
\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X}
\]

Eq. 3

where \( M_2 \) is the nanoparticle amount of compartment 2 which is the compartment of interest; \( Q \) is the blood flow; \( C_1 \) and \( C_2 \) nanoparticle \textit{concentrations} of compartments 1 and 2, respectively; \( k_p \) is the partition coefficient; and \( X \) is the permeability coefficient. An example flow diagram illustrating nanoparticles traveling from the arterial compartment towards a peripheral tissue compartment utilizing the above distribution equation is seen in Figure 2.5:

![Flow diagram](image)

Figure 2. 5. A flow diagram illustrating nanoparticles traveling from arterial blood towards a peripheral tissue.

Note that the term \( \frac{X}{1 + X} \) from equation 1 shows up twice in this flow diagram indicating the application of permeability coefficient for nanoparticles entering as well as leaving the tissues.

The distribution equation is the product of the permeability term, the difference in nanoparticle concentrations between the 2 compartments, and the blood flow.

The reason for the use of the permeability term \( \frac{X}{1 + X} \) and not the just the permeability coefficient \( X \) by itself is a result of the derivation process where the capillary compartment is now considered a quasi-compartment due to the
assumption that the time nanoparticles remain in the capillaries are negligible. This derivation and assumption have been made by Li and colleagues. Because our model adopts a membrane-limited model (where permeation of nanoparticles across membranes is considered rate-limiting), the permeability component of the distribution equation is used. For compartments that contain phagocytizing sub-compartments, nanoparticle-specific absorption and desorption components are added to the distribution equation:

\[
\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X} - \left[ \left( V_2 \times C_2 \times k_{ab} \right) \left( 1 - \frac{M_{2,m}}{M_{2,\text{cap}}} \right) \right] \left( k_{de} \times M_{2,m} \right)
\]

Eq. 4

where \( k_{ab} \) [per minute] is the maximum uptake rate constant; \( k_{de} \) [per minute] is the desorption rate constant; \( V_2 \) [liter] is volume of compartment 2; \( M_{2,m} \) is the nanoparticle amount within PC sub-compartment of compartment 2; and \( M_{2,\text{cap}} \) is the PC uptake capacity of compartment 2. The desorption rate for these colloidal nanoparticles have been reported to be much lower than the absorption rates. It may be possible that the desorption rate constants reflect the notion that some nanoparticles are detached from the cell membrane before they are even internalized rather than reflecting the actual process of macrophages releasing nanoparticles after internalization. Wilhelm et al. performed kinetic analyses and modelized uptake kinetics on the interactions of the membranes of HeLa tumor cells and mouse RAW macrophages on superparamagnetic nanoparticles by...
accounting for adsorption, desorption, and internalization. Their study utilizes a Langmuir adsorption to describe the interactions prior to internalization of nanoparticles into cells but does not include mathematical expressions for any possible release of nanoparticles once they have internalized. While our mathematical framework of macrophage uptake does account for nanoparticle desorption, we have taken the desorption term out of the model structure during calculation to simplify the model. The absorption term in our model represents the combined effect of surface adsorption and desorption.

For excretory compartments, a term for renal or biliary excretion is used

$$\frac{dM_2}{dt} = Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X} - \frac{dM_{ex}}{dt}$$

Eq. 5

$$\frac{dM_{ex}}{dt} = (M_l \times CL_b + M_k \times CL_r)$$

Eq. 6

where $M_{ex}$ is the amount of nanoparticles either in the liver tissue or the kidney's capillary blood; $CL_b$ [per minute] is the clearance to feces from the liver tissue; and $CL_r$ [per minute] is the clearance to urine from the kidney's capillary blood.

Parameter estimation

We employed Matlab’s ode15s to numerically integrate our high-dimensional system of differential equations. The numerical output was fit to the observed data points using maximum likelihood estimation of the model.
parameters. For the optimization process, we used a SimBiology® built-in scatter search global optimization algorithm which selects a subset of points from a pool of points. The search then evaluates each point and iteratively explores different directions to find better solutions which then replaces the old solution with a better one. Specific iteration steps may be found at the MathWorks® help center on sbiofit. Once the global optimization reaches a stopping criterion (convergence of the likelihood function or max iterations reached), it then runs a local optimization process which utilizes a hybrid gradient descent and Gauss-Newton method, Levenberg-Marquardt algorithm (LM). The LM algorithm updates the parameters when it is nowhere near the local minimum (in the steep region) but uses a Gauss-Newton method to reduce the sum of squared errors to find the minimum.

Global sensitivity analysis

For GSA, we used the Sobol method to determine the contribution of an input parameter or its interaction with another parameter to the output model variance. It does this by decomposing the model output variance into individual input parameter variances. The input parameter summands of variances consist of terms of increasing dimensionality to account for not only a parameter’s influence, but also its interaction with other parameters. Briefly, the fraction of influence parameter $X_i$ has on model output, or the first-order Sobol sensitivity index is defined as:
\[ S_i = \frac{V_{X_i}(E_{X_{-i}}(Y|X_i))}{V(Y)}; \]

Eq. 7

where \( X_i \) is the \( i^{th} \) factor and \( X_{-i} \), the matrix of all factors but \( i^{th} \). \( V(Y) \) is the total variance. \( V_{X_i}(E_{X_{-i}}(Y|X_i)) \) is the expected value of the reduction in model variance if \( X_i \) is fixed where \( Y \) is the model output. For example, a parameter's first-order Sobol index of 0.9 indicates that 90% of the output variance is caused by variation in that particular input parameter. The total index, which includes first-order and all other parameter interactions is defined as:

\[ S_{Ti} = \frac{E_{X_{-i}}(V_{X_i}(Y|X_{-i}))}{V(Y)}; \]

Eq. 8

where \( E_{X_{-i}}(V_{X_i}(Y|X_{-i})) \) is the expected variance left if all but factor \( i^{th} \) is fixed. It is customary to report values of first-order and total-order indices due to Sobol indices being computationally expensive. These two values should provide us with enough information on whether an input parameter is sensitive as well as all other interactions associated with. Any residual amount \( S_{Ti} - S_i \) equates to the interaction contribution (or lack thereof). Only 1\(^{st}\) order indices are presented in this paper since there are negligible differences between the total and 1\(^{st}\) order indices. While these values are different at different time points, the peak Sobol indices were chosen and plotted. Even though peak values do not provide us with information on how sensitive each parameter is throughout the course of drug biodistribution, they provide guidance on which parameters are the least sensitive overall.
Initial simulation parameter values were used with 10% upper and lower bounds. Sobol indices were calculated using `sbiosobol` on Matlab® using the method described in Saltelli 133.

Parameter Sieving

By performing GSA using Sobol indices, we can compare the percentages of contribution of influence a variation in one parameter value has, as well as the parameter’s interaction with other parameters and their influence on the model output. The results of this GSA matrix allow us to sieve out parameters that are relatively insensitive. These parameters have very little effect on output values when they are varied. Sieving out these insensitive parameters does not mean that we exclude them in the model structure. Rather, they are kept fixed at values obtained from various literature sources and are not involved in the parameter estimation process. We hypothesize that this would help to reduce the margins of error for parameter estimation. For instance, if a parameter could be varied multiple-fold without much affecting the output value and ultimately the goodness of fit, then this presents a parameter identification problem during parameter estimation.

While there does not seem to be any guidance on what index value is considered insensitive for the purpose of holding parameters constant, in order to sieve out insensitive parameters, the Sobol indices cut-off value was arbitrarily set at 2%. As a result of the sensitivity analysis, seven different parameters were held...
constant after parameter sieving allowing 16 parameters to be estimated in the reduced model compared to that of 23 in the full model. The calculated log-likelihood of the reduced model was higher than the full model. This was not aligned with our expectation by virtue of the fact that the reduced model had less parameters to tune to obtain an optimized fit. However, due to the large parameter space, it is possible that the simulation had reached a stopping criterion before a global optimum was found. Both the AIC and corrected AIC scores for the reduced model were lower than that of the full model. To ensure that a lower AIC score could be achieved based on rewarding more parsimonious models even at the expense of goodness-of-fit, we used the estimated parameter set of the reduced model as the initial starting parameter set in the full model.

ASSOCIATED CONTENT

Equations

Blood compartments

Venous:
\[
\frac{dC_{\text{ven}}}{dt} = \frac{1}{V_{\text{ven}}} \left[ rX \cdot Q_r \cdot (C_r/K_p) + stX \cdot Q_{st} \cdot (C_{st}/K_p) + liX \cdot Q_{li} \cdot (C_{li}/K_p) \right. \\
+ kX \cdot Q_{li} \cdot (C_k/K_p) + bX \cdot Q_b \cdot (C_b/K_p) + hX \cdot Q_h \cdot (C_h/K_p) \\
\left. - luX \cdot Q_{lu} \cdot (C_{ven}) \right]
\]

Arterial:

\[
\frac{dC_{\text{art}}}{dt} = \frac{1}{V_{\text{art}}} \left[ luX \cdot Q_{lu} \cdot (C_{lu}/K_p) \right. \\
- \left. rX \cdot Q_r \cdot (C_r) - pX \cdot Q_p \cdot (C_p) \right. \\
- \left. [stX \cdot Q_{st} \cdot (C_{st})] - [liX \cdot Q_{li} \cdot (C_{li})] - [spX \cdot Q_{sp} \cdot (C_{sp})] \right. \\
- \left. kX \cdot Q_{li} \cdot (C_k) - [bX \cdot Q_b \cdot (C_b)] - [hX \cdot Q_h \cdot (C_h)] \right]
\]

Non-peripheral compartment

Lungs:

\[
\frac{dC_{\text{lu}}}{dt} = \frac{1}{V_{\text{lu}}} \left[ luX \cdot Q_{lu} \cdot (C_{ven}) - luX \cdot Q_{lu} \cdot (C_{lu}/K_p) \right. \\
\left. - \left( V_{\text{lu}} \cdot C_{lu} \cdot a_{lu} \left( 1 - \frac{C_{lu,m}}{m_t} \right) \right) \right]
\]

Lung PC cell compartment:
\[ \frac{dC_{tu,m}}{dt} = \frac{1}{V_{tu}} \left[ (V_{tu} \cdot C_{tu} \cdot a_{tu}) \left( 1 - \frac{C_{tu,m}}{m_t} \right) \right] \]

Peripheral compartments

Heart:
\[ \frac{dC_h}{dt} = \frac{1}{V_h} \left[ hX \cdot Q_h \cdot C_{art} - \left( hX \cdot Q_x \cdot \left( \frac{C_h}{K_p} \right) \right) \right] \]

Brain:
\[ \frac{dC_b}{dt} = \frac{1}{V_b} \left[ bX \cdot Q_b \cdot C_{art} - \left( bX \cdot Q_b \cdot \frac{C_b}{K_p} \right) \right] \]

Spleen:
\[ \frac{dC_s}{dt} = \frac{1}{V_s} \left[ spX \cdot Q_s \cdot C_{art} - \left( V_s \cdot C_s \cdot a_s \left( 1 - \frac{C_{s,m}}{m_t} \right) \right) - \left[ liX \cdot spX \cdot Q_{pv} \cdot \frac{C_s}{K_p} \right] \right] \]

Spleen PC cells:
\[ \frac{dC_{s,m}}{dt} = \frac{1}{V_s} \left[ (V_s \cdot C_s \cdot a_s) \left( 1 - \frac{C_{s,m}}{m_t} \right) \right] \]

Kidneys:
\[ \frac{dC_k}{dt} = \frac{1}{V_k} \left[ kX \cdot Q_k \cdot C_{art} - \left( V_k \cdot C_k \cdot a_k \left( 1 - \frac{C_{k,m}}{m_t} \right) \right) - \left[ kX \cdot Q_k \cdot \frac{C_k}{K_p} \right] - f \cdot C_k \right] \]
Kidney PC cells:
\[
\frac{dC_{k,m}}{dt} = \frac{1}{V_k} \left( V_k \cdot C_k \cdot a_k \left( 1 - \frac{C_{k,m}}{m_t} \right) \right)
\]

Liver:
\[
\frac{dC_{li}}{dt} = \frac{1}{V_{li}} \left[ liX \cdot Q_{li} \cdot C_{art} - \left( V_{li} \cdot C_{li} \cdot a_{li} \right) \left( 1 - \frac{C_{li,m}}{m_t} \right) \right] - \left[ liX \cdot Q_{li} \cdot C_{li} / K_p \right]
\]
\[
+ \left[ liX \cdot px \cdot Q_{pv} \cdot C_p / K_p \right] + \left[ liX \cdot spX \cdot Q_{pv} \cdot C_s / K_p \right] - f \cdot C_{li}
\]

Liver PC cells:
\[
\frac{dC_{li,m}}{dt} = \frac{1}{V_{li}} \left( V_{li} \cdot C_{li} \cdot a_{li} \left( 1 - \frac{C_{li,m}}{m_t} \right) \right)
\]

Stomach:
\[
\frac{dC_{st}}{dt} = \frac{1}{V_{st}} \left[ stX \cdot Q_{st} \cdot C_{art} - \left( stX \cdot Q_{st} \cdot C_{st} / K_p \right) \right]
\]

Pancreas:
\[
\frac{dC_p}{dt} = \frac{1}{V_p} \left[ px \cdot Q_p \cdot C_{art} - \left( liX \cdot px \cdot Q_{pv} \cdot C_p / K_p \right) \right]
\]

Remainder:
\[
\frac{dC_r}{dt} = \frac{1}{V_r} \left[ rX \cdot Q_r \cdot C_{art} - \left( rX \cdot Q_r \cdot C_r / K_p \right) \right]
\]
Excretory compartments

Feces:

\[ \frac{dC_{fe}}{dt} = \frac{1}{V_{fe}} [f \cdot C_{lt}] \]

Urine:

\[ \frac{dC_{ur}}{dt} = \frac{1}{V_{ur}} [f \cdot C_k] \]

Summary of formulations for the experimental dataset obtained from Takeuchi et al. 

<table>
<thead>
<tr>
<th>Gold nanoparticle Formulation</th>
<th>Diameter [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10nm PEG2000</td>
<td>28.5 +/- 8.4</td>
</tr>
<tr>
<td>10nm PEG5000</td>
<td>50.9 +/- 18.7</td>
</tr>
<tr>
<td>20nm bare</td>
<td>21.9 +/- 5.9</td>
</tr>
<tr>
<td>50nm bare</td>
<td>53.9 +/- 15.7</td>
</tr>
</tbody>
</table>

Table 2. 5 Mean volume nanoparticle diameter of each of the formulations from the Takeuchi study.

Not listed here are the polydispersity index (PDI) and zeta potential (ZP) which are measures of size distribution and surface charges, respectively.

Model output for full model

The lack of standard deviation in some plots is due to that fact that we do not have the actual backend datasets obtained from Takeuchi and colleagues and
relied on manually digitizing the data points. Some of the standard deviation bars were not visually differentiable from data of other formulations that were overlayed on the plots. Also, if the standard deviation is too small to recognize visually, then it was not was recorded.
Figure 2.6a. 10nm PEG2000
Figure 2.6b. 10nm PEG5000
Figure 2.6c. 20nm bare
Figure 2.6d. 50nm bare
Original data points were extracted using ImageJ. Solid lines represent predicted data. Dashed lines for all non-excretion plots represent predicted amounts of nanoparticles in the PC cells compartments. Dots represent observed data. For the excretion plots, solid line represents predicted values for nanoparticles in the feces and dashed lines represent predicted values for nanoparticles in the urine.
Parameter Estimates

Figures 2.7-2.8. show parameter estimations for simulations for all 4 different formulations (10nm PEG2000, 20nm PEG5000, 20nm bare, and 50nm bare colloidal gold nanoparticles) with empirical data originally obtained from Takeuchi 6.
Figure 2. 7. Parameter estimates and uncertainties for all four different formulations for the full model.
Figure 2.8. Parameter estimates and uncertainties for all four different formulations for the reduced model.
CHAPTER 3:

TIME-COURSE SENSITIVITIES TO OVERCOME PARAMETER ESTIMATION UNCERTAINTIES

ABSTRACT

Physiologically-based Pharmacokinetic (PBPK) models for nanoparticles often contain a high-dimensional parameter space, which can lead to challenges in estimating parameters without large uncertainties. This hinders our ability to use models to effectively analyze and elucidate physiological patterns regarding nanoparticle formulation and characteristics. In this study, we used previously published experimental biodistribution data for four different nanoparticle formulations to investigate parameter sensitivities. We performed time-course sensitivities using the Sobol method to uncover which parameters are sensitive and which are insensitive, and whether their sensitivities are temporally dependent. By examining the time-course sensitivities for all estimable parameters, we were able to infer on drug delivery strategies. Our results indicated that nanoparticle uptake capacity rates were insensitive for all four formulations for all compartments. Permeability coefficients for the pegylated nanoparticles were found to be temporally dependent in the venous blood compartment. These results provided us with the ability to rationalize drug delivery strategies, for example, co-administering a drug locally to enhance lung permeation to increase blood circulation times for pegylated nanoparticles.
INTRODUCTION

Physiologically-based Pharmacokinetic (PBPK) modeling can be used as a priori and in silico tool to predict the biodistribution of nanoparticle drugs. While the pharmacokinetics of drugs in general can be understood by obtaining experimental results, the resources needed to perform these studies are extensive. For example, a pharmacokinetic evaluation and optimal dosing regimen for antibiotics development can be cost-intensive. Furthermore, the lack of previous human studies data combined with its ethical implications to obtain new ones, increase our interest in mathematical modeling to better understand drug pharmacokinetic profiles. Therefore, it is not surprising that many have pursued mathematical modeling and simulation of newly developed nanomedicines and materials.

Since nanotechnology may be used to carry drugs to their destinations, and their pharmacokinetics may be altered depending on physical characteristics, it follows that the advantages of coupling nanoparticles with an active pharmaceutical ingredient can be of great benefit in the field of drug delivery, especially for drugs with unfavorable absorption, distribution, metabolism, and excretion (ADME) profiles.

However, mathematical models such as the likes of PBPK are multi-compartmental and involve complex sets of ordinary differential equations. When working with models involving new drugs or nanoparticle formulations, we cannot fully rely on published drug/nanoparticle parameter values and therefore will need to estimate parameters by fitting model output to experimental data.
Estimation issues can thus arise due to the high-dimensional parameter space associated with PBPK models. Each nanoparticle formulation requires estimation of a new set of parameters to take into account the differences in particle surface, morphology, size, and any functionalization. A manipulation causing a change in one or more of these characteristics may illicit a different innate immune response leading to different biomedical applications. Furthermore, changes in their physical characteristics will also affect their general absorption, distribution, metabolism, and excretion (ADME). A problem still remains and that is the ability to accurately estimate nanoparticle-specific parameters for use in future a priori studies and drug regimenting.

In this investigation, we estimate parameters by fitting temporal model output to time series data. The uncertainty in parameter estimates results from a high-dimensional parameter space and wide bounds on possible parameter values, leading to a complex objective function with multiple local minima. The uncertainties further pose issues in analysis making it difficult to investigate how certain physiological or nanoparticle changes can alter parameters. While there is a lack in physiological meaning that comes with parameter estimation issues, we show in this paper that performing time-course sensitivity analysis on our model for all formulations can provide more insights on the importance of various parameters at different times leading to the discovery of formulation-dependent relationships and possible rationale for drug target intervention.
Methods

Empirical Data Source

Our empirical data source was obtained from Takeuchi et al. with biodistribution data for four different nanoparticle formulations \(^{115}\). The naming convention relates to the authors’ intended sizes and compositions of each of the formulations rather than the actual measured size and surface characteristics. The sizes for each of the formulations are summarized in the supplementary material section (Table 3.3). All of the initial nanoparticle concentration units in micrograms of gold (Au) nanoparticle per gram of tissue have been converted to percent of initial dose [%ID] in order to facilitate comparisons between different organ compartments. The authors did not provide specific dosage amounts in terms of how much nanoparticles were initially injected. Therefore, we determined the initial dose based on performing curve fitting of the blood compartment and calculating its y-intercept. We chose the 10nm PEG2000 formulation due to it having the highest r-squared values as well as not having a sharp fall in concentration compared to its non-pegylated counterparts. As a result, our determined initial dose was 206.8 µg of Au injected.

In order to obtain the data from the Takeuchi source, we determined each of the data points in the biodistribution plots using an image processing software, ImageJ, a public domain software produced by the National Institutes of Health that is not subject to copyright protection.
Figure 3.1. Multi-compartmental model structure showing the 7 different compartments used in this paper. (Urine and feces not counted as compartments).

The “remainder” compartment is not fitted to experimental data points. It accounts for a number of different compartments such as bones, muscles, and peripheral organs not included in the model structure. Here, blood travels from the spleen into the liver via the hepatic portal vein instead of directly returning to the venous compartment.

Our 7-compartment model is not fit to data from the heart, brain, pancreas, or stomach because the experimental data for %ID in each of these organ compartments do not surpass 0.2% with large standard deviations, suggesting that the measured nanoparticle concentrations cannot necessarily be relied on to indicate the pharmacokinetics of the particles in these compartments.
Unlike some PBPK models that use only a single blood compartment, our model takes into account both the venous and arterial compartments. In our preliminary results for a single blood compartment model, we found that the predicted results did not fit well with the biodistribution data points in the lungs compartment. This may be attributed to the time latency that takes place for blood to travel initially from the venous compartment (after IV administration) to the peripheral organs. Our 2-blood compartment model prioritizes chronological movement of blood which allows the lungs to be the first-in-line to receive nanoparticle distribution.

Initial parameter values were determined by a combination of organ-specific parameters obtained from the PK-Sim® 8 database, an open source software within the Open Systems Pharmacology Suite, and nanoparticle-specific parameters obtained from various literature sources. We made an initial assumption that different colloid nanoparticles will have the same nanoparticle-specific parameters since some of the nanoparticle-specific parameters that were initially used in our model were obtained from the literature and were not for gold nanoparticles. Albeit this is an assumption not consistent with our investigative question of whether or not pegylated and non-pegylated gold nanoparticles have different biodistribution profiles. Some parameters undergo allometric scaling due to only having been found for rats and not mice. Uptake capacities were taken from non-mice sources. Due to the uncertainty of many of our initial parameters, they were allowed to have wide boundary constraints (upper and lower bounds of 1,000,000 fold) during parameter estimation. The
fact that wide constraints are allowed in some of our parameters for estimation, finding a global optimum for our objective function is expected to be a challenge. A summary of the rest of the parameters used may be found in 147.

Mathematical framework

The derivation of the distribution equation has been shown by Li and colleagues 33,77 and summarized in a PBPK tutorial paper 147. Briefly, we present here the governing differential equation calculating the concentration of nanoparticles in any of the compartments.

\[
\frac{dC_2}{dt} = \frac{1}{V_2} \left[ (Q \times \left( C_1 - \frac{C_2}{k_p} \right) \times \frac{X}{1 + X} - \left( V_2 \times C_2 \times a \left( 1 - \frac{C_m}{m_t} \right) - (d \times C_m) \right) \right]
\]

Eq. 1

where,

- \( C_1 \) and \( C_2 \) [µg/L] denote concentrations of the first and second compartments, respectively;
- \( C_m \) [µg/L] denotes nanoparticle concentration currently in macrophages;
- \( V_2 \) [L] denotes the volume of compartment 2 (compartment currently being measured);
- \( Q \) denotes organ-specific blood flow;
- \( X \) term represents nanoparticle-specific permeability having values between 0 and 1;
- \( k_p \) denotes nanoparticle-specific partition coefficient;
- \( a \) denotes nanoparticle-specific maximum absorption rate for macrophage uptake;
- \( d \) denotes nanoparticle-specific desorption rate for release from macrophage;
$m_t$ [µg/L] denotes uptake capacity in a Phagocytizing (PC) cells.

The equation describes time-dependent nanoparticle concentration in a compartment 2 where nanoparticles are traveling from compartment 1 to compartment 2. Since this model has been modified to: (1) contain only a reduced amount of compartments; (2) take into account the hepatic portal system regarding the spleen’s connection to the liver via the portal vein; and only integrating PC cell compartments in the lungs, liver, spleen and liver, the specific equations used for each of the compartments and sub-compartments are shown in the supplementary information section.

Since the term $\frac{X}{1+X}$, which represents permeability of nanoparticles having physiological limits of only between 0 and 1, our calculation of $X$ becomes additionally challenging. As $\frac{X}{1+X}$ converges to 1, $X$ can have a wide range of large integer solutions. Therefore, we have chosen to estimate the whole term as $cX$ where $c$ represents the compartment which $X$ is the permeability of.

Modeling and simulation
Our modeling and simulation takes place on Mathworks® Matlab version R2020a with the help of the associated Simbiology application. We used ode15s, a stiff differential equation solver. We used a global scattersearch and a local Levenberg-Marquardt algorithm for our parameter estimation with 1000 as the maximum number of iterations. As for the parameters used, nanoparticle-specific and organ specific parameters values are listed in Tables 1 and 2.
<table>
<thead>
<tr>
<th>Organ specific parameters</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ volumes based on % of body weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>liter</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart</td>
<td>liter</td>
<td>9.5E-5</td>
</tr>
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<td>Spleen</td>
<td>liter</td>
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</tr>
<tr>
<td>Venous blood</td>
<td>liter</td>
<td>0.000524818</td>
</tr>
<tr>
<td>Blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>l/min</td>
<td>5.47E-3</td>
</tr>
<tr>
<td>Heart</td>
<td>l/min</td>
<td>2.80E-4</td>
</tr>
<tr>
<td>Brain</td>
<td>l/min</td>
<td>1.30E-4</td>
</tr>
<tr>
<td>Spleen</td>
<td>l/min</td>
<td>9.00E-5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>l/min</td>
<td>1.30E-3</td>
</tr>
<tr>
<td>Liver</td>
<td>l/min</td>
<td>3.50E-4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>l/min</td>
<td>5.20E-5</td>
</tr>
<tr>
<td>Stomach</td>
<td>l/min</td>
<td>1.10E-4</td>
</tr>
<tr>
<td>Portal vein</td>
<td>l/min</td>
<td>1.75E-3</td>
</tr>
</tbody>
</table>

Table 3. 1. Organ-specific parameters.

Organ-specific parameters and volumes were obtained from and calculated by PK-Sim® 8 database, an open source software within the Open Systems Pharmacology Suite using values determined for a typical 20g mouse \(^{122}\).
<table>
<thead>
<tr>
<th>Description</th>
<th>Lungs</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Pancreas</th>
<th>Brain</th>
<th>Stomach</th>
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</thead>
<tbody>
<tr>
<td>Nanoparticle-specific parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partition units</td>
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<td>0.1</td>
<td>0.0</td>
<td>0.15</td>
<td>0.15</td>
<td>0.1</td>
<td>0.15</td>
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</tr>
<tr>
<td>Permeability coefficient</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.001</td>
<td>0.03</td>
<td>0.0000</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Max uptake rate constant for PC</td>
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<td>generic</td>
<td>generic</td>
<td>generic</td>
<td>0.11</td>
<td>generic</td>
<td>generic</td>
<td>generic</td>
</tr>
<tr>
<td>Excretion rate constant</td>
<td>N/A</td>
<td>N/A</td>
<td>1.1</td>
<td>6.56×10^-3</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Uptake capacity</td>
<td>1.4</td>
<td>N/A</td>
<td>7.0</td>
<td>9.524×10^9</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3.2. Nanoparticle-specific parameters.

Nanoparticle-specific parameters were obtained from various sources. Partition coefficients were taken from 34. Max uptake rate for PC cells and excretion rate constants were taken from 77. Uptake capacity original values were reported in micrograms of nanoparticles per gram of tissue 77.

Global sensitivity analysis
Due to the fact that our multi-compartmental model has a high-dimensional parameter space with some parameters having wide bounds on possible values, our estimated parameters cannot be relied on to give accurate physiological values. Even if we obtain high $r$-squared values for all of the fitted curves, this only means that our objective function may have converged at the local level. Due to the fact that many other 'local' maxima could exist for the objective function, other solution sets with high loglikelihood for parameter estimates are possible. Ideally, we would want our parameter estimation to have no uncertainties in order that we might use these estimations to help draw physiological conclusions. In light of these uncertainties, one may still analyze complex models that involve parameter uncertainties using sensitivity analysis. Here, we use the Sobol method as a global sensitivity analysis (GSA). The variance-based Sobol GSA was calculated using \textit{sbiosobol} with 10% upper and lower boundaries of the initial average estimated parameter values. The calculation of Sobol indices for this paper uses Saltelli and colleagues' published work\textsuperscript{130}. Briefly, according to\textsuperscript{131,149}, the first-order Sobol sensitivity index is defined as:

$$S_i = \frac{V_{X_i}(E_{X_{-i}}(Y|X_i))}{V(Y)};$$

\text{Eq. 2}

where $X_i$ represents the $i^{th}$ factor;

$X_{-i}$ is the matrix of all factors but $i^{th}$;
$V(Y)$ is the total variance;

$V_{X_i}(E_{X_{-i}}(Y|X_i))$ is defined as the expected value of the reduction in model variance when $X_i$ is fixed and $Y$ is the model output.

The calculated Sobol indices are customarily reported as first-order and total order indices due to being computationally expensive $^{132}$. The total order Sobol index (includes the first-order, second-order, and $n^{th}$ order) is defined as:

$$S_{Ti} = \frac{E_{X_{-i}}(V_{X_i}(Y|X_{-i}))}{V(Y)}$$  \hspace{1cm} \text{Eq. 3}$$

where $E_{X_{-i}}(V_{X_i}(Y|X_{-i}))$ is the expected variance left when all factors but the $i^{th}$ is fixed $^{131}$.

The sum of all first-order indices should equal to 1 for a particular model output. If there is a difference between the total order and first-order indices, this indicates that there are interactions between parameters. The time-course sensitivities show sensitivity indices over the course of simulation study which is 48 hours for our datasets.

RESULTS

Model Output: Predicted and observed data

The experimental data sets obtained from Takeuchi et al. include biodistribution data for 4 different formulations of gold nanoparticles: (1) 10 nm gold
nanoparticles coated with polyethylene glycol MW 2000 or 10nm PEG2000; (2) 10 nm gold nanoparticles coated with polyethylene glycol MW 5000 or 10nm PEG5000; (3) 20 nm non-coated bare gold nanoparticles or 20nm bare; and (4) 50 nm non-coated bare gold nanoparticles or 50nm bare. Our simulated results, for the most part fit very well with the observed data. Our model performs well for the blood, lungs, spleen, liver, as well as excretion compartments (see Figures 2-5). The lowly perfused organ compartments such as heart, pancreas, brain, and stomach were not included in the model structure of this paper. As seen from the Takeuchi data sets, biodistribution in these organ compartments show high standard deviation as well as concentrations not greater than 0.2% ID.

**Biodistribution in the lungs and their PC cells**

According to our model output, only simulated results for the lungs compartment for the 10nm PEG2000 formulation show visibly different amounts of phagocytized nanoparticles compared to that of its total amount in the compartment shown in Figure 3.1. This suggests that the nanoparticle concentration for 10nm PEG2000 formulation in the lungs is not solely a result of PC cell uptake. The phagocytized nanoparticle concentration for other organ compartments coincided with their respective total concentration, although the phagocytized nanoparticle concentration differs slightly from the total amount in the 20nm bare formulation. Because the inner surfaces and lining layer of the lungs act as the first lines of defense, the macrophage activities in inhaled delivery in both these locations have been studied. The study of
macrophage uptake in the lungs in response to intravenous delivery of nanoparticles also exists $^{153}$. Our results regarding phagocytized nanoparticles in the lungs and the other macrophage rich organs are based on the assumption that these organs do have endocytic cells capable of adhering to and internalizing nanoparticles.

*Spleen, liver, and kidneys and their PC cells*

For the spleen, liver, and kidneys, the phagocytized nanoparticle concentrations coincide with their total nanoparticle concentrations for all four formulations. The model indicates that the presence of nanoparticles in these tissue compartments are mostly due to cell (macrophage) uptake. However, as mentioned above, the simulated results for the lungs compartment (for 10nm PEG2000 and 20nm bare formulations) also show the presence of nanoparticles in the tissue without being in the PC cell sub-compartment. It may be possible that phagocytic activity in the lungs due to IV delivery are not as strong as in the spleen, liver, or kidneys.

*Biodistribution in the venous compartment*

Regarding the difference between biodistribution profiles among the different formulations, non-pegylated formulations express a sharper rise in nanoparticle concentrations in the lungs. Notably, for the 50nm bare gold nanoparticles, the sharp increase in concentration takes place early on within the first hour and slowly continues to increase in concentration. This may be attributed to the fact that concentrations in the blood fall rapidly, thereby causing all other organ
compartments to take on the rapid influx of nanoparticles. The swift decline in the nanoparticle concentration in the blood for non-pegylated formulations, especially in 50nm bare particles, may, in part, be due to recognition and phagocytosis by monocytes where these particles are then taken into tissues. Pegylation, the process of attaching or coating nanoparticles with a layer of polyethyleneglycol (PEG) has been used to cause nanoparticles to evade macrophage uptake.\textsuperscript{154,155} It has even been shown that partial pegylated Janus particles—two-faced particles with different functionalities on different sides, evade macrophage internalization just as well as fully pegylated ones.\textsuperscript{156} Meanwhile, the non-pegylated particles are more easily recognized by phagocytizing cells, and therefore, show a sharper fall in particle concentrations in the blood. It is interesting to see that the permeability coefficient of the lungs (luX) is temporally sensitive for the non-pegylated formulations in the venous compartment for the first 3 hours or so. On the other hand, the luX is only sensitive in the first 30 min or so for the 20nm bare formulation and almost completely insensitive for the 50nm bare formulation. This is consistent with the discussion that non-pegylated nanoparticles cause a greater immune response when it comes to being uptaken by cells. At this point, any small changes in the permeability coefficient for the lungs does not seem to have much of an effect on the bare nanoparticle concentrations in the venous compartment. Since monocytes can be recruited into the lungs, or alveolar spaces, under both homeostasis and inflammation for effective clearance of foreign materials,\textsuperscript{157,158} it could be that captured nanoparticles may now also have another route to enter into the lung tissue,
further reflecting luX sensitivities. Another reason for the luX short-lived sensitivities in the venous compartment for the non-pegylated nanoparticles may be due to their short circulation time—which is a result of non-specific binding of proteins.

_Uptake capacity_

Our simulated results indicate that phagocytic uptake in all organ compartments, for all four formulations, did not reach their full capacity, \( m_t \). The nanoparticle concentration in PC cells, depicted in Figures 2-5 as dashed lines, not only did not seem to reach a plateau, but also continued to rise. This is consistent with the fact that uptake capacity parameters, \( m_t \), are insensitive for all four formulations. Interestingly, the fact that uptake capacity parameter values are high and that small changes will not influence the model output may be an indication of the biological system’s resilience to small drops in levels of PC cells. This ‘resilience’ is the body’s way of withstanding tolerable environmental or physiological changes. Investigating insensitive parameters such as \( m_t \) helps to elucidate the biological system in question. In disciplines such as precision or personalized medicine, insensitive parameters may also allow more room for imprecision with regards to drug formulation and engineering.
Figure 3.2. Overlaid predicted and observed biodistribution plots for (a) 10nm PEG2000, (b) 10nm PEG5000, (c) 20nm bare, and (d) 50nm bare gold nanoparticle formulations.
Figure 3.2b.
Figure 3.2c.
Figure 3.2d.
For data points showing no standard deviation implies that the standard deviations from the original source were obscured and overlapped with other data points which could not reliably be measured. In some cases, the standard deviation values are too small to meaningfully show up on the regenerated biodistribution plots.

Temporally-dependent sensitivities (TDS) vs temporally-independent sensitivities (TIS)

As we have hypothesized earlier, setting wide upper and lower boundaries for parameter estimation will impose a challenge in finding the objective function’s global optimum. We have found that among all four of the formulations, uptake capacity parameters (relating to the maximum amount of nanoparticles that can be taken up by phagocytizing cells) are insensitive throughout the 48-hour time course.

Here we define temporally-dependent sensitivities with maximum change in sensitivity index ($\Delta S$) as mildly dependent with $0.3 \leq \Delta S \leq 0.5$; and $\Delta S > 0.5$ as highly dependent. Any sensitivity index with $\Delta S$ having values less than 0.3 will be regarded as temporally-independent. In other words, for sensitivities to be considered mildly temporally-dependent, the difference in sensitivities across the time series must be at least 30%.

**Venous compartment**

For the blood compartment, sensitivities for LuX are temporally dependent for all four formulations. However, in non-pegylated formulations, changes in LuX sensitivities occur much quicker. Notably, in 50nm bare gold nanoparticles, LuX index rises and drops quickly within less than the first 30 minutes. Sensitivities for
liX, however, are temporally independent with low peak values of approximately 0.2 for pegylated formulations. For the non-pegylated formulations, liX sensitivities are highly temporally dependent with a sharp rise and fall at around 2 hours before rising again for the 50nm bare formulation. While sensitivities for rX and partition (remainder) are both temporally dependent, their indices rise much quicker early on around the first couple of hours and gradually decrease over time for the non-pegylated formulations. Specifically, for the 50nm bare formulation, the time course sensitivity for rX displays a high value around 0.5 from the beginning and quickly drops, and then similar to its counterpart in the 20nm bare formulation, rises again and gradually falls. Whereas, for the pegylated formulations, their indices gradually rise at around 4 hours.

Figure 3.3. Time course sensitivities for the permeability coefficients for the lungs (luX), liver (liX), remainder (rX), and partition coefficient for the remainder compartment, for all four different formulations in the venous blood compartment.

The legend represents the first-order Sobol indices ranging from 0-1. The x-axis extends over a period of 48 hours.
**Lungs compartment**

For the lungs compartment, luX is of particular importance because it is a parameter that is directly involved the nanoparticle’s permeability into and out of the lungs. Yet, it is the sensitivity of the partition coefficient that peaks at almost 1.0 and falls back down to 0.3-0.4 within the first 2.5 hours for the pegylated formulations. For non-pegylated formulations, however, sensitivities for luX rise and fall quickly within the first hour. This indicates that the model output for the lungs compartment is more sensitive to the partition coefficient when nanoparticles are pegylated, while it is more sensitive to luX within the first hour or so when nanoparticles are bare.

However, when we look at the PC cells in the lungs, luX does not seem to have an influence for the pegylated formulations and certainly not much of an influence for the non-pegylated ones. Even though the partition coefficient sensitivities in this sub-compartment for the pegylated formulation do not rise as sharply compared to their counterparts in the lungs compartment, their values still range from 0.3 to 0.4 indicating a sizeable influence on the nanoparticle concentration in PC cells in the lungs.

**Spleen compartment**

For the spleen compartment, sensitivities for spX and max absorption (spleen) are most notable for all formulations. For the non-pegylated formulations, however, spX and max absorption (spleen) parameters are less sensitive than for
the pegylated ones. Also, in non-pegylated formulations, liX gets progressively much more sensitive over time with $\Delta S = 0.6$ to $0.7$.

Regarding the PC cells in the spleen, spX is highly sensitive with peak values of approximately $1.0$ for all four formulations. Sensitivities for spX is more temporally dependent in pegylated formulations. Similar to the spleen compartment, sensitivities for liX in this sub-compartment increase gradually in the non-pegylated formulations, albeit, much less temporally dependent.

*Kidneys compartment*

Sensitivities for liX in the kidneys compartment for the non-pegylated formulations are also temporally dependent with $\Delta S = 0.6$ to $0.7$. However, they are not as noticeable in the pegylated formulations. It would not be surprising that kX values in the kidneys compartment would be highly influential towards the model output. However, this is only the case for the 10nm PEG2000 formulation. Time-course sensitivity for kX for this formulation was too low to even pass the threshold to be presented as part of the results. It is noted that, unlike the spleen, the kidneys compartment is not connected to the liver via the hepatic portal vein. Rather, blood does return directly to the venous compartment. However, nanoparticles an also be excreted into the urine compartment.

In the kidney’s PC cells, kX parameters are more sensitive here having peak indices of $0.5$ for the 10nm PEG2000 formulation and $1.0$ for the non-pegylated formulations. The partition coefficient, and max absorption (kidneys) parameter are sensitive (Sobol index = 0.5) in the 10nm PEG5000 formulation. Since max
absorption (kidneys) is the maximum rate of absorption by phagocytizing cells in the kidneys, it is not surprising that this parameter would be sensitive. However, this is not the case for the 10nm PEG2000 and the non-pegylated formulations in this sub-compartment.

Liver compartment
In the liver, not surprisingly, max absorption (liver) parameter is sensitive and temporally dependent. Permeability coefficients for the remainder compartment are sharply sensitive where they rise and fall quickly within the first 30 minutes to 1 hour for the non-pegylated formulations. This behavior is similarly seen for the non-pegylated formulations in other compartments as well. The partition coefficients for the non-pegylated formulations are not sensitive until around 30 minutes to 1 hour where they rise to 0.3 to 0.4 and fall back to zero after 25 hours.

Unlike the liver compartment, liX in PC cells in the liver are highly sensitive for all four formulations and their sensitivities showed mild to no temporal dependence. Interestingly, max absorptions (liver) in all four formulations are insensitive even though this is the parameter that controls the maximum rate in which nanoparticles are phagocytosed. It is noted that this parameter is more sensitive in the liver compartment.

Remainder compartment
In the remainder compartment, luX is temporally dependent and immediately peaks and falls back down at around 4 hours for the pegylated formulations. Its permeability coefficients (rX) for all but the 50nm bare formulation are insensitive.

**Excretory**

In the feces compartment, max absorption (liver) and the rate of excretion in feces are sensitive in the non-pegylated formulations but are insensitive in the pegylated ones. In the urine compartment, kX parameters are sensitive and temporally independent for all but the 10nm PEG5000 formulation. The lack of sensitive kX in 10nm PEG5000 is compensated by the sensitive partition coefficient and rate of excretion into urine parameters.

**DISCUSSION**

Cell uptake sensitivities relating to charge and size

The measured polydispersity indices for all of the 4 formulations are small indicating that the synthesized particles have narrow size distributions.

Additionally, Takeuchi et al. also measured the zeta potentials of these nanoparticles: (1) -12.1 mV for 10nm PEG2000; (2) -5.1 mV for 10nm PEG5000; (3) -42.3 mV for 20nm bare; and (4) -40.8 mV for 50nm bare. Briefly, the zeta potential is the electrokinetic potential difference between the stagnant fluid layer surrounding the particle and the fluid milieu in which the particle is dispersed in 159.
The use of zeta potential in the analysis of nanoparticle biodistribution is important. The higher the magnitude of the zeta potential of the nanoparticle formulation, the more stable the nanoparticles are in the dispersion by virtue of the particles having strong enough charges to repel each other and overcome attractive forces. The interactions of these particles in the dispersion, however, may not accurately reflect their stability in the body. Size and charge of nanoparticles do affect macrophage uptake. Cell uptake of nanoparticles can be broken down into adsorption and internalization where zeta potential can affect initial adsorption and particle size can affect the internalization or endocytic process. It is interesting to note that the macrophages' interactions with nanoparticles have been shown to be influenced by both the macrophage and nanoparticle’s charge. The measured magnitude of zeta potential for the bare gold nanoparticles are much higher than that of the pegylated ones. The zeta potential of the higher molecular weight pegylated formulation (10nm PEG5000) is the smallest out of the 4 formulations. When we consider only the charges, we might not be able to fully comment on phagocytic activity of macrophages since a large magnitude of zeta potential indicates particle stability from each other, that is, particles repel each other and do not flocculate. As far as macrophage’s interaction with particles are concerned, the influence of surface charges may vary amongst different types of phagocytizing cells. However, we are able to comment on whether or not the rate of the maximum absorption, $a$, of nanoparticles—which considers both adsorption and internalization, is a sensitive parameter. Looking at the compartments that
contain PC cells (lungs, kidneys, spleen, and liver), only parameters \( a \) for: PC cells in lungs, spleen, and kidneys (20nm bare) are mildly sensitive (0.25-0.35 Sobol indices); and PC cells in kidneys (10nm PEG5000) and liver (temporally dependent) are highly sensitive (0.5 or greater Sobol indices) with the \( a \) in the liver being the most sensitive. This might indicate that the influence of charge or size on phagocytosis, as we have discussed above, seems to occur prominently in the liver for all four formulations. This means that if charge or size do affect the rate of cell uptake, it is mainly seen in the liver. For the kidneys, \( a \) is only sensitive in the larger pegylated nanoparticles where as it is not sensitive in non-pegylated formulations. Interestingly, maximum absorption rate of the liver seems to have an effect on nanoparticle concentrations in the feces. This may be attributed to the fact that the only route for nanoparticles to travel to the feces compartment originates from the liver according to our proposed model.

Rationale for drug delivery and drug target intervention

Interesting key drug targets are strategic physiological points that have outcomes which can easily be affected through intervention using small molecules \(^{164}\). In other words, drug intervention discovery can arise by looking at outcomes that are easily amendable by a parameter. In our investigation, we suggest that this is accomplished by performing sensitivity analysis.

Hypothetically, in a scenario where a drug can increase the permeability coefficient value with respect to an output value and enhance delivery of a therapeutic agent to that respective organ compartment, it would make
necessary sense to perform *in silico* experiments to predict the dose responses for that organ. If the permeability coefficient for that compartment is highly insensitive throughout its sensitivity time course, then the effectiveness of using permeation enhancing agents, for example, with the purpose of altering that parameter in order to increase drug permeation into that compartment, would not be great. On the other hand, engineering of a controlled release enteric (oral) dosage would benefit greatly from time-dependent sensitivity analysis results that suggest the highly sensitive parameters related to permeation in the gastrointestinal tract. Of course, there are at least 2 underlying assumptions: (1) the time course sensitivity profile of that compartment is unaffected by the delivery of this drug, or (2) the time-course sensitivity profile of that compartment is still relevant up until the point of delivery to allow for the intended strategy to take effect.

As shown in the results section, pegylated formulations have longer circulation time in the blood with half-lives of almost 2 hours whereas non-pegylated formulations have half-lives of less than 30 minutes. This may be a phenomenon that occurs in pegylated nanoparticle drugs as a result of reduction in non-specific binding and opsonization allowing nanoparticles to continue to freely circulate. This information about circulation times can easily be obtained through conventional *in vivo* pharmacokinetics study. However, as we look at the time-course sensitivities, we see that the permeability coefficients for the lungs (luX) in the venous compartment for pegylated nanoparticles are highly sensitive and temporally dependent (Figure 3.6). By looking at the formulation-dependent
sensitivities for luX, we see that sensitivities are much greater in one type of formulation compared to that of the other. Therefore, if we wanted to influence circulation time in the formulation that has highly sensitive luX, we can direct our drug development process to consider enhancing permeation into the lungs locally, for example, via inhaled drug delivery.

Co-administration of drugs is an effective and studied strategy in clinical practice where one drug influences the pharmacokinetics of the other drug \(^{165}\). Time-course sensitivity analysis can thus assist in the strategy of co-administration of drugs by elucidating critical targets not necessarily noticed via conventional experimental or even simulated results. To test this hypothesis, future studies can target and influence physiological parameters that have been determined to be highly sensitive. One such activity may be the co-administration of another drug to affect the pharmacokinetics of the first drug at only specific sites. Local delivery of drugs may be considered in conjunction with IV administration. Local delivery may be an effective way to directly deliver drugs to the desired area without waiting for that drug to undergo distribution. This avoids the possibility of time-course sensitivity profile to be changed as a result of a change in the system (namely, a value in a parameter), and thereby nullifying the effectiveness of the strategy. However, the time-course sensitivity profile may still be affected subsequent to the local delivery.
CONCLUSIONS

While uncertainties in parameter estimations can exist in complex biological systems, performing global sensitivity analysis using the Sobol method on our model can be helpful in providing parameter insights. We initially proposed that calculating the time-course sensitivities for all estimable parameters will provide insights on the importance of parameters at different times and suggest drug target intervention possibilities. Using these time-course sensitivities, we were able to comment on maximum absorption rate and rationale for drug delivery strategies.

Regarding maximum absorption rate, for example, we noticed that absorption in the liver is highly sensitive for all four formulations. If we make the assumption that nanoparticle charge or size can affect absorption (since this is related to adsorption and internalization), we can formulate a hypothesis that altering these physical characteristics can affect macrophage uptake in the liver more so than in other organs. Regarding drug delivery strategy, for example, we can formulate the rationale of increasing circulation time in the blood of an IV administered drug by locally co-administering a permeation enhancer via inhaled delivery in the first hour for the pegylated formulations. This is because the permeability coefficient for the lungs is sensitive in the venous compartment for the first hour for the pegylated formulations. It is important to note that once a drug is co-administered with the intent to change a parameter value (ie. permeability coefficient), the time-course sensitivity profile, at least for the compartment of interest, is altered. How co-administering a drug or altering a parameter or set of parameters affects
the remaining time-course sensitivity profile of a compartment is a question of future investigation.

Without time-course sensitivities, we would not be able to meritoriously suggest this strategy. This paper has thus presented a method of analysis to discover insights on how to utilize time-course parameter sensitivities to inform specific drug delivery strategies.

SUPPLEMENTARY MATERIAL

Blood

Venous:
\[
\frac{dC_{ven}}{dt} = \frac{1}{V_{ven}} \left[ \left( rX \cdot Q_r \cdot \left( \frac{C_r}{K_p} \right) \right) + \left( liX \cdot Q_{li} \cdot \left( \frac{C_{li}}{K_p} \right) \right) + \left( kX \cdot Q_{li} \cdot \left( \frac{C_k}{K_p} \right) \right) - \left( luX \cdot Q_{lu} \cdot \left( C_{ven} \right) \right) \right]
\]

Arterial:
\[
\frac{dC_{art}}{dt} = \frac{1}{V_{art}} \left[ \left( luX \cdot Q_{lu} \cdot \left( \frac{C_{lu}}{K_p} \right) \right) - \left( rX \cdot Q_r \cdot \left( C_r \right) \right) - \left( liX \cdot Q_{li} \cdot \left( C_{li} \right) \right) - \left( spX \cdot Q_{sp} \cdot \left( C_{sp} \right) \right) - \left( kX \cdot Q_{li} \cdot \left( C_k \right) \right) \right]
\]
Non-peripheral compartment

Lungs:
\[
\frac{dC_{lu}}{dt} = \frac{1}{V_{lu}} \left[ l u X \cdot Q_{lu} \cdot (C_{ven}) - \left[ l u X \cdot Q_{lu} \cdot \left( \frac{C_{lu}}{K_p} \right) \right] - \left[ (V_{lu} \cdot C_{lu} \cdot a_{lu}) \left( 1 - \frac{C_{lu,m}}{m_t} \right) \right] \right]
\]

Lung PC cell compartment:
\[
\frac{dC_{lu,m}}{dt} = \frac{1}{V_{lu}} \left[ (V_{lu} \cdot C_{lu} \cdot a_{lu}) \left( 1 - \frac{C_{lu,m}}{m_t} \right) \right]
\]

Peripheral compartments

Spleen:
\[
\frac{dC_{s}}{dt} = \frac{1}{V_s} \left[ s p X \cdot Q_s \cdot C_{art} - \left[ (V_s \cdot C_s \cdot a_s) \left( 1 - \frac{C_{s,m}}{m_t} \right) \right] - \left[ l i X \cdot s p X \cdot Q_{pv} \cdot \frac{C_s}{K_p} \right] \right]
\]

Spleen PC cells:
\[
\frac{dC_{s,m}}{dt} = \frac{1}{V_s} \left[ (V_s \cdot C_s \cdot a_s) \left( 1 - \frac{C_{s,m}}{m_t} \right) \right]
\]

Kidneys:
\[
\frac{dC_{k}}{dt} = \frac{1}{V_k} \left[ k X \cdot Q_k \cdot C_{art} - \left[ (V_k \cdot C_k \cdot a_k) \left( 1 - \frac{C_{k,m}}{m_t} \right) \right] - \left[ k X \cdot Q_k \cdot \frac{C_k}{K_p} \right] - f \cdot C_k \right]
\]

Kidney PC cells:
\[
\frac{dC_{k,m}}{dt} = \frac{1}{V_k} \left[ (V_k \cdot C_k \cdot a_k) \left( 1 - \frac{C_{k,m}}{m_t} \right) \right]
\]

Liver:
\[
\frac{dC_{li}}{dt} = \frac{1}{V_{li}} \left[ l i X \cdot Q_{li} \cdot C_{art} - \left[ (V_{li} \cdot C_{li} \cdot a_{li}) \left( 1 - \frac{C_{li,m}}{m_t} \right) \right] - \left[ l i X \cdot Q_{li} \cdot \frac{C_{li}}{K_p} \right] + \left[ l i X \cdot s p X \cdot Q_{pv} \cdot \frac{C_s}{K_p} \right] - f \right]
\]

Liver PC cells:
\[
\frac{dC_{li,m}}{dt} = \frac{1}{V_{li}} \left( V_{li} \cdot C_{li} \cdot a_{li} \left( 1 - \frac{C_{li,m}}{m_t} \right) \right)
\]

Remainder:
\[
\frac{dC_r}{dt} = \frac{1}{V_r} \left[ rX \cdot Q_r \cdot C_{art} - rX \cdot Q_r \cdot \frac{C_r}{K_p} \right]
\]

Excretory compartments

Feces:
\[
\frac{dC_{fe}}{dt} = \frac{1}{V_{fe}} \left[ f \cdot C_l \right]
\]

Urine:
\[
\frac{dC_{ur}}{dt} = \frac{1}{V_{ur}} \left[ f \cdot C_k \right]
\]
Figure 3.4c
Figure 3.4g
Figure 3.4h
Figure 3.4j

50mm bare

10mm PEG5000

20mm bare

10mm PEG2000

Reminder
Figure 3. 4. Time-course sensitivities (Sobol indices) for parameters for each of the compartments calculated in our model.

Parameters that have Sobol indices of at least 0.02 are not excluded from being shown in these sensitivity plots. Some parameters are shown even though they have peak sensitivities less than 0.02 in order to used for analysis and comparison.

### Gold nanoparticle Formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Diameter [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10nm PEG2000</td>
<td>28.5 +/- 8.4</td>
</tr>
<tr>
<td>10nm PEG5000</td>
<td>50.9 +/- 18.7</td>
</tr>
<tr>
<td>20nm bare</td>
<td>21.9 +/- 5.9</td>
</tr>
<tr>
<td>50nm bare</td>
<td>53.9 +/- 15.7</td>
</tr>
</tbody>
</table>

Table 3. 3. Diameters of the formulations developed in the Takeuchi datasets.

According to the authors, particle sizes were determined using dynamic light scattering.

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