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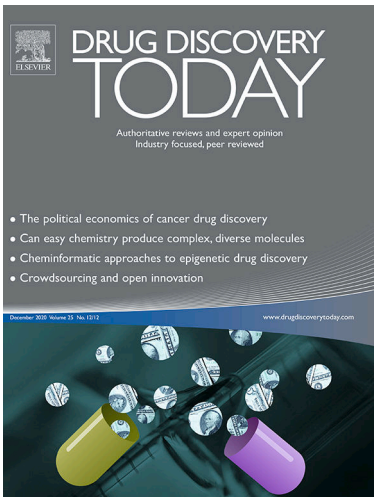

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Opportunities and challenges of physiologically based pharmacokinetic modeling in drug delivery

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Keywords: PBPK modeling, oral formulation, injectable formulation, nanomedicine, inhalation, transdermal formulations.
Teaser: This review summarizes the methodology, applications, opportunities, and challenges of the PBPK modeling in various drug delivery systems.

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Physiologically based pharmacokinetic (PBPK) modeling is an important *in silico* tool to bridge drug properties and *in vivo* PK behaviors during drug development. Over the recent decade, the PBPK method has been largely applied to drug delivery systems (DDS), including oral, inhaled, transdermal, ophthalmic, and complex injectable products. The related therapeutic agents have included small-molecule drugs, therapeutic proteins, nucleic acids, and even cells. Simulation results have provided important insights into PK behaviors of new dosage forms, which strongly support drug regulation. In this review, we comprehensively summarize recent progress in PBPK applications in drug delivery, which shows large opportunities for facilitating drug development. In addition, we discuss the challenges of applying this methodology from a practical viewpoint.

Introduction: the PBPK method

Drug PK evaluates drug exposure inside the body, associated with any following effective and toxic responses. The same drug with different DDS might result in disparate PK profiles, leading to distinct positive and adverse effects. Thus, PK issues should be considered throughout drug development to assure the efficiency and safety of products.

The PBPK method is an advanced PK modeling approach that predicts the *in vivo* disposition of drugs. The PBPK model is built based on a huge number of drug physicochemical and absorption, distribution, metabolism, and elimination (ADME) attributes, such as lipophilicity, solubility, pKa, molecular weight, and plasma unbound fraction, and physiological parameters, including blood flow, tissue volume, vessel surface area, transporters, and enzymes expression level.[1] In the PBPK model, parameters and compartments are physiologically meaningful. Thus, the PBPK model is interpretable, discriminating it from the classical PK model, wherein the compartments do not represent specific organs. Thus, the PBPK model can simulate the PK profiles of a drug in plasma and other organs, which can then be further combined with pharmacodynamic (PD) simulation.[2,3]

The idea of PBPK was first introduced by Teorell in 1937.[4] He constructed a PK model based on physiological parameters and five compartments representing drug circulation, deposition, and elimination.[5,6] During the 1980s, the rapid development of computer science and refined *in vitro* experiments made it possible to construct better PBPK models. This method was further applied to investigate issues in clinical pharmacology, such as specifying the dose for patients according to their PK response, predicting drug–drug interactions (DDIs), and extrapolation from experimental animal data to humans. Investigations of all these issues involve PBPK methods.[7] Subsequently, this field has undergone sustained and remarkable development, especially in recent years.[6] Currently,

simulation results are usually included in submissions to regulatory agencies, such as the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA), influencing dosage scheme decisions.[8] In addition, the EMA and FDA have compiled guidance to encourage PBPK use in submissions.[9,10]

In recent years, this progress has stimulated interest in including the PBPK method in drug delivery research.[6] This trend should also be consolidated by the popularization of newly advanced drug development approaches, such as the quality-by-design (QbD) strategy,[11] model-informed drug development (MIDD),[12] or model-informed drug discovery and development (MID3),[13] and computational pharmaceutics.[14] As a mechanically based method, PBPK modeling naturally shows potential to interpret the influence of pharmaceutical properties on the *in vivo* disposition of drugs, which is helpful for drug development.

The uptrend in PBPK applications has also benefited from upgraded modeling tools.[15,16] These include standard commercial software, such as GastroPlus (Simulations Plus), Simcyp (Simcyp), and PK-Sim (Bayer Technology Services), and platforms on which users can customize their model structure, such as MATLAB (MathWorks) and STELLA (Cognitus).[5] Software integrating all necessary modules makes building a PBPK model much easier.

Currently, the PBPK model has been used for nearly all types of formulation. It has been proven to be a useful interpretive and predictive tool for oral forms, addressing issues such as the effect of release pattern, food, and particle size on PK profiles. Many new drug applications (NDAs) to the FDA incorporate PBPK simulations.[17] PBPK applications are expanding to other formulations, such as injections, inhalations, eye drops, and transdermal formulations. Moreover, many complicated delivery systems (e.g., nanoparticles (NPs), liposomes, and microspheres] and advanced therapeutics (e.g., therapeutic proteins, antibody–drug conjugates (ADCs), and RNA molecules] have also been simulated to provide increased knowledge of their PK behaviors.

In this review, we comprehensively summarize applications of PBPK modeling in drug delivery (Table 1), introducing the modeling methods and how they are used to address issues during drug formulation development. These applications show numerous opportunities for this methodology. We also discuss challenges associated with the use of PBPK modeling.

Applications of PBPK in oral formulations

Basic principles of modeling

The basic components of a PBPK model for oral formulations are shown in Figure 1. Generally, PBPK modeling requires many predefined parameters and mathematical equations, which is the outcome of concerted efforts of investment in *in vitro* experiments and modeling. Primarily, it is important for an oral model to correctly describe processes of dissolution, transit along the gastrointestinal tract, and absorption of formulations.

Formulation dissolution is often measured by *in vitro* tests. The dissolution profile can be input unchanged to the PBPK model or depicted by one type of dissolution equation. The earliest dissolution equation is the Noyes–Whitney equation, put forward in 1897. This model assumes a thin and stagnant diffusion layer at the interface between the product and dissolution media. Since then, other equations based on Noyes–Whitney theory have been introduced.[18,19] These models further consider the shapes and sizes of dissolving particles and the diffusion layer thickness, making modeling more flexible. Another commonly used dissolution form is the *Z*-factor equation (e.g., Equation 1)[20]:

$$\frac{dw_t}{dt} = \frac{D\Gamma N^{\frac{1}{3}}}{\delta\rho^{\frac{2}{3}}} w^{\frac{2}{3}}(C_s - C) = z w^{\frac{2}{3}}(C_s - C) \quad [1],$$

where w_t is the amount dissolved at a certain time point; w is the amount remaining to be dissolved; Cs is the saturation solubility; C is the drug concentration; D, Γ, N, δ, and ρ are the diffusion coefficient, shape factor, number of undissolved particles, diffusion layer thickness, and particle density, respectively, and are reduced to *Z*-factor, which refers to the dissolution rate. *Z*-factors can be obtained by fitting to experimental dissolution profiles and then used as input to models.

For drugs transferring and distributing along the gut, one popular model is the 'advanced compartmental absorption transit' (ACAT) model integrated into GastroPlus software.[21] It parameterizes the transit time for each intestinal segment in proportion to the segment length. Another method uses a distribution function depending on time and position in the gut to represent the intestinal drug concentration.[22] Then, the distributed drug is absorbed under the control of permeability through the gut wall, which is often detected by Caco-2 cell layers and the rat's single-pass perfusate assay, or predicted based on their molecular physicochemical properties.[23,24]

After absorption, a drug undergoes distribution,[25-29] metabolism, and excretion.[26,30] If behaviors of dissolution or absorption of products are of most interest, systemic disposition can be simplified to be represented by classical compartmental models (Figure 1). However, if other issues, such as the concentration in target tissues, are also important, whole-body PBPK models should be considered. The most basic equation for drug distribution between plasma and tissue assumes that organs are 'well stirred' and the distribution is limited by blood perfusion (Equation 2)[31]:

$$V_T \frac{dC_T}{dt} = Q_T C_{ART} - Q_T \frac{C_T \times R}{K_{T-P}} \quad [2],$$

where V_T is the tissue volume; C_T is the drug concentration in the tissue; C_{ART} is the plasma drug concentration; Q_T is the tissue blood flow; $K_{T\text{-}P}$ is the tissue to plasma partition coefficient, which can be either fitted to observed data or calculated from other models, such as Poulin–Theil[26,28] or Rodgers–Rowland[32,33] theory; and R is the blood to plasma drug concentration coefficient. The first term describes the drug mass flux into the organ, whereas the second indicates the drug mass streaming out of the organ. The difference in the mass distributes into the tissue.

In metabolic and excretory organs, such as the liver and kidney, drug metabolism and excretion processes can be modeled as a first-order clearance or Michaelis–Menten function if the kinetics of a drug being degraded by enzymes or transporters is determined.[1,2] It is suggested that a PBPK model for intravenous (i.v.) administration that represents the distribution, metabolism, and excretion behaviors of the drug is built before modeling the oral formulation.[2,34] A set of available i.v. data is helpful to determine the related parameters.

After building and validation against profiles from PK studies, the PBPK model is able to establish the relationship between input parameters and PK behaviors of formulations. For example, PBPK was used in a study of the amorphous solid dispersion (ASD) form of carbamazepine, wherein polymers of hydroxypropyl methylcellulose acetate succinate (HPMCAS) and/or Soluplus were contained.[35] Although the ASD containing both HPMCAS and Soluplus was higher in solubility than that including HPMCAS alone, the former only showed improved C_{max} whereas its area under the curve (AUC) was not markedly improved. The explanation based on the PBPK simulation is that the form with lower solubility was more absorbed in distal intestinal segments, whereas solubilities for all formulations are enough for full absorption. Thus, PK profiles with different C_{max} but similar AUC occur.

Further interest in formulations might include determining critical parameters and how their changes affect PK behaviors. This can be conducted by parameter sensitivity analysis (PSA), which alters one parameter within a range while fixing other parameters to simulate a series of PK profiles. PSA has been used in investigations about, for example, the effect of excipients on drug absorption.[36] The presence of excipients can vary the solubility, permeability, and activity of metabolism enzymes and transporters of drugs. The PSA result shows that the effect of parameter changes depends on the biopharmaceutical classification system (BCS) type of drugs. BCS I drugs are prone to be sensitive to changes in enzyme activity, whereas BCS II and III drugs are more sensitive to transporter activity changes. [36]

Another popular application of qualified PBPK models is the virtual bioequivalence (BE) test. PBPK software can generate virtual subjects by assigning physiological parameters from certain distributions. PK profiles of test and reference formulations can be simulated and compared in this virtual population. In a BE study of warfarin,[37] 1200 individuals were generated to simulate formulations stored in various environments. Simulation results from 30 individuals were extracted to conduct the BE comparison, which was repeated 100 times. A human BE study further consolidated the virtual BE result.

Applications of PBPK models have covered nearly all types of oral formulation. Related studies include various ASDs,[38–40] a silibinin-cyclodextrin (CD) complex,[41] an itraconazole/CD/polymer ternary formulation,[42] flubendazole/ maleic acid co-crystals,[43] carvedilol NPs,[44] bicyclol sustained-release (SR) forms,[45] and osmotic pumps.[46] These applications primarily show the ability of PBPK modeling to predict PK behaviors. However, the development of oral formulations often needs to consider many factors impacting behaviors of product dissolution, distribution, and absorption. PBPK modeling is often used to address the effect of these factors, as discussed below.

Factors influencing the dissolution and modeling method

Special dissolution pattern designed Some drugs might need to be designed as controlled-release forms to obtain the desired PK profile. This requires a deep understanding of the relationship between *in vitro* dissolution and *in vivo* dissolution. The core of modeling such formulation relies on inputting proper dissolution data. *In addition*, the dissolution test design should also be elaborated to correspond to the goal of the formulation.

Jung *et al.*[47] constructed a PBPK model with STELLA software to simulate the SR form of flurbiprofen. Given the special dissolution pattern designed, the release profile was first tested in the medium of fasted state-simulated gastric fluid (FaSSGF) for 1 h, then tested in the medium of fasted state-simulated intestinal fluid (FaSSIF) for 24 h. Different dissolution conditions mimicked disparate environments for dissolution. The combined release profile was input to the PBPK model, which was further validated by two sets of clinical data.

PBPK models constructed in a similar way could be used to find acceptable dissolution profiles. In the case study of venlafaxine designed as a hydrochloride openable matrix,[48] the dissolution profiles were tested in two stages (pH 1.5 for 1.5 h then pH 6.8 for 22.5 h), which corresponded to the SR period. This model suggested that a lag time of less than 4 h would result in bioequivalence with the reference osmotic pump formulation. Another example of the PBPK model for self-microemulsifying DDSs (SMEDDS) of simvastatin[49] also loaded a dissolution profile tested in conditions simulating the altering gastrointestinal environment. This model further considered the simvastatin metabolism pathway via CYP3A4 in the gut wall because presystemic metabolism has been proven to largely impact its bioavailability.[50] This model suggested that a formulation fully releasing at 3 h would result in much higher bioavailability than an immediate-release formulation because the SR formulation could be mainly absorbed in the distal intestine, where CYP3A4 is less enriched.

A model validated against various situations is suitable for constructing *in vitro* and *in vivo* correlations (IVIVCs) for oral formulations. One study reports how to construct IVIVCs for the SR formulation of sildenafil with a semimechanical population PK model.[51] This model disintegrates dissolution into two parts, the dissolution fraction and the dissolution rate. The fraction is described by the Hill equation, and the rate is represented by the Michaelis–Menten equation. The parameter standing for the maximum dissolution rate in the Michaelis–Menten equation (V_{max}) was fitted to *in vitro* dissolution profiles and *in vivo* PK data for one immediate-release form and three SR forms. Thus, a correlation between *in vitro* and *in vivo* V_{max} was constructed, and the R^2 of IVIVC reached 0.9942 for SR forms. Another example of IVIVC is seen in ranitidine hydrochloride delivered by a floating and prolonged-release system.[52] This PBPK model has been validated against five different forms.

Effect of food and other agents Food effect is *a* common concern in drug formulation development *because* food easily changes the solubility and dissolution rate of drugs and impacts their PK behaviors. However, the PBPK model can reliably predict the potential food effect, outperforming other assessment methods based on the dose number by BCS, the solubility limited absorbable dose by *the*

Developability Classification System (DCS), gut bioavailability, and the dose proportionality in single-dose PK stud*ies*.[53] The key step for modeling the food effect is integrating dissolution data tested in the fed condition.

In the study of the food effect on nanosized aprepitant modeling,[54,55] the dissolution profiles were tested in four types of medium [FaSSGF, FaSSIF, fed state-simulated gastric fluid (FeSSGF), and fed state-simulated intestinal fluid (FeSSIF)], which were fitted to dissolution equations and led to four *Z*-factors and solubilities. These factors were used in the PBPK model to simulate dissolution in different situations. Another example of PBPK modeling for a type of BCS II drug[56] also takes a similar setting. The food effect is modeled for amorphous and crystalline forms. A virtual BE test conducted with 32 individuals in a crossover manner confirmed that the two formulations were bioequivalent in the fed condition.

One important mechanism of food effect is mediated via bile salt. More excreted bile salts in the fed condition solubilize lipophilic drugs to form micelle complexes. Considering the bile salt factor brings modeling closer to the physiological condition. In the PBPK model for a venetoclax ASD formulation built with Simcyp software,[57] the solubility in the fed condition was scaled up via the micelle to buffer the partition coefficient. This parameter was obtained by fitting data to dissolution profiles tested in FaSSIF and FeSSIF. The eventual PBPK model accurately predicted the clinical data in fasted and fed conditions.

In addition to food, other agents are likely to be taken concomitantly with intended drugs. Acid-reducing agents (ARAs), such as proton pump inhibitors, especially affect the absorption of weakly basic drugs. A recent study investigated the ability of PBPK methods (with GastroPlus and Simcyp) to assess the effect of ARAs.[58] The modeling setting increased the gastric pH to a predefined level and loaded a pH-dependent solubility to mimic the effect of ARAs. The result showed that current PBPK method can only qualitatively predict the positive effect of ARAs. However, the predictivity might be underestimated in this study because the too few model drugs with positive ARA effect were selected. In addition, the effect of alcohol has also been researched by the PBPK method.[59] Similarly, solubility and dissolution profiles tested in the presence of ethanol are required by this model.

Particle size The particle size of drugs is a critical quality attribute.[11] Generally, the smaller size often leads to accelerated dissolution rate, increased saturated solubility, and decreased unstirred diffusional layer.[60] The size effect can be modeled either via the dissolution rate fitted to experimental data or via equations with particle size explicitly represented. Such equations include the Wang–Flanagan models[18] and the Johnson model,[19] which are integrated into commercial software, such as GastroPlus, Simcyp, and PK-Sim. The PBPK model is a powerful tool to find the optimum particle size of *a* formulation and set specification.[17]

In a study for one BCS II drug,[61] the PBPK model for four batches with different particle sizes (23, 43, 57, and 85 μm) was built via a fitted diffusion rate. The predicted PK profiles of particle size at 23, 43, and 57 μm were similar, whereas particle size with 85 μm showed a moderate reduction in C_{max} and AUC. However, drug plasma concentrations at 12 h for all forms were almost equal. For this therapy, the drug concentration in a sustained period is more important than C_{max}. Thus, the batch at the size of 85 μm should also be acceptable. This example sets the size specification in relation to clinical concerns, embodying the advantage of PBPK usage.

The PSA method is often used to analyze the size effect, which benefits from using dissolution models containing particle size. In a case study about the painkilling effect of the free acid and the salt form of ibuprofen,[62] a diffusion layer model bridges the particle size and dissolution. The predicted PK data were linked to a PD model to predict the pain score. PSA showed that, when the particle size was around 50 μm, the free acid and the salt form performed similarly. However, an increase in particle size impacted the performance of the free acid significantly but had minimal influence on the salt form because of the difference in their dissolution rates. A similar method has also been adopted in investigations of bitopertin[63] and a phosphoinositide 3-kinases inhibitor[64] to identify the desired particle size using PSA. However, the algorithm of PSA only assesses the sensitivity to one parameter independently and sometimes might lead to a biased result. In the case study of the phosphoinositide 3-kinases inhibitor,[64] the size reduction process could not enhance the bioavailability because of the increased excretive effect of the P-glycoprotein transporter in this situation.

When dealing with particles in nano size, enhanced absorption because of entrapment of particles in apical microvilli of the intestinal wall should be considered. This can be achieved by adding one parameter, such as the 'NP factor effect', to the dissolution model to scale up the absorbable drug mass. This parameter is built in in software such as GastroPlus and has been applied to study the size effect of compound X,[65] which also incorporates the PSA of size on the drug absorption fraction under fasted or fed conditions.

Precipitation Precipitation of dissolved drugs in the gut lumen is another important issue. PBPK modeling can simultaneously incorporate dissolution and precipitation processes in simulations for oral formulations. The parameters for precipitation models are also determined from dissolution tests.

For ASD forms, dissolved drug molecules exist in a thermodynamically metastable state in the gastrointestinal tract. In this situation, ASD is prone to precipitate. Kambayashi *et al.*[66] combined dissolution and precipitation processes into STELLA software to build a PBPK model for the ASD form of a drug molecule named T2CP. The precipitation process was mechanically represented by equations describing nucleation and particle growth steps.[67] The parameters needed were fitted to dissolution profiles obtained in FaSSGF and FaSSIF medium. Thus, the PBPK model structure allowed precipitation in both the stomach and intestine. After validation, this newly developed model predicted several PK profiles, suggesting a non-proportional relationship between plasma concentration and dosage, which is reasonable support for dosage selection in further clinical studies.

Apart from mechanical models, other model forms are seen in applications to simulate formulation precipitation, such as first-order equations[68] and zero-order equations.[69] The precipitation rate constant is built in in commercial software and is usually used to simulate precipitation. This method was applied to compare the absorption behavior of ciprofloxacin HCl salt between formulations of IR tablets, suspensions, and sachets.[70] PBPK model, which does not consider the precipitation process, matched well to the observed data for tablets and suspensions, whereas a model containing precipitation predicted the clinical data for sachet better. This indicated that precipitation might occur for the sachet but not for the suspension or tablet.

Physical stability The matrix state of formulations can change *in response to long-term* storage or in unfavorable conditions. This change might impair the solubilization effect and the consequent absorption. Dissolution profiles tested for the 'impure' formulation

can be input to PBPK models to simulate the effect of physical stability issues. One example is the study of the impact of crystallinity on the bioavailability of *the* ASD form of tacrolimus.[71] In this case, dissolution profiles of a series of ASDs with crystallinity from 0% to 100% were used to build the PBPK model. The virtual BE test show*ed* that crystallinity exceeding 10% or 40%, dependent on test conditions, would result in nonbioequivalent formulations (beyond 80–125%). This strategy was also adopted in *a* BE study for the warfarin sodium-isopropyl alcohol (IPA) complex.[37]

Another modeling method considers the impure formulation as a mixture of multiple submatrices with different dissolution behaviors. In a study of prasugrel HCl,[72] solubilities of pure prasugrel HCl and its free base were first derived from human studies and then combined to calculate the solubility for 'mixed matrix' according to the matrix composition. PBPK results showed that <20% free base fraction ensured BE with the pure form. In addition, in the case study of pioglitazone HCl,[61] the predicted PK of the formulation was the combined results of the free base and the salt form.

Salt form choice The salt form needs to be carefully chosen when developing salt drugs. PBPK modeling can predict PK profiles based on the solubility and dissolution behavior of salt forms, which provides insight into the *in vivo* performance of intended salt forms and facilitates salt form choice. One reported PBPK model for danirixin HBr salt form[73] was developed based on a model for its free base with substituted solubility and particle size. This model predicted a higher performance and a minimal interaction with ARA for the HBr salt form. This prediction supported the choice of salt type and was confirmed by a subsequent clinical trial.

Another study for phenytoin showed the advantage of incorporating the PBPK method at the early stage of the salt screen.[74] In this study, PSA indicated that solubility at 0.3 mg/ml was appropriate for phenytoin because a further increase in solubility gained no additional bioavailability. This was proven by the fact that different salt forms of phenytoin, with the solubility range from 1.2 mg/ml for piperazine salt to 73.4 mg/ml for sodium salt, all resulted in similar PK profiles. Thus, putting significant efforts into the salt screen for phenytoin might not be economical.

Issues of drug transit along the gut and absorption Compared with a large number of reports relating to issues about dissolution, studies focusing on issues of drug transit along the gut are less common. An important factor altering gastrointestinal transit time is the food effect. However, this influence can be automatically integrated into PBPK models if commercial software is used. In addition, the transit time might also need to be additionally considered for simulating multiple-unit (pellet) formulations.[75] The complicated transit behavior could be identified with PBPK models. Kambayashi et al.[76] used a PBPK model to simulate the diclofenac multiple-unit. Gastric emptying was modeled by the Weibull equation, the two pending parameters of which were determined by fitting to the observed PK data in fasted and fed conditions.

Drug molecules permeating through the gut wall is the last major step of absorption. Excipients in formulations can change the absorption behavior of drugs via multiple mechanisms. The influence of transporters and metabolism enzymes expressed in enterocytes has been researched by PBPK modeling and PSA.[36] Drug molecules binding to excipients is another potential mechanism impeding absorption. A typical example is the form of CD, introduced by Dahan *et al.*[77] In this case, although an increasing content of CD can entrap more progesterone molecules, leading to higher solubility, the decreasing fraction of free progesterone lowers the absorbable concentration. This influence on absorption can be modeled via an adjusted permeability of progesterone. Sun *et al.* [78] used the adjusted permeability and solubility according to the progesterone and CD molar ratio in a formulation to build a PBPK model with GastroPlus. Wang and Ouyang[79] then modified this model by adding the transit of CD to the model structure with MATLAB. Thus, this new PBPK model was able to calculate both solubility and permeability in real-time during simulation. This model was validated against *in vivo* data of progesterone[79] and andrographolide.[80] There are few published reports of PBPK models considering transits of excipients. However, models with such consideration are more mechanically based for drug delivery. This model type might be more applicable for formulations with relatively clear interactive mechanisms among their ingredients.

Applications of PBPK in complex injectable products

There are many PBPK applications for injections. In fact, it has been suggested that an i.v. model for small-molecule drugs should be built and validated to determine the important distribution and elimination behaviors initially in the modeling task for oral formulations[2]. Thus, here we focus more on PBPK applications in complex injectable products for complicated compounds,[81–83] proteins,[84–86] nucleic acids,[87] T cells,[88] and other special substances.

The commonly used delivery systems for these active pharmaceutical ingredients (APIs) include lipid formulations, nano-DDSs, and microspheres, which show more complicated PK behaviors than simple formulations. Liposome drugs often lead to a higher solubility, prolonged retention, and more selectivity to target sites.[89] Liposomes are believed to take the characteristic of enhanced permeability and retention (EPR),[90] leading to preferential binding to tumor tissues, and most approved liposome products are for cancer treatment.[91] The distinct PK characteristics of NPs include the ability to be absorbed intact by the gastrointestinal tract, transportation in the lymphatic pathway, uptake and clearance by the mononuclear phagocytic system (MPS), including macrophagocytes in the liver and spleen, and accumulation in organs by via the EPR effect.[92–94] Microsphere forms feature on their porous structure design, which can release loaded drugs in controlled or sustained patterns.[95,96]

Given the distinct PK features of these formulations, their PBPK simulations are often conducted on self-defined models with software such as MATLAB. Using a compartmental or lumped PBPK model (without compartments defined for specific organs) is an effective way to investigate the core attributes of most interest. By contrast, a whole-body PBPK model (a typical structure is shown in Figure 2) is required to analyze the drug distribution among other organs. In addition, a PK model linked to additional compartments for PD modeling is also commonly seen for injections.

Liposomes and nanomedicines loading small-molecule drugs

PBPK models for liposomes[97] and nano-DDSs[92–94,98–100] are common. A typical model drug is the antitumor drug, doxorubicin,[101] which is frequently used in reported studies.

An early lumped model for doxorubicin liposomes was reported by Harashima *et al.*[82] in 1999. The model contained sections of blood, tumor extracellular compartment, and tumor cell. In the blood and extracellular compartment, free doxorubicin was released from the liposome at a first-order rate, and both forms were metabolized in distinct pathways. Only free drug could enter tumor cells to exert its anticancer effect. The simulation result quantitatively showed that only a moderate release rate could balance the retention of liposomes in circulation and drug concentration in tumor cells to achieve the optimal cancer-killing effect. Hendriks *et al.*[102] further divided tumor cells into cellular and nuclear compartments to reflect the binding of free doxorubicin to DNA. Observed PK data from different cell lines, tumor types, and administrated doses were normalized to fit parameters to build the model. PSA showed that different tumor types (represented by blood flow and microvessel density) significantly influenced the efficacy of liposomal doxorubicin. The result explained why the delivery efficiency of liposome forms is higher in mice than in human, consistent with *in vivo* study results.[103,104] A lumped model was also applied to doxorubicin in LC-Dox-PoP liposomes for chemophototherapy.[105] In this case, a scaling factor multiplied with the original doxorubicin influx rate constant to reflect permeabilization effect resulting from a laser. The results showed that administration of liposomes led to a 12.5-fold increase in vascular permeation. A whole-body PBPK model for doxorubicin has also been reported.[106]

Other liposomal drugs, such as amphotericin B[81] and docetaxel,[83] have also been studied with PBPK models. Research into liposomal docetaxel[83] introduces details of how to construct a whole-body model, choose a suitable organ model, fit the parameter and covariate matrix, and conduct statistical analyses.

A typical PBPK application in nanomedicine is for antiretrovirals.[107] This is a whole-body PBPK that contains all the main organs and an intact gastrointestinal compartment. In addition, this model includes a muscle compartment for intramuscular administration. The injected antiretrovirals are first released into the capillaries in the muscle and then enter the circulating system. The PBPK model was first validated against data from oral treatment. The dosage scheme of intramuscular administration was then optimized to achieve effective plasma concentration. The predictions suggested monthly intramuscular administration as a possible scheme to replace traditional daily dosing for dolutegravir, emtricitabine, efavirenz, rilpivirine, raltegravir, and tenofovir.

There are other various PBPK applications for nanoformulations to provide understanding about such formulations. The model for SNX-2112, an Hsp90 inhibitor for cancer therapy, highlights the release of nanocrystals,[108] and the model for temoporfin simulates the effect of nano- and macroparticles caused by drug deposition at the injection site on *in vivo* performance.[109] Behaviors, such as organ selectivity and retention in lymphatic tissue, have also been simulated for Z-GP-Dox (a doxorubicin derivative) nanomicelles[110] and dexamethasone formed in polyethylene glycol (PEG)-based NPs.[111] In addition, a model for cisplatin encapsulated in gold NPs [112] tries to interpret the influence of the shape and targeting-head modification in NPs on their PK behaviors.

Microbeads are another form the PK behaviors of which have been investigated with PBPK modeling.[106] Microbeads with diameters of hundreds of micrometers can embolize microvascular vessels after regional i.v. injection and release loaded drugs into nearby tissues.[113] In the whole-body porcine PBPK model for doxorubicin microbeads,[106] the liver was divided into the treated compartment and nontreated compartment, and the microbead was injected into the treated liver part. Combined with *in vitro* release test data, this model predicted the disposition of doxorubicin. The model was then scaled to a human model with hepatocellular carcinoma by changing the physiological parameters to reflect conditions in patients. Additionally, a model for liposomal doxorubicin has also been constructed to help further investigation.

Protein and antibody drugs

Therapeutic proteins, such as monoclonal antibodies (mAbs)[84,85] and ADCs,[86] are becoming increasingly essential in the pharmaceutical industry and clinical practice. Modeling of such agents should deepen our understanding of this dosage form and help dose selection for clinical use.[114] Compared with small molecules, proteins have distinct PK features that need to be considered,[115] such as lymphatic distribution and circulation, lower permeability through vascular endothelium, and specific binding to target and neonatal Fc receptors (FcRns). FcRn binding in endosomes assists antibodies to return to the extracellular space via exocytosis. This process protects antibodies from catabolism and extends their circulation. Chen and Balthasar[116] modified a catenary PBPK model better to describe the binding kinetics of antibodies to FcRn. Meanwhile, Boswell *et al.*[117] reviewed quantitative technologies to analyze the compartmental tissue distribution of antibodies, especially the concentration in interstitial space, developing the tissue model for antibodies. A generic whole-body PBPK model for therapeutic proteins is integrated into PK-Sim software (Figure 3).[85] This model simulates the entry of proteins into interstitial space with a two-pore model and considers lymphatic distribution, endosomal clearance, and recycling mediated by FcRn.

An excellent study of PBPK modeling for mAb was reported by Hu and D'Argenio.[118] Their model structure further incorporated the subcutaneous injection site compartment to enable simulations of subcutaneous and i.v. administration. More importantly, this complicated model involved four mAb-dependent parameters to describe pinocytosis into endothelial space, the diffusion-convective transport ratio, lymphatic clearance, and lymphatic uptake. After fitting to 20 i.v. and 12 subcutaneous pieces of data, the parameters showed positive correlations with the positive charge metric in complementarity-determining regions of mAbs, which is a physicochemical property dependent on mAb type. Applying this relationship predicted the PK profiles of three new mAbs successfully. The simulation result also indicated that mAbs are mostly absorbed via the lymphatic route, and PSA showed that PK is highly influenced by lymph flow. A similar insight was also reported by another PBPK application,[119] which identified the isoelectric point of mAbs as a critical attribute influencing their lymphatic clearance.

The application of PBPK modeling in ADC can be seen in studies of ado-trastuzumab emtansine (T-DM1), T-DM1 composites of mAbs, trastuzumab, and the small-molecule microtubule inhibitor, emtansine. The simulation was achieved by linking a model for macromolecules to a model for small molecules through a disassociation process.[120] Cilliers *et al.*[121] used a PBPK/PD model to research the effect of the additional use of trastuzumab on the depth achieved by T-DM1 in tumor tissue. The assumption was that blank

antibodies and trastuzumab compete for receptors with T-DM1 on the tumor surface, leading to deeper penetration of T-DM1. This assumption was supported by immunofluorescence tests and PBPK modeling results. Thus, this research showed a possible way to enhance the efficacy of T-DM1 and avoid dosage increases and consequent adverse effects.[121]

PBPK modeling is also applied to antigen research. One example is the Ag85B-ESAT-6 (H1) antigen vaccine delivered by a type of immunostimulatory liposome.[122] The PBPK model only contains the popliteal lymph node, muscle, plasma, and a compartment indicating the rest of the body. The results showed that antigen degradation rate and the fraction leaving the muscle significantly affect antigen concentration in muscle and lymph node of mice, while the same two factors only made a difference in the lymphatic concentration in humans. This reflects the difference among species in the immune response to vaccines.

Nucleic acids therapy

In 2018, the FDA approved the first small interfering (si)RNA-based therapeutic, ONPATTRO® (patisiran), to treat polyneuropathy with hereditary transthyretin-mediated amyloidosis, marking the first use of treatment that delivers exogenous nucleic acids into the human body.[123] Later, the huge success of the mRNA vaccines BNT162b2[124] and mRNA-1273[125] against severe acute respiratory syndrome-coronavirus 2019 (SARS-CoV-2) further solidified the efficiency of nucleic acids. There is an urgent need to grasp the PK behaviors of these novel formulations to facilitate their further development and, thus, modeling methods should be included in such investigations.[87]

A typical example is ALN-PCS or inclisiran, a siRNA enveloped by lipid NPs. It downregulates the level of circulating proprotein convertase subtilisin-kexin type 9 (PCSK9). Given that PCSK9 binds to the low-density lipoprotein (LDL) receptor in tissues and promotes their degradation, the downregulation of PCSK9 restores LDL receptors, accelerating the clearance of LDL from plasma and reducing the risk of hyperlipidemia.[126–128] Sokolov *et al.* reported a PK/PD model for anti-PCSK9 therapeutics [siRNA (ALN-PCS and inclisiran) and mAbs counterparts (alirocumab and evolocumab)], which contains PCSK9, LDL, and other hyperlipidemia biomarkers.[129] The effect of siRNA or mAbs is reflected by the altered turnover rate of PCSK9. The simulation result shows that the maximum degree to which PCSK9 be downregulated is ~80%, and the effect of siRNA is limited by its selectivity to the liver.

Another refined PK/PD model was built for mRNA therapy for Crigler Najjar syndrome type 1.[130] This disease features the accumulation of unconjugated bilirubin in the brain. In this model, the mRNA, hUGT1A1-modRNA, encoding uridine-diphosphate-glucuronosyltransferase family 1 member A1 (UGT1A1), is delivered via lipid NPs (LNPs). LNPs in plasma are eliminated as well as endocytosed by the targeted compartment. After endocytosis, mRNA is released from the endosome and translated to UGT1A1. The enzyme catalyzes bilirubin into mono- or diglucuronide forms, which are quickly eliminated. These processes are modeled in agreement with the therapeutic mechanism. By considering the physiological parameters of rats and humans, the simulation of rats can be used to predict the dosage scheme for first-in-human studies.

Cell therapy and other applications

Recently, the first approved chimeric antigen receptor T cells (CAR-T) therapy, KYMRIAH, and its followers[131] entering clinical studies showed the potential of treatment with gene-transfected cells. Expanding applications of cell therapies require more knowledge of the PK properties of exogenous cells. The mathematic model for cells has been investigated for nearly two decades. Ganusov and Auerbach[132] reported a kinetic model for lymphocytes to describe the migration of lymphocytes from the thoracic duct to all major organs and to estimate average residence times. Recently, one whole-body PBPK model of T cells for tumor therapy was built by Khot *et al.*[88] This model represents rats, containing all the main tissue compartments, lymph flow, and tumor compartments parametrized for melanoma. Some basic parameters were identified by fitting to PK data of exogenous chromium-51-labeled T cells extracted from the spleen. Although the simulated cells are not the same as engineered T cells that are used in clinics, this model is still valuable for building a more refined PBPK model in the future.

Besides the formulations discussed above, specific objects and agents have also been investigated via the PBPK method. Many applications focus on kinetics of oil molecules. For example, ASO3,[133] the adjuvant product for influenza vaccine, was simulated by Tegenge and Mitkus.[134,135] In this model, ASO3 of oil–water emulsion at the injection site cracks to release the free oil molecules, squalene and (a)-tocopherol, augmenting the immune response to the influenza vaccine that would be injected later. Based on the concentration of the two oils, this model can suggest an appropriate interval time between injections of ASO3 and the influenza vaccine. In addition, the effect of a 'lipid sink'[136] to treat the overdose of lipophilic drugs, such as anesthetics and psychical drugs, has also been investigated. Administration of empty liposomes[137] and lipid emulsions[138] could result in the redistribution of the drugs from organs to plasma, as captured by PBPK models.

PBPK model for endogenetic antibodies, such as anti-PEG antibodies, has been reported by McSweeney *et al.*[139] PEG is commonly used to form nano-DDSs to extend their circulatory time,[140] and anti-PEG antibodies accelerate the elimination of PEG-contained medicine and reduce the therapeutic effect in some patients. This model predicts an alarming threshold of antibody concentration of 500 ng/ml. Furthermore, this model and the following experiment proved that pre-infusion of free PEG, especially at 40 KDa, to saturate antibodies, can prolong the circulation of NPs.[141]

Another type of PBPK application is for contrast agents for diagnosis. An early such work reported in 1999 was the model for octafluoropropane gas-loaded microspheres.[142] Octafluoropropane gas is mainly eliminated via exhalation. Thus, the PBPK model contains a lung compartment in a piston-like structure that can force the gas out with a physiological breathing rhythm. This model performed well in validation and identified the appropriate dose of contrast agents in clinical practice. A later application was for dendrimer NPs containing Gd^{3+} for tumor diagnosis.[143] The PBPK model indicated that binding to plasma protein is a key factor influencing the distribution of the imaging contrast.

Applications of PBPK in inhalations

Inhalation formulations are another commonly used delivery system. The delivered drugs are first distributed in the airway and enter deep lung tissue. Then, the drugs are possibly absorbed into the circulation via alveolar gas–blood exchange. Feasible PBPK model for inhalation formulations, including the model structure and physiological factors, were reviewed by Borghardt *et al.*[144] Furthermore, Radivojev *et al.*[145] summarized and highlighted pharmaceutical factors in the dissolution test impacting the IVIVC for inhalation formulations. To the best of our knowledge, investigations focusing on optimizing the formulation design with the help of PBPK are limited. Current applications focus more on optimizing predictive *in vitro* and *in silico* tools. These might, in turn, benefit future inhalation formulation development.

Hassoun *et al.* assessed the predictive power of a dissolution apparatus, the PreciseInhale and DissolvIt system, with the PBPK method.[146] This novel dissolution system can automatically produce aerosol for drug delivery and dissolves drugs within a small media volume. Thus, it is more convenient than conventional dissolution apparatus. Using flixotide as the model drug, the PBPK simulation results suggested that the dissolution rate derived from this new apparatus is closer to the *in vivo* situation.

Boger and Wigström[147] constructed a PBPK model to deepen the association of the dissolution process with pharmaceutical and physiological properties for inhalation formulations. This model considers particle size distribution and can simulate the concentration along radial and longitudinal in the airway. Additionally, Boger and Friden presented a refined PBPK/PD model for inhaled products.[148] This model divides the lung into 24 airway generations, and each generation has its distinct blood flow, volume, and permeability (Figure 4). This model was shown to predict the PK/PD profile for salbutamol very well.

Combining PBPK and computational fluid dynamics (CFD) modeling reveals another way to simulate the PK for inhalation forms. The CFD method can simulate drug particle deposition in virtually constructed geometric models of the respiratory tract, which can be used to identify key factors influencing the drug disposition.[149] Vulović *et al.*[150] combined the CFD and PBPK method into an amiloride HCl dry powder model. The CFD model is used to obtain parameters, such as the mass median aerodynamic diameter, geometric standard deviation, and emitted dose, which describe aerosol dynamic properties. Combining this information with PK parameters derived from oral and i.v. formulation, PBPK can predict plasma amiloride concentration after inhalation.

Another novel example of PBPK coupling with CFD modeling evaluates the influence of breathing patterns on nasal tissue concentrations in rats.[151] First, researchers measured the breath profiles of rats, then divided the profiles into segments, with each representing a cycle of breath. The k-means algorithm was used to classify these segments into three categories: normal breaths, exploratory sniffs, and intermediate breaths. Three groups of data were manipulated to derive the average profile for each breath pattern. Three types of typical profiles were combined according to their natural frequency to obtain a typical breath pattern to be used in CFD. The 3D airway model was constructed based on X-ray micro-CT information. The result calculated by CFD modeling was then served as input to the PBPK model to predict its disposition in nasal tissue.

Applications of PBPK in transdermal formulations

Skin is an important administration site for many drugs and topical anesthetics.[152] PBPK modeling is an influential tool for assessing transdermal products, as suggested by the FDA.[153] Many strategies have been used to design PBPK models for skin. For example, Dancik *et al.*[154] described the skin as a homogenous tissue wherein drug is convected in a vertical direction. Ibrahim *et al.*[155] used a two-pore model in endothelium combined with lymph to predict the preferred clearance pathway in the skin for small and large molecules. Recently, Chen *et al.* [156] divided skin into layers of stratum corneum, epidermis, and dermis, with each layer further divided into a number of grids (Figure 5). The drug distributes within the grids under the control of alternative permeabilities. Although the transdermal PBPK model is still evolving, this method has been successfully applied to drug development.

One application of this approach is for therapeutic patches for Alzheimer's disease. Mittapelly *et al.* modeled skin patches loaded with memantine and donepezil.[157] The relationship between the *in vitro* permeation through rat skin and the *in vivo* absorption was established with the PBPK model, using parameters calculated from basic properties. They compared permeabilities and PK profiles of patches with and without enhancers, such as oleic acid, R-limonene, and ethylene vinyl acetate, and identified marginal conditions enabling good absorption. In addition, Nozaki *et al.* have developed a PK model simulating patches with different rivastigmine doses loaded.[158] This model was used to find the optimal strategy to achieve the required steady plasma concentration.

Given that PBPK modeling requires diverse parameters, a tool that can predict those parameters will promote the modeling tasks. The newly reported online platform 'BIOiSIM' (VeriSIM Life) associates machine learning (ML) methods with the PBPK model, which was used to simulate buprenorphine and oxycodone transdermal patches.[159] ML optimization predicts parameters, such as the blood–plasma partition coefficient, tissue–plasma partition coefficient, and transdermal permeability, which enable PBPK modeling. The transdermal model comprises three 'well-stirred' compartments representing the stratum corneum, epidermis, and dermis, and is connected to a whole-body model. Thus, both the local and systemic exposure to the two opioid analgesics drugs can be assessed.

A well-developed transdermal model, the multiphase multilayer MechDermA model, has been integrated into the Simcyp software. This model was used to support the approval of a generic diclofenac sodium topical gel as an Abbreviated New Drug Application (ANDA), reported by FDA scientists.[160] Formulation-specific parameters, such as pH, viscosity, and droplet size, were considered in this model. The main contribution of the model is that the result of the virtual BE test on drug exposure at the target tissue could be used to replace the *in vivo* BE study on the clinical end point recommended in the current product-specific guidance. However, for this goal, the model should be validated against data of various drugs to prove the validity of the diclofenac model and the modeling platform.

Applications of PBPK in ophthalmic formulations

Ophthalmic drug products are often used to treat ophthalmological diseases, such as inflammation, infection, and intraocular hypertension. Although PBPK has been used in ophthalmic formulation investigation for several decades, and some models have been reported,[161–163] only a few recent models are able to study the impact of formulation-related properties on their physiological endpoints.

Walenga *et al.*[164] constructed a complex mathematic model for cyclosporine emulsion, considering formulation properties such as viscosity, surface tension, and osmolality. Cyclosporine is used to treat keratoconjunctivitis sicca, such as dry eye disease,[165] by forming a film of tear outside the corneal. Thus, a long tear film breakup time is expected. This model can evaluate the impact of physicochemical properties of formulation on tear film breakup time and has been used to assess BE between a reference formulation (Restasis) and similar in-house formulations.

Le Merdy *et al.* used the Ocular Compartmental Absorption & Transit (OCAT) model in GastroPlus to investigate the interplay among physiological factors, formulation properties, and the PK behavior of a dexamethasone ophthalmic suspension.[166] This model includes formulation features, such as particle size distribution and viscosity, and predicts the dexamethasone concentration in the cornea, conjunctiva, aqueous humor, and plasma. This model was validated to PK data of formulations with various particle size distributions and viscosities. The simulation results showed that PK profiles were more sensitive to viscosity than to particle size distribution because viscosity determines dexamethasone retention in local eye tissue. Later on, this model was combined with the Higuchi release model to simulate the performance of two ointment formulations of dexamethasone and fluorometholone.[167] The PSA result indicated the importance of the Higuchi release constant influencing the PK and suggests specific measurement of this property *in vitro* because of the large gap between theoretically calculated and experimental values.

Future perspectives for PBPK modeling in drug delivery

Opportunities for PBPK modeling

The above sections comprehensively summarize the applications of PBPK modeling in different areas of drug delivery. As the paradigm of drug development shifts to the QbD strategy, there should be numerous opportunities for the PBPK method (Figure 6).[168]

One of the most significant opportunities is to apply PBPK modeling to the regulation and development of oral formulations. Many such applications have been submitted to, and positively commented on by, the FDA.[17] One example is modeling of particle size, which is identified as a crucial material attribute. The PSA function can model the influence of changes in the size; the shift in BE due to size changes can be simulated by the virtual BE method; and, based on the simulated PK results, the changes in ADME properties and clinical outcomes can be assessed. Eventually, these results help to establish the clinically relevant drug product specifications and define the space for the size change. A similar framework can be applied to the issue of polymorphic form. One can use the *Z*-factor to reflect the influence of the change in polymorphic form on dissolution and to conduct analysis. Critical process parameters, such as the milling method, can also be assessed in this way because they influence particle size and dissolution. In addition, submissions also include applications to address issues of food effect and gastric pH.[17] A PBPK framework for predicting food effect was recently assessed and achieved a primarily good result (23 of 30 compounds with high or medium prediction confidence).[169] The FDA guidance also encourages companies to use the PBPK method to investigate the DDI with acid-reducing agents[170]. These applications should be of interest to the pharmaceutical industry because they are related to pre- and postapproval changes, risk-based biowaiver requests,[171] and generic drug development.[12] In addition, other issues, such as the SR design, precipitation, salt forms, physical stability, gastrointestinal transit, and absorption, can also be mechanically interpreted by the PBPK modeling, as discussed above, which can promote the development of oral products.

The success of PBPK in oral formulations relies on predictive dissolution tests, which are intended to indicate the *in vivo* dissolution. However, there are still gaps between dissolution in the two situations and, thus, the PBPK method can be used to assess and improve dissolution test methods. The deconvolution method in PBPK can derive an *in vivo* dissolution profile from a PK profile by simultaneously considering other impacting factors, such as permeabilities and metabolism rates. The deviation between the *in vitro* and *in vivo* dissolution reflects defects in the dissolution test, which helps to establish an IVIVC on the dissolution.[172] Petrakis *et al.*[68] performed a PSA and suggested that a dissolution profile tested in the environment reflecting the lower intestine would result in a better simulation of compound HA. Beloica *et al.*[173] compared the PBPK result to the observed PK data and proved that reciprocating cylinder apparatus and the change of biorelevant dissolution medium were important to test the dissolution of SR ibuprofen. Gao *et al.*[174] used the PBPK model to validate a dissolution test performed in two compartments at four different pHs and a series of different rotation speeds mimicking the stomach and intestine of a rat. Interestingly, Cascone *et al.*[175] used a PBPK model to develop an artificial stomach dissolution apparatus, which mimicked the volume, shape, and contraction of the real stomach. The aim to develop experiment methods via PBPK validation should also be applied to other DDSs in the future.

Although current PBPK models of other formulations are generally less mature than for oral models, they have provided insights for various agents and products, and their influence shows potential for their future use. Typical examples of advanced injections include modeling of small molecules in two forms,[44,47] modeling of macromolecule drugs with lymph flow and the subcutaneous compartment,[85,118] and extending the model to simulate PD behaviors.[82,102,105,129,130] The agents investigated include small molecules, proteins, lipids, nucleic acids, and contrast agents. These models mechanistically present the PK and PD of formulations. Some of them identified crucial parameters[118] in formulations or show promise to suggest the dose used in human studies.[107,130] Although PBPK models for inhaled, transdermal, and ocular formulations might be less popular, they still show the ability to capture PK behaviors in some cases. Hassoun *et al.*[146] showed that a predictive dissolution test is useful when modeling inhalations, similar to the situation with oral formulations. The two examples of ocular formulations show that the PBPK strategy can capture forms of solution and suspension and has some predictivity when extrapolating to ointments, although the Higuchi release constant should be measured in future studies.[166,167] As for transdermal formulations, scientists from the FDA have reported the first approval of an ANDA generic topical formulation, which involved PBPK modeling[176] and they have encouraged sponsors and applicants to include results, such as virtual BE analysis, in submissions.[153] This indicates that PBPK models for transdermal formulations have the potential to influence decision-making and, thus, such applications should be more popular in the future.

Consistent with this increasing trend in PBPK applications, funded grants and projects have also been initialized. At the 8th International Symposium in Quantitative Pharmacology, scientists from the FDA reported numerous Generic Drug User Fee Amendment (GDUFA) II Grants/Contracts involving PBPK models, mainly aiming toward BE studies for all types of formulation, development of predictive platforms for complex routes delivery, and biowaivers for BCS III drugs. In addition, the NanoSolveIT project, funded by the European Commission Horizon 2020, will also integrate PBPK into its cloud-platform to simulate the exposure and toxicity of nanomaterials.[177]

Challenges of PBPK modeling

PBPK modeling in drug delivery is expected to produce more accurate results for more types of formulations at a higher speed. However, currently there are plenty of challenges impeding this goal. Figure 6 summarizes potential challenges from a practical view.

The first step in PBPK modeling is to develop an available platform and model structure. Commercial PBPK software, such as GastroPlus, Simcyp, and PK-Sim, have largely relieved the need to build models. However, it is better to develop programming skills to construct models because further improvements of existed models are always needed. For example, the dynamic change in pH caused by acid-reducing agents is different from current modeling strategies, which increase the gastric pH to a certain level.[58] The interaction between drugs and the intestinal microenvironment, such as bile salts in the fed state, should be more carefully considered when modeling.[169] In addition, excipients (e.g., CD) would alter the absorption of oral formulations.[36,79] However, there is generally no transit route for excipients in PBPK models. Other factors that could be integrated into models include, but are not limited to, charge metrics of mAbs,[118] proliferation rate and cellular kinetics for CAR-T cells therapy,[178] heat, [179] and skin integrity[153] for transdermal formulations, and Higuchi release constant for ocular ointments.[167] Associating other computational tools with the PBPK method is another way to build a more mechanistically based model. A typical tool is CFD modeling, which is believed to largely promote PBPK modeling for inhalations,[149] as highlighted by numerous GDUFA II Grants/Contracts.

In addition, PK/PD modeling should also benefit from involving more physiological processes in models to describe the effect of drugs more mechanistically. Nanavati and Mager[180] reported a Boolean network model to simulate the synergistic effects of bortezomib and vorinostat to treat multiple myeloma. This model includes 79 proteins linked by 225 connections, revealing the dynamic relationship among crucial biomarkers, such as pNFκB, p53, p21, and cell stress. When connected to the PK models of bortezomib and vorinostat, this model can make *in vivo* predictions. Scientists from Pfizer reported a disease model in the context of the pandemic of SARS-CoV-2,[181] in which viral dynamics, immune responses, and tissue damage are represented. The immune response involves pathways of many cytokines, such as interleukins, TNF-α, and interferon-γ. This model simulates an immune response with proper intensity leading to a positive defense against viral infection and an uncontrolled response bringing about inflammation and alveolar damage. The submodels above represent some typical cellular signal pathways with the potential to migrate to other PK/PD models.

The second step in modeling is to collect parameters required by the intended model structure. This step is often challenging. PBPK models require numerous parameters, and the identification of many crucial parameters relies on fitting to available PK data, which contradicts the intention of prediction by modeling. In addition, the data quality of collected parameters is limited by the experiment method. Thus, on the one hand, the experimental equipment should be improved as more crucial factors are identified. A typical example is 3D-printed airways used for assessing the disposition of inhaled drugs.[182] On the other hand, the experimental design also needs to be upgraded to improve data quality. For example, the dissolution test as a key role for modeling oral formulations and, therefore, the project Oral biopharmaceutics tools (OrBiTo) was initialized partially to improve the usage of dissolution tests for PBPK models. An important area is the decision tree developed to select the most appropriate experimental set used in dissolution test for a given drug/formulation/prandial state combination,[183] resulting in high-quality dissolution data.

If experimental data are not accessible, one can resort to predictive methods to estimate parameters. Some early reported models for permeability prediction using quantitative structure–activity relationship (QSAR) methods[23,24] have been integrated into PBPK software. However, these models usually derive from databases containing only tens of molecules; thus, models that are more robust are expected. The ML method can establish the relationship between structures and activities based on more data and has been applied to pharmaceutical intentions. [184,185] AI should facilitate PBPK modeling in the future, as exemplified by the BIOiSIM platform.[159] Finally, the PK modeling of products of proteins and nucleic acids in humans is challenging and predictive methods, such as allometric scaling, is usually effective in these cases.[186]

The third step of PBPK modeling is to simulate a treatment and validate against observed data from PK studies to assess the confidence of the model. However, it is not always the case to find enough qualified data to validate models, especially for specific populations (e.g., pregnant women, children, and patients) and samples that are hard to acquire (e.g., ocular samples). Models that cannot be adequately validated have low confidence and limited influencing power.[8] In this situation, validation against animal data or drawing conservative conclusions based on the model is optional. Other factors challenging validation are intra- and intersubject variability and the physiological difference between the general public and specific populations. Although commercial PBPK software generally considers this variability by randomly generating virtual subjects with parameters such as height, body weight, and organ volume sampled from predefined distributions, this might not be enough because other factors, such as protein and cytokine expression levels, also vary among subjects. However, ML should be an effective tool to manipulate this high-dimensional data. ML is able to incorporate prior knowledge about cellular response to predict clinically relevant outcomes.[187] Additionally, an ML-based model outperformed a traditional population-PK/PD model for trastuzumab emtansine treatment.[188] The ML model was based on the Neural Ordinary Differential Equations methodology, which maintains the kinetic relationship among dose, PK, and PD, and sets aside enough space for the model to fit the training data.

If the validation is not satisfying, optimization of the model is needed. Defects in model structure, experimental methods, and predictive tools can cause low model performance. A lack of understanding of these factors impedes model optimization. Therefore,

the guidance or framework of modeling, which is based on adequately validated results, is most helpful.[34,169,189] However, available guidance mostly focuses on oral formulations, and models for other formulations need to be validated more extensively in the future. a lack of understanding of the properties of formulations and their interaction with the environment, such as food and excipients, also challenges model optimization. The underlying mechanisms should be explored through experiments or computational tools, such quantum mechanics and molecular dynamic simulations.[185,190] Along with this trend, some basic logics in the PBPK modeling can also be improved, such as the PSA, which currently analyzes the sensitivity of one parameter while the others are fixed. As more mechanisms are developed, interdependent parameters can be rationally linked and the sensitivity could be analyzed in this context.

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Declaration of interests

No competing interests.

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Figure 1. Basic principles of physiologically based pharmacokinetic (PBPK) modeling on oral formulations. Abbreviations: ADME, absorption, distribution, metabolism, and elimination; BE, bioequivalence; PSA, parameter sensitivity analysis.

Figure 2. Typical model structure of a whole-body physiologically based pharmacokinetic (PBPK) model. Redrawn from [83].

Figure 3. Generic whole-body physiologically based pharmacokinetic (PBPK) model for therapeutic proteins in PK-Sim. Redrawn from [85].

Figure 4. Physiologically based pharmacokinetic (PBPK) model for inhalation formulations. **(a)** Whole-body PBPK model. **(b)** Lung tissue is divided into 24 airway generations of the tracheobronchial and alveolar region. **(c)** The structure for each airway generation. Redrawn from [148]. Abbreviation: ELF, epithelial lining fluid. Abbreviation: IV, intravenous.

Figure 5. Structure of a skin penetration model. Redrawn from [156].

Figure 6. The learn-and-confirm approach, opportunities, and challenges of physiologically based pharmacokinetic (PBPK) modeling in drug delivery.

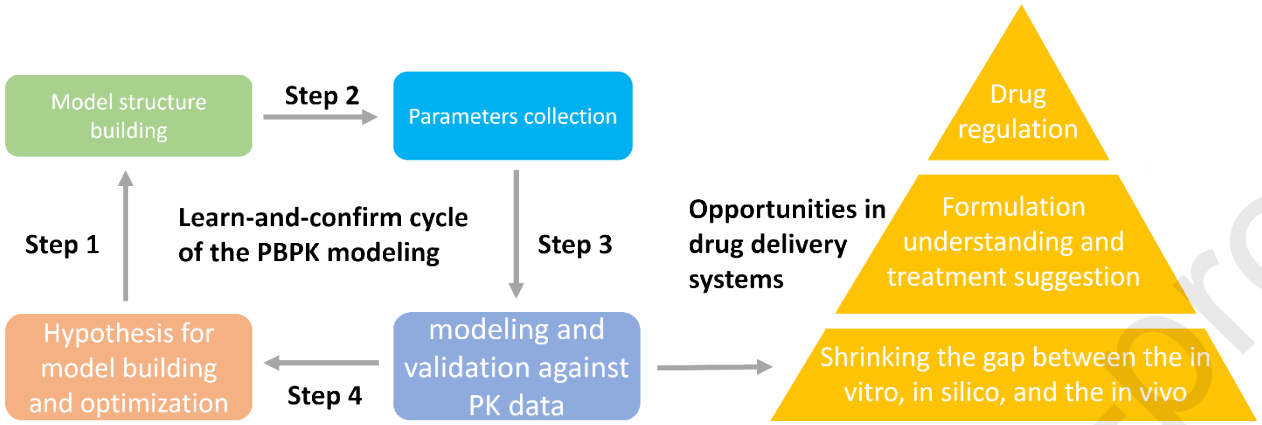
Table 1. PBPK applications in drug delivery development

Drug or agent (BCS class if specified)	Formulation	Route	PBPK software	PBPK applications	Refs
Carbamazepine (II)	Solid dispersion	Oral	GastroPlus	Predict and use PSA to analyze impact of solubility on solid dispersion PK behaviors and compare with co-crystal form	35
Warfarin	Sodium salt and isopropyl alcohol (IPA) complex	Oral	GastroPlus and Simcyp	Whether loss of IPA will make formulation fail in BE	37
Lacidipine (II)	Solid dispersion	Oral	GastroPlus	Use virtual BE test to prove bioequivalence of two solid dispersion formulations to market drug	38
Silibinin	CD	Oral and i.v.	NONMEM	Predict PK behavior for silibinin and CD complex	41
Itraconazole (II/IV)	Ternary complexes with CD and polymer	Oral	STELLA	Validate model for market formulation and predict PK profile for itraconazole-CD-Soluplus ternary complex	42
Flubendazole	Co-crystal with maleic	Oral	GastroPlus	Predict PK profile of co-crystal and compare with its crystal alone	43
Carvedilol (II)	Nano DDS	Oral	GastroPlus	Use PSA to analyze influence of dissolution process on PK behavior	44
Bicyclol	Oral SR preparation	Oral	GastroPlus	Predict PK profile of bicyclol SR tablets in human	45
Flurbiprofen (II)	Nano DDS	Oral	STELLA	Compare efficacy for IVIVC of two *in vitro* release tests	47
Venlafaxine hydrochloride (I)	Oral SR preparation	Oral	GastroPlus	Use PSA to investigate effect of lag time of drug dissolution on PK profile	48
Simvastatin (SV), simvastatin acid (SVA) (II)	Lipid-based formulation	Oral	GastroPlus	Simulate intrinsic process of SV and its metabolite SVA to determine influence of formulation-related parameters and optimal intestinal region for absorption	49
Sildenafil (II)	Oral SR preparation	Oral	Berkeley Madonna	Build IVIVC for dissolution rate of sildenafil SR form	51
Ranitidine hydrochloride (III)	Oral SR preparation	Oral	GastroPlus	Simulate and compare PK profiles from formulations with different composition	52
Ten TKIs	Not specified	Oral	MATLAB	To identify whether their absorption is solubility limited and affected by food	53
30 drugs	Not specified	Oral	GastroPlus and Simcyp	Assess predictivity of PBPK methods of food effects	169
Aprepitant (IV)	Nano DDS	Oral	STELLA	Determine effect of particle size on food effect of aprepitant	54
Aprepitant (II/IV)	Nano DDS	Oral	Simcyp	Simulate food effect of aprepitant	55
Not specified (II)	Amorphous	Oral	GastroPlus	Assess effect of food on BE result by virtual population simulation	56
Venetoclax (iv)	Solid dispersion	Oral	Simcyp	Investigate effect of food and drug–drug interactions with CYP3A inducer and inhibitor on venetoclax ASD	57
Tapentadol, darunavir, saxagliptin, erlotinib	Not specified	Oral	GastroPlus and Simcyp	Assess predictivity of PBPK method of effect of acid-reducing agents	58
Ibuprofen (II)	Oral SR preparation	Oral	Simcyp	Predict PK behavior of ibuprofen SR form in presence of ethanol	59
Pioglitazone, losartan, and other three unspecified compounds	Not applicable	Oral	GastroPlus	Simulate PK profiles under influence of pH, particle size, free form, and dissolution rate	61

Ibuprofen (II)	Salt form	Oral	Simcyp	Predict and compare PK and pain relief PD profiles of ibuprofen free acid and salt forms and use PSA to evaluate influence of size change on their *in vivo* fate and effect	62
Not specified (II)	Size reduction	Oral	GastroPlus	Simulate PK behaviors with different gastric pH, and to prove gastric pH change has lower effect on nanosized form	64
Not specified (II/IV)	Size reduction	Oral	GastroPlus	Investigate food effect and conduct PSA on particle size	65
T2CP	Solid dispersion	Oral	STELLA	Combine *in vitro* dissolution and precipitation processes to construct PBPK model for T2CP solid dispersion for further investigation	66
HA (II)	Salt form	Oral	STELLA	Estimate two different values of precipitation rate in proximal and distal gut, showing changeable precipitation process of HA sodium salt along gastrointestinal tract	68
Fenofibrate (II)	Lipid-based formulation	Oral	STELLA	Use PSA to show that dissolution rate of SMEDDS is enough for its absorption	69
Ciprofloxacin	Salt form	Oral	Simcyp	Determine precipitation process in different ciprofloxacin forms and use PSA to analyze impact of supersaturation, precipitation, and population on PK profiles	70
Tacrolimus	Solid dispersion	Oral	GastroPlus	Predict PK profiles for formulations with different degrees of crystallinity transformation to analyze impact of crystallinity of formulation on BE result	71
Prasugrel	Salt form	Oral	Simcyp	Simulate C_{max} for prasugrel HCl salt form containing different fraction of free base prasugrel and conduct virtual BE tests	72
Danirixin	Salt form	Oral	GastroPlus	Predict and evaluate effect of PPI on PK profiles of danirixin salt form and compare with pure drug	73
Phenytoin (II)	Salt form	Oral	GastroPlus	Predict PK profiles for different phenytoin salt forms with different solubility and demonstrate value of PBPK method in salt screen	74
Efavirenz (II)	Solid dispersion	Oral	PK-Sim	Derive *in vivo* dissolution profiles and investigate effect of surfactant on dissolution of ASD formulation	172
Ibuprofen (II)	Oral SR preparation	Oral	Simcyp	Find best dissolution test condition of SR products for IVIVC	173
Abbott Model Compound	Cosolvent and solid dispersion	Oral	GastroPlus	Validate newly developed dissolution apparatus	174
Diclofenac (II)	Oral SR preparation	Oral	Not specified	Compare PK profiles from dissolution in artificial stomach method and conventional method	175
			STELLA	Identify optimal constants for diclofenac gastric emptying behavior in fed state	76
Doxorubicin	Liposome	i.v.	Not specified	Use PSA to find optimum release rate of drug from liposome to kill tumor cells	82
				Use PSA to find differences in effect of liposomal doxorubicin among different tumor properties and between mice and humans	102
			ADAPT	Simulate permeabilizing effect of light on tumor	105
Amphotericin B	Liposome	i.v.	MATLAB	Whole-body PBPK model might be helpful for further investigations	81
Docetaxel	Liposome	i.v.	NONMEM	Whole-body PBPK model might be helpful for further investigations	83
Antiretrovirals	Nano DDS	Intramuscular	MATLAB	Simulate optimal intramuscular dose strategy to achieve effective intrinsic exposure	107

Temoporfin	Nano DDS	i.v.	STELLA	Understand behavior of temoporfin after injection and analyze clearance pathways	109
Z-GP-doxorubicin	Lipid-based formulation	i.v.	MATLAB	Obtain PK parameters of formulation and use partition coefficients to compare their difference	110
Dexamethasone	Nano DDS	i.v.	MATLAB	Understand NP behavior	111
Cisplatin	Nano DDS	i.v.	Berkeley Madonna	Describe and compare PK behavior of several NPs	112
Doxorubicin	Microbead	i.v. route in liver region	Berkeley Madonna	Predict doxorubicin PK profile after treatment with DEBDOX in pigs and humans with hepatocellular carcinoma	106
24 mAbs	Protein	i.v. and subcutaneous	ADAPT	Successfully identify relationship between PK behaviors and charges of mAbs	118
Ten mAbs	Protein	Subcutaneous	MATLAB	Successfully identify isoelectric point as critical parameter impacting PK of mAbs	119
T-DM1	Protein	i.v.	MATLAB	Identify effect of additional trastuzumab on degree to which T-DM1 permeates into tumor	121
Ag85B-ESAT antigen	Protein in liposome	Intramuscular	MATLAB	Use PSA to find factors most influencing muscular antigen concentration	122
ALN-PCS and inclisiran	siRNA in LNPs	i.v., subcutaneous	R software	Compare PK/PD properties between siRNA and mAbs	129
T cells	Cells	i.v.	ADAPT V software	More in-depth insights into PK behaviors of exogenously administered T cells	88
Squalene	Lipid-based formulation	Intramuscular	Vensim PLE Plus	Use simulated PK profile to understand better its immunodynamic effect on influenza vaccines	134
(a)-tocopherol	Lipid-based formulation	Intramuscular	Vensim Professional	Use simulated PK profile to understand better its immunodynamic effect on influenza vaccines	135
Liposome (as therapeutic agent)	Liposome	i.v.	MATLAB	Therapeutic effects of liposome	137
PLD and free PEG	Nano DDS	i.v.	MATLAB	Investigate effect of anti-PEG antibodies on PK of PEG-drugs	139,141
Octafluoropropane	Microsphere	i.v.	Mathcad Plus 6.0	Evaluate safety of octafluoropropane in humans	142
Gd+3	Nano DDS	i.v.	MATLAB	Analyze distribution characteristic of NPs	143
Fluticasone propionate	Inhalation	Inhaled	Java	Verify predictive efficacy of dissolution tests using PreciseInhale and DissolvIt systems	146
Salbutamol	Inhalation	Intratracheal instillation	MATLAB	Construct salbutamol PBPK/PD for further investigation	148
Amiloride HCl	Inhalation	Inhaled	GastroPlus	Construct predictive method by combining CFD and PBPK	150
Acetaldehyde	Inhalation	Inhaled	Not specified	Combine CFD and PBPK model to predict acetaldehyde distribution and metabolism in airway after inhalation	151
Memantine and donepezil	Patches	Transdermal	MATLAB	Identify factors impacting PK behavior of memantine and donepezil transdermal formulation and build correlation of *in vitro* permeation and *in vivo* absorption	157
Rivastigmine	Patches	Transdermal	Winnonlin	Simulate PK profile of combined use of old and new patches	158
Buprenorphine and oxycodone	Patches	Transdermal	BIOiSIM	Assess both local and systemic exposure to two opioid agonists	159
Diclofenac sodium	Topical gel	Transdermal	Simcyp	Good practice of virtual BE modeling for regulatory decision making	176
Cyclosporine	Emulsion	Ocular	MATLAB	Analyze impact of viscosity, surface tension, and osmolality on tear film break-up time	164

Dexamethasone	Suspension	Ocular	GastroPlus	Analyze impact of viscosity, particle size distribution on PK behavior	166
Dexamethasone and fluorometholone	Ointment	Ocular	GastroPlus	Conduct PSA on application area, application time and Higuchi constant	167



Challenges of the PBPK modeling in drug delivery

Steps	Challenges	Strategies
Step 1	Shortage in skills on programming for building model structures.	Multidisciplinary talents training. Develop easily-used and qualified PBPK platforms.
	The gap between available models and physicochemical and physiological processes.	Measure or estimate more physiological data. Integrate more physiological processes and tools to build more mechanistically-based models.
Step 2	Limitation in experiments to get desired parameters.	Improve in vitro experimental design. Conduct preliminary *in vivo* studies and bridging work.
	Limitation in predictive tools to estimate desired parameters.	Develop easily-used and qualified *in silico* tools for properties prediction.
Step 3	Available *in vivo* study data is relatively few. Difficulty in sampling in studies.	Conduct more refined PK studies. Draw conservative conclusions. Simplify the model or investigate in animal models.
	Intra- and inter- subjects variability. Physiological difference between normal people and special populations.	Identify causes of variability. Extrapolate findings among species and populations. Integrate multi-omics information into modeling.
Step 4	Shortage in knowledge about formulations' physicochemical properties, PK/PD behaviors, and their representation by *in silico* models.	Multidisciplinary talents training. Conduct more basic research. Develop and propagate standard frameworks of model development and optimization.

Highlights

- PBPK modeling bridges drug properties and PK behaviours in drug discovery and development.
- The PBPK is often used in oral formulation development, to address critical issues.
- The PBPK is mainly applied to complex injectable products as an explanatory tool.
- The PBPK is also tried for inhaled, transdermal, and ophthalmic formulation.
- Both experimental and computational improvements would facilitate the PBPK in drug delivery.

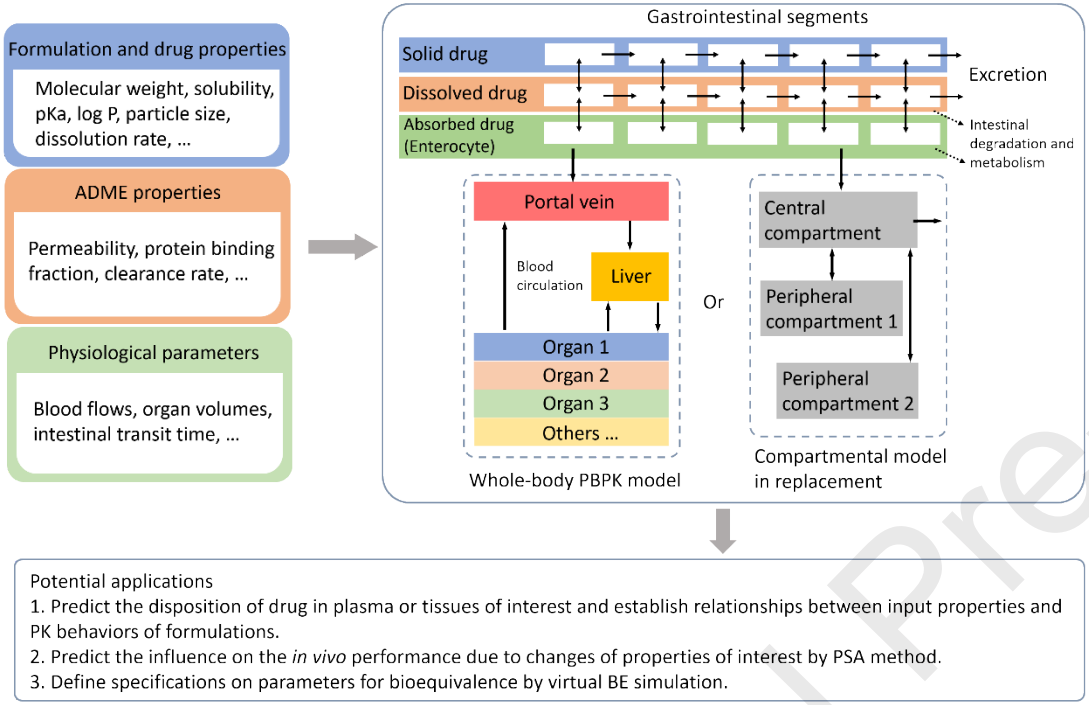
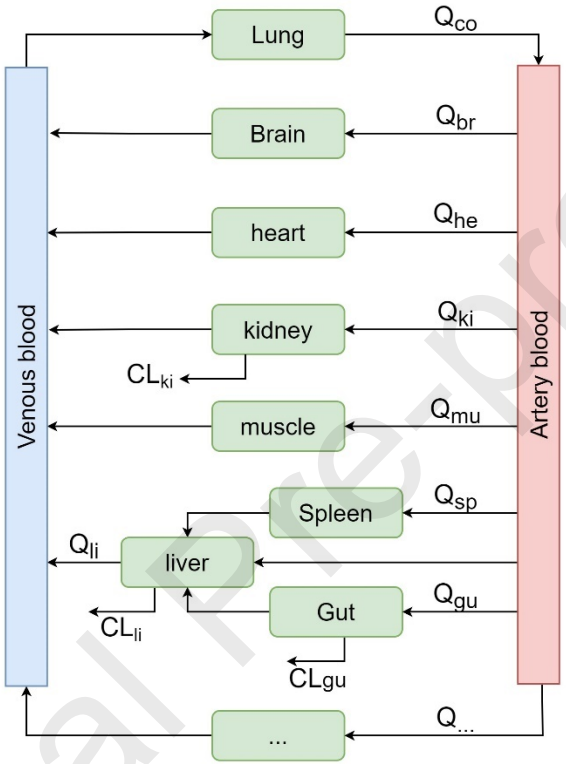


Fig. 1.



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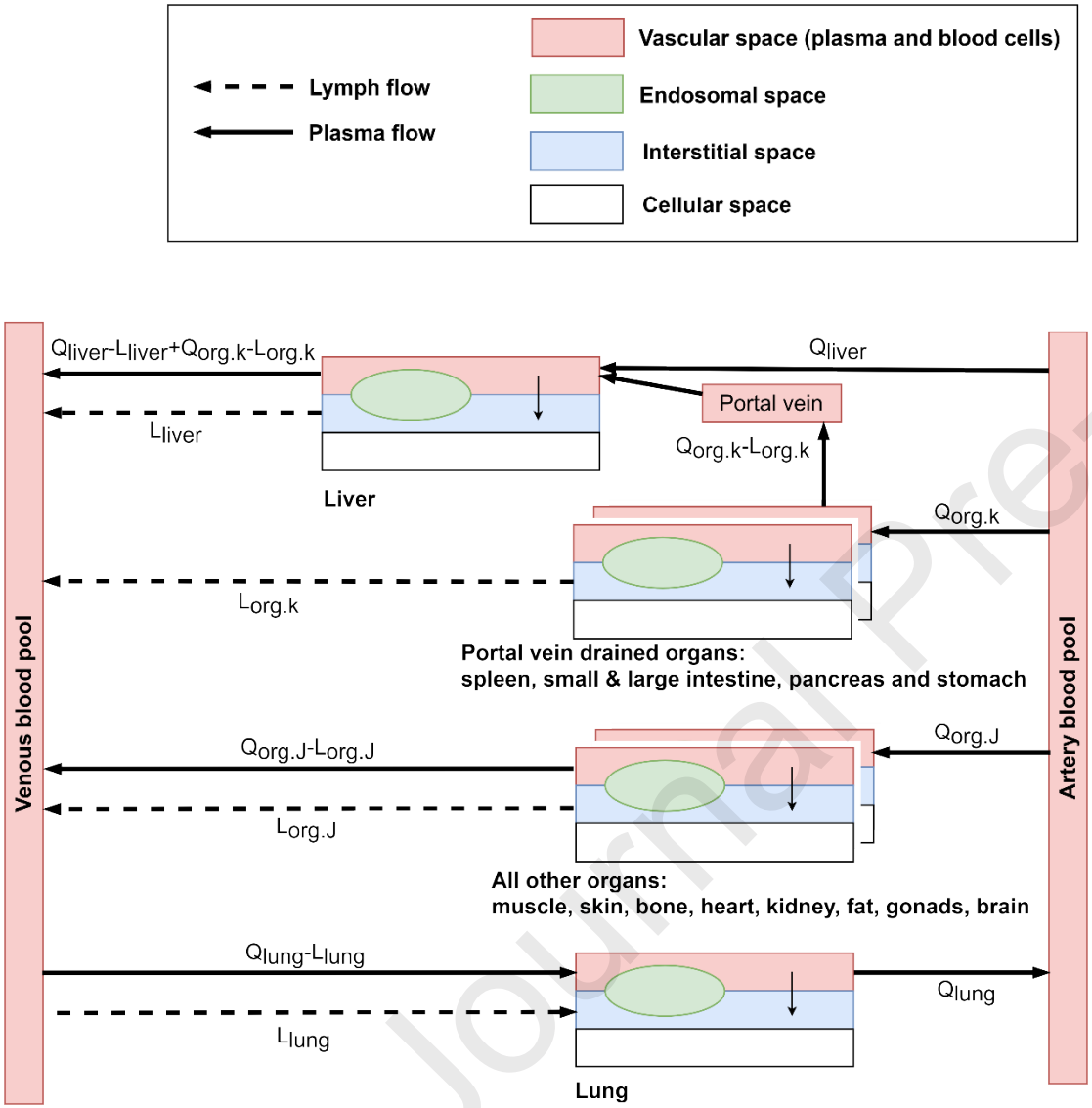

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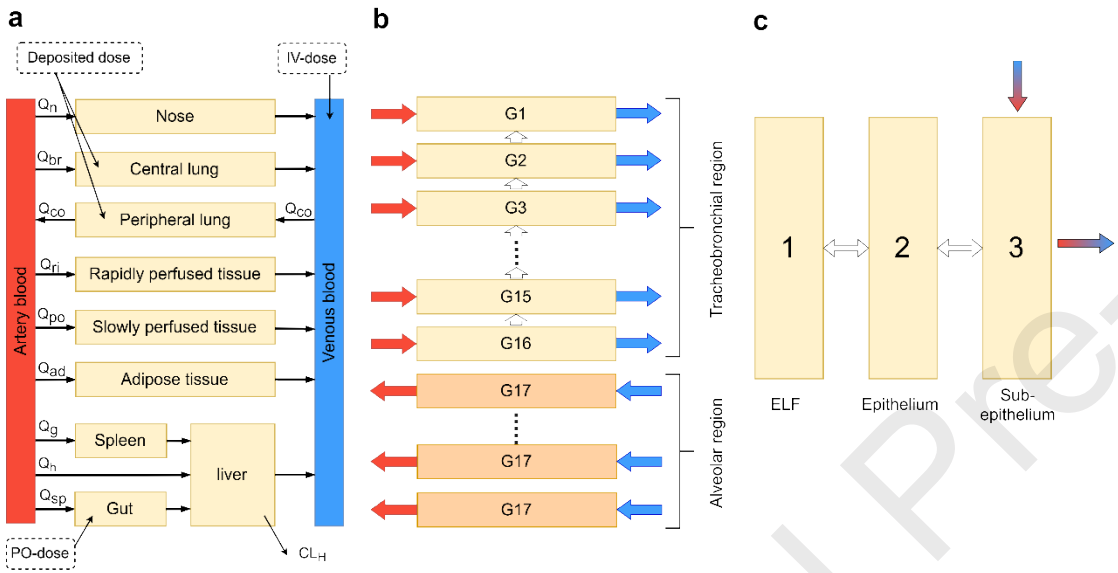

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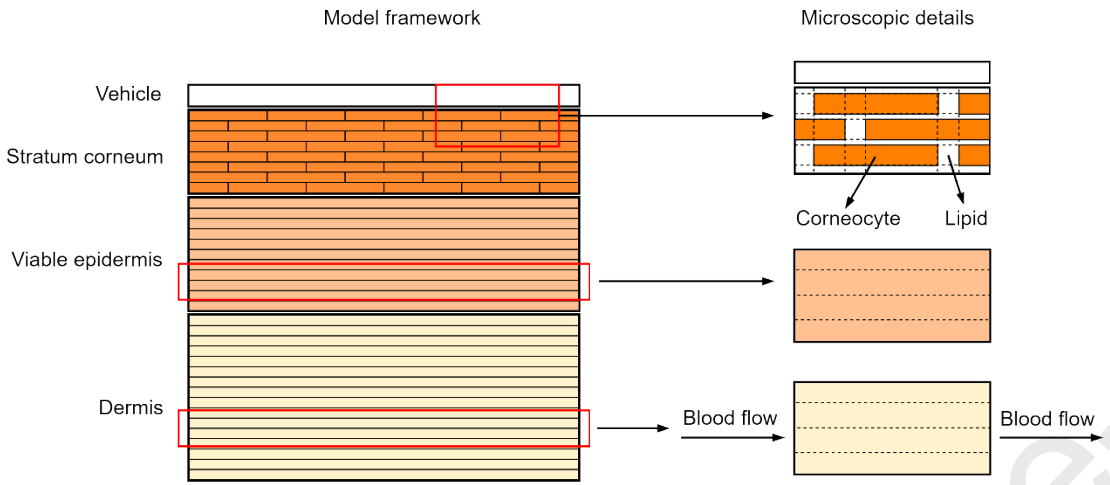

Fig. 5.

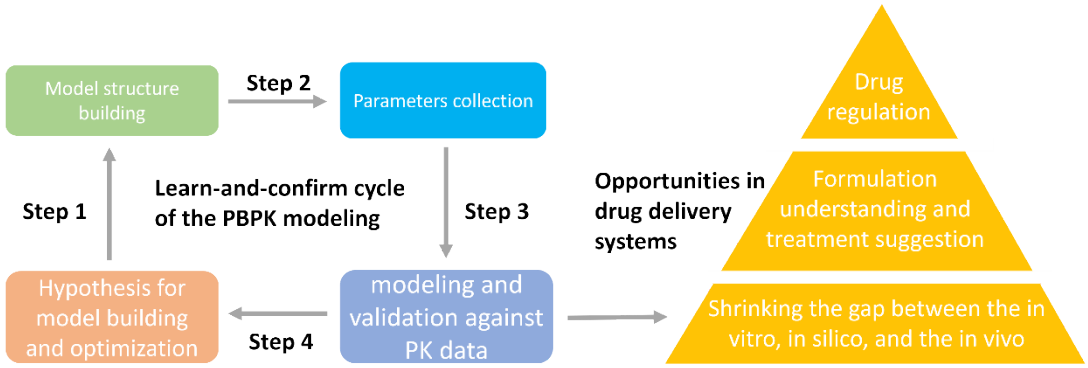

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Fig. 6.

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