Modeling and Simulation of Hepatic Drug Metabolism: In Silico Hepatic Intrinsic Clearance

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Abstract

The Functional Unit Representation Method (FURM) initiates a new procedure for the design of in silico models that are flexible, adaptable and most importantly reusable. We conduct experiments using FURM and an agent-based experimental framework to generate validated in silico hepatocytes that are capable of clearing simulated test drugs in such a way that the experimental outputs are indistinguishable from the real *in vitro* data. We call this experiment in silico hepatic intrinsic clearance (IS-HIC). A similarity measure along with an optimization method, known as Nelder and Mead simplex method, was used to automate the evolution process of in silico hepatocytes and drugs. The final output of the simulation is indistinguishable from in vitro published data. We also show that FURM models are good candidates for being *reused* in other simulations.

Keywords: hepatic drug clearance, stochastic modeling, biological systems, model reuse, optimization

1. INTRODUCTION

Computer simulations can increase the effectiveness of drug discovery and development [2]. Pharmacokinetics, tracking the time course of drug metabolism, distribution, and elimination, is an important aspect of that research. There are two main approaches used by researchers to perform pharmacokinetics simulations. The first, known as physiologically based pharmacokinetic (PBPK) modeling, uses mathematical equations similar to those used by engineers, i.e. it formulates the system based on chemical rate laws governing species concentration and locations, leading to a set of linear or non-linear differential equations. PBPK models are used to represent the flow of fluids and the chemicals that they contain through the "pipes and tanks" of the body [2-6]. The resulting simulations are deterministic, i.e., the trajectory of the system is along a fixed path from its initial state. Such simulations assume that the concentration (in the simulated biological system) is a continuous (real) value. This assumption is valid in many situations where the volumes are large and the contents are well-mixed and the number of molecules of interest is also large, which is not always the case. As a consequence the discrete nature of change at low concentrations needs to be taken into account [7].

The second approach uses *stochastic modeling* to keep track of every single or group of molecules or cells in the system. Concentrations are assumed to be discrete values, which are stochastically changed in response to reaction events. A limitation of stochastic algorithms is that they are computationally expensive. An overview of approaches used for modeling stochastic phenomena in cellular systems is presented in [7]. There are additional technologies to predict drug metabolism, including rule-based tools, electronic models and homology models (surveyed in [8]).

The Functional Unit Representation Method (FURM) is introduced in [1] as a method to simulate biological systems. It seeks more flexible, adaptable and evolvable biological models,

whose base components are the modular functional units that are designed to be easily decomposed for reuse in other biological models. The first demonstration example of this class of models is the ISL [1].

The primary goal of this work is to show how easily one can decompose an existing ISL and reuse its component submodels to build new models. The consequential purpose of this report is to provide validated in silico hepatocytes for use in simulating *in vitro* experiments. They, in turn, can be reused in other models. We use FURM to implement flexible in silico biomimetic experiments that produce data that can be used to calculate a measure that corresponds directly to intrinsic drug clearance, the standard measure of drug metabolism. That measure is the in silico hepatic intrinsic clearance (IS-HIC). The experimental framework reuses the agents from our previous model, the in silico Liver (ISL) [1, 9], that were used to represent hepatocyte metabolic functions within that larger system. Using a *stochastic* approach, the ISL simulates the clearance of drugs in the liver, and is validated against data obtained from experiments on isolated perfused rat livers. The ISL is implemented in a discrete-event manner using the Swarm [10] multi-agent software platform. The primary functional unit of the ISL is a component that maps to the liver lobule, which is a network of interconnected tubes called sinusoids through which blood flows after entering the liver. The primary functional unit of IS-HIC is the *hepatocyte*. In the simulation hepatocytes are represented using agents while compounds are mobile objects. The simulated hepatocytes interact with a simulated test drug within a 2D space. The IS-HIC framework measures the in silico clearance (*Cl*) of drug objects by the in silico hepatocytes.

Clearance is one of the most useful pharmacokinetic parameters. It is a measure of the ability of a tissue or an organ or such as liver or a kidney to irreversibly remove a compound from blood via metabolization or elimination [4]. *In vivo*, when that removal is due to metabolism, then the observed clearance is a function of blood flow rate to the tissue or organ and the intrinsic metabolic clearance, CL_{int}, a flow independent measure of the tissue or organ's ability to metabolize available compounds. It is the proportionality constant relating the rate of drug metabolism (V) and drug concentration at the enzyme site (C_E) [5]. The intrinsic metabolic clearance is the basis for the extrapolation of *in vitro* to *in vivo* measures of metabolism.

In the section that follows we briefly describe the implementation of the IS-HIC as a FURM framework, using components borrowed directly from the ISL and its framework. Next we briefly discuss the optimization method that has been used in this work. We then describe the experiments that were conducted to validate the in silico hepatocytes and the results of those experiments. Our concluding observations are presented in the Discussion.

2. METHODS

In the IS-HIC context FURM and its framework uses three models: an articulated[*], functional unit model (ArtModel), an accepted mathematical model—the reference model (RefModel), and *in vitro* experimental data (DatModel) for validating the ArtModel. These three models are executed by an *experiment agent* (Exper Agent) in cycle. The ExperAgent is responsible for: managing the resources required for each experiment, controlling the models, taking data from the models, progressing from one experimental setup to the next, scoring each model against some performance measure, and acquiring telemetric data from the experiments.

2.1 The Reference Model (RefModel)

RefModel is any accepted mathematical model that describes *in vitro* hepatic clearance under specific conditions. As long as there is a local equilibrium between drug and metabolic enzymes, metabolic elimination of that drug by metabolism generally follows first order kinetics. The

[*] We use articulated to emphasize that that the IS-HIC model is assembled from components that are designed to fit together and, once assembled, to function as a whole.

metabolic rate decreases in proportion to the local drug concentration as given by equation (1) [12], where k is a first order rate constant.

$$\frac{dC}{dt} = -kC(t) \qquad (1)$$

Intrinsic clearance is a measure of enzyme activity, and is independent of physiological factors such as the liver blood flow or drug binding in the blood. In vitro, the intrinsic clearance of a drug is commonly expressed by equation 2, where C_s is the concentration of the unbound drug at the enzyme site [12].

$$\textit{Rate of metabolism: } V = CL_{int}\, C_s \quad (2)$$

Rate of metabolism is generally defined by the Michaelis-Mention enzyme kinetics relationship [12]. When the drug concentration is much smaller than K_m (the Michaelis-Mention constant), CL_{int} becomes:

$$CL_{int} = V/C_s \qquad (3)$$

What we need for our mathematical reference model, is an equation that describes the concentration of the unchanged drug as a function of time. Researchers use the following equation to express the concentration of unchanged drug for in vitro experiments, when keeping the initial drug concentration much smaller than K_m [11-12]:

$$\frac{dC}{dt} = -CL \times D \times C(t) \qquad (4)$$

where C(t) is the concentration, CL the in vitro clearance of the drug and D the cell density.

By solving differential equation (4) the amount of drug remaining after incubation time T is expressed as follows:

$$C(T) = C(0) \times e^{-CL \times D \times T} \qquad (5)$$

The basic equations explained above cannot be applied to all drugs [4], but they are applicable to the drugs and conditions selected for this study, and so we use equation 5 to express *in vitro* clearance and to be the reference model for IS-HIC.

2.2. The Articulated Model (ArtModel)

The IS-HIC ArtModel consists of three basic components, *solute objects, hepatocytes* and a *WanderSpace*. The solute objects and hepatocyte agents are the exact same as the corresponding components used in the ISL [9].

Solute objects: solute objects are the mobile objects representing a group of molecules of the chemical compound (the test drug) as they move around in the reaction mixture. These in silico objects can be parameterized to make their behavior similar to that of a drug *in vitro*. The more relevant parameters are as follows. SoluteBindingProb: The probability that a specific type of drug object will bind to a Binder within a hepatocyte. SoluteBindingCycles: The number of steps that a drug object remain attached to a Binder; embraneCrossing: indicates whether a compound can or cannot enter hepatocytes. A solute object is destroyed once it is "metabolized." Metabolites are not currently tracked.

Binder: Objects within hepatocyte agents that can both bind and metabolize compounds. Each Binder represents a fraction of all subcellular components that can bind or sequester a drug object, and includes a fraction of all metabolizing enzymes.

***Hepatocytes*:** These objects are the agents representing the isolated hepatocytes suspended in the *in vitro* cell culture media. They are capable of taking in, binding and metabolizing drug objects. The parameters are: BindersPerCellMin, BindersPerCellMax: Minimum and maximum binders per cell, respectively. MetabolizationProb: The probability that the cell metabolizes an attached solute object at the end of its binding period.

WanderSpace is a fine-grained space, representing the *in vitro* cell culture, in which solute objects and hepatocytes interact with each other. SpaceWidth, SpaceLength: Width and length of WanderSpace. HepDensity: The density of hepatocytes in the space. TotalSoluteMass: The total compound mass initially added to WanderSpace.

Functioning of the IS-HIC ArtModel: First, hepatocyte agents are placed randomly in the WanderSpace as fixed objects. Drug (and other) mobile objects are then randomly placed within the space (external to all hepatocytes) based on an initial concentration (see Figure 1). The mobile objects move randomly, while the hepatocytes have the opportunity to "take up" and then metabolize nearby solute objects.

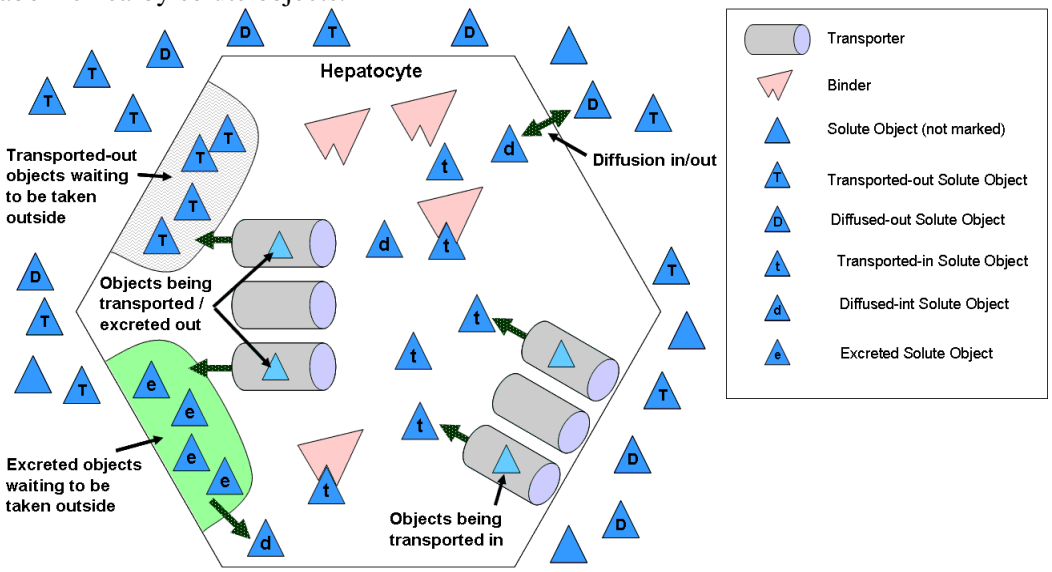


Figure 1. An illustration of in silico hepatocyte interaction with solute objects within the WanderSpace

As the simulation progresses, the number of solute objects decreases as a consequence of metabolism. At intervals this number was counted, normalized and scaled to represent the concentration of the unchanged drug. Figure 2 illustrates how IS-HIC keeps track of each solute object in the space.

2.3. The Data (DatModel)

The DatModel represents the real biological system. It contains the data obtained from *in vitro* experiments, and is used to validate the in silico model (ArtModel). The validation process involves measuring the output of the in silico model and comparing it with the data provided by DatModel. In this work the data was obtained from Fig. 2 of [11], which depicts the time course for nine unchanged compounds in cell culture media containing freshly isolated rat hepatocytes different compounds. The data points were carefully obtained from the graphs using computer design tools. The DatModel interpolates the data points using a linear interpolation method to estimate the drug concentration at each time step of the simulation.

2.4. The Similarity Measure

After each in silico experiment a *similarity measure* algorithm compares the output of that experiment with the data provided by DatModel and assigns a score to the output based on the degree of their similarity. Several similarity measure algorithms are surveyed in [13]. The similarity measure used in this work was the measure calculated by the "global standard deviation" method [13], minus the magnitude of an error vector:

$$Similarity\ Score = SS_{gsd}^{10}$$

where SS^{10}_{gsd} is the similarity score by the global standard deviation method with a wider envelope: $upper_i = m_i (1 + sd) + 10$, $lower_i = m_i (1 – sd) – 10$, where m_i and sd are the nominal mean and standard deviation of the time series (for additional detail see [13]). This score is calculated by counting the number of observations of the candidate time series that fall within the envelope and dividing that by the total observations in the series [13], as a result $SS^{10}_{gsd} \in [0,1]$.

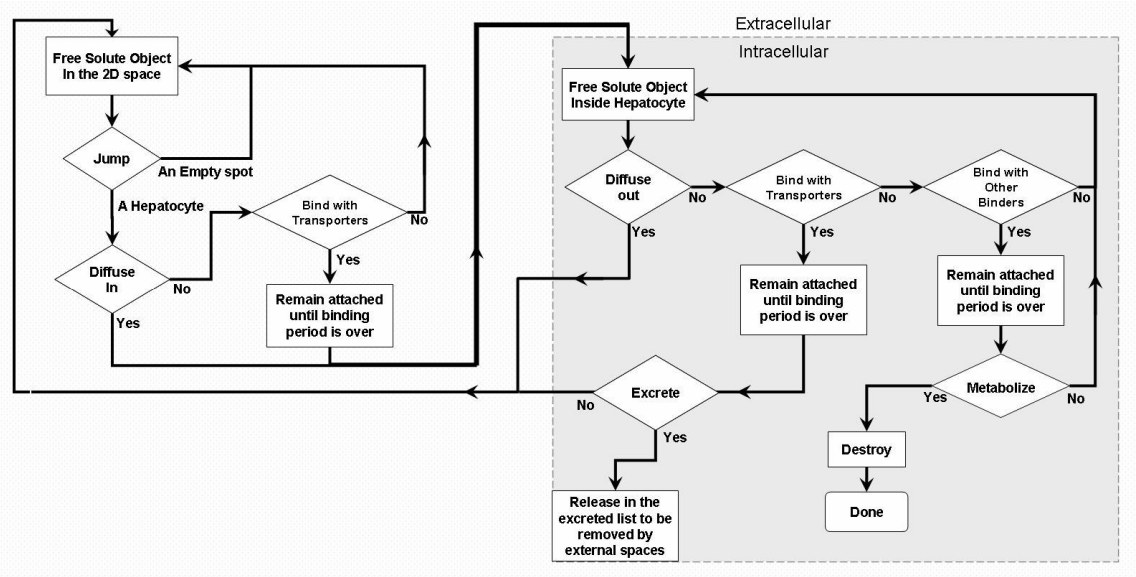


Figure 2. Movement of a solute object in IS-HIC

2.5. Reusability of FURM Models

Reusing models of one simulation in other ones can increase the efficiency of software development and in most cases decreases the costs of development, especially when models have been expensively and slowly written and developed. There has been a wide range of effort by engineers designing reusable models [25-28]. The spectrum of software reuse can range from code scavenging to full model reuse [23]. Utilizing a high level *component-reuse* strategy, FURM, is making progress toward methods that enable arbitrary interpretation and reuse of any given component in the system's framework including both the cases of multiple models for the same referent and multiple referents for any given model [24].

IS-HIC as an example of reusing FURM models, demonstrates the effortless reusability of previously developed FURM components, while persisting their original assumptions. In fact IS-HIC provides means of optimizing some of the ISL models (i.e. Hepatocyte and Solute) in absence of other models pressure (e.g. the blood flow in the sinusoidal space, etc.) which, in turn, can be used in other simulations including ISL itself.

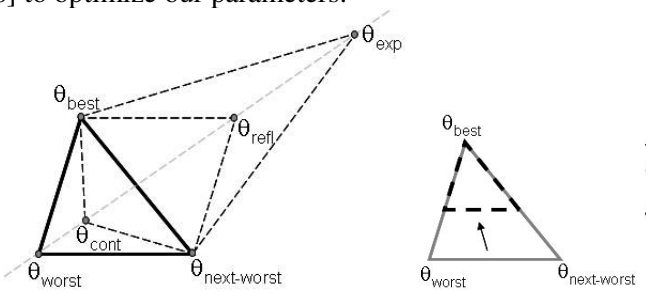
3. MODEL OPTIMIZATION

By model optimization we mean finding a set of model parameter values for ArtModel which produce the maximum possible similarity score. Several simulation optimization methods are surveyed in [17], including the Nelder and Mean simplex search method.

The Nelder and Mead algorithm, introduced in [18] for the first time, has been used widely to solve parameter estimation problems for almost 40 years. Despite its age it is still the method of choice for many practitioners in the fields of statistics, engineering and the physical and medical sciences because it is straightforward to code and easy to use. Particularly, it's been used widely by researchers for simulation optimization [18-22]. It belongs to a class of methods which do not

require derivatives and which are often claimed to be robust for problems with discontinuities or where function values are noisy. This property makes it a good candidate for optimizing our stochastic in silico simulation.

There are several different versions and extensions of this optimization algorithm. We used the one described in [16] to optimize our parameters.



(a) **(b)**

Figure 3. The simplex four basic operations: (a) reflect θ_{refl}, contract θ_{cont}, expand θ_{exp} (b) Shrink

There are four basic operations used in this algorithm: reflect, contract, expand and shrink each of which is depicted for a 2D simplex in Figure 3. θ_{best},θ_{worst} and $\theta_{next\text{-}worst}$ are the best, worst and next worst vertex of the simplex. The general heuristic in this search method is to move away from the worst point toward the best.

4. EXPERIMENTS AND RESULTS

Eight chemicals from [11] (Diazepam, FK079, FK480, Quinotolast, Zidovudine, Diltiazem, FK1052, Acetaminophen) were chosen as our test drugs to be cleared in silico by simulated rat hepatocytes. *In vitro*, each compound (at a concentration of 1000 μM) had been incubated for various time periods at 37°C with freshly isolated rat hepatocytes. The cell density in the reaction mixture is reported 0.5×10^6 (cells/ml) for Diazepam, FK480, FK1052, Quinotolast and Diltiazem, 2×10^6 (cells/ml) for Zidovudine, 4×10^6 (cells/ml) for FK079 and 1×10^6 (cells/ml) for Acetaminophen.

Calculation of parameters. Because the Hepatocytes and the test drug are in the same volume of mixture,

$$\begin{aligned} C_1 &= \frac{P_1}{V} \\ C_2 &= \frac{P_2}{V} \end{aligned} \Rightarrow \frac{P_1}{P_2} = \frac{C_1}{C_2} \qquad (6)$$

For example consider FK1052:

$$\frac{P_1}{P_2} = \frac{1000(pmol/ml)}{0.5\times10^6(cells/ml)} = \frac{1000(fmol)}{500(cells)}$$

To encompass 500 cells we need 0.001ml of the mixture (V = cells/cell-density = $500/.5\times10^6$ = 0.001ml). Assuming each spot in the WanderSpace corresponds to 350×10^{-9} ml of the mixture, for 0.001ml we will need 2857 spots. So we chose the WanderSpace to be 53 by 54 (=2862). Other parameters were chosen as follows: HepDensity = 500/2857 = 0.175, TotalSoluteMass = 1000 fmol, MetabolizationProb = 0.5, BindersPerCellMin = 5, BindersPerCellMax = 10. Table 1 summarizes all the parameters values.

The parameters SoluteBindingProb and SoluteBind-ingCycles were found using the simplex method described in the previous section.

Figure 4 shows the output of the IS-HIC ArtModel using above parameterization along with *in vitro* clearance profiles of Diazepam, FK079, FK480, Quinotolast, Zidovudine, Diltizem, FK1052, and Acetaminophen. The simulation results are in a good agreement with both the mathematical model

and the in vitro data. Table 1 shows the final values of parameters which resulted in satisfactory similarity scores.

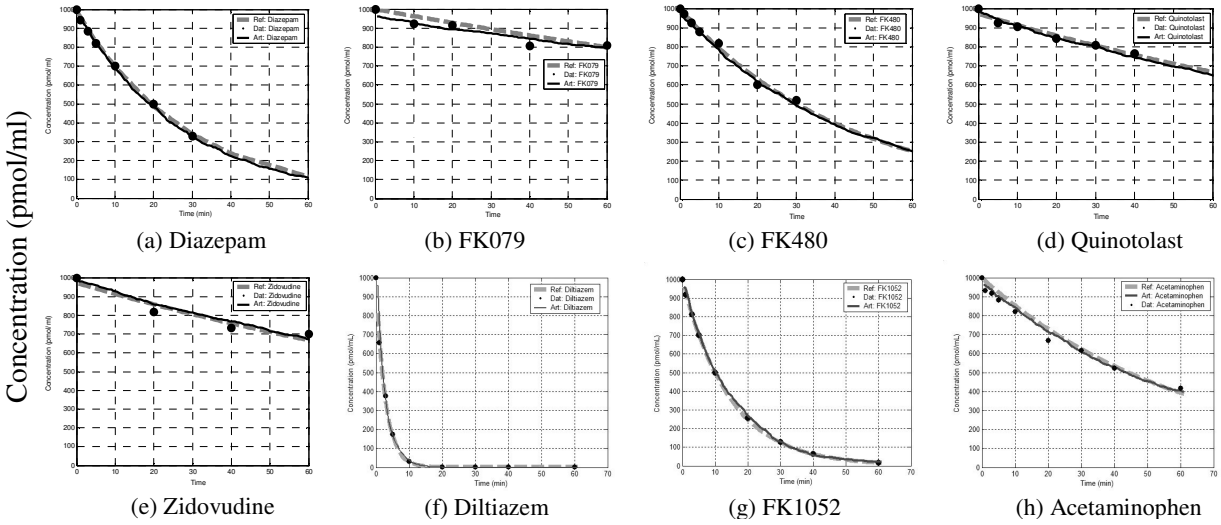


Figure 4. ArtModel outputs against RefModel and DatModel: (a) Diazepam, (b) FK079, (c) FK480, (d) Quinotolast, (e) Zidovudine, (f) Diltiazem, (g)FK1052, (h) Acetaminophen

5. CONCLUSION AND DISCUSSION

In this paper we first, demonstrated the reusability of the existing FURM-developed models to perform new in silico experiments, persisting their original assumptions; second, provided verified in silico FURM components representing hepatocytes and compound of interest that can be used in other in silico experiments. We used ISL components [9] to perform a totally new in silico experiments that provided measures of IS-HIC (in silico Hepatic Intrinsic Clearance). The IS-HIC is much simpler and faster than the ISL, because it has only three main elements: hepatocytes, test drugs and the suspension media. We validated hepatocyte models that are capable of clearing test drugs in such a way that the outputs are indistinguishable from actual *in vitro* data.

The resulting IS-HIC provides a clean measure of in silico drug clearance which does not depend on the blood flow in the liver sinusoids. However, in silico hepatocytes that have been newly parameterized and validated as above for drug *X* can be used to replace the existing hepatocytes within a separately validated ISL producing a new ISL model that can be used to estimate the expected hepatic clearance of *X*.

The stochastic nature of our models required us to use a non-gradient approach to optimizing the simulation. These methods include Nelder-Mead simplex method which has been a very popular direct search method in simulation optimization. Although this multidimensional optimization algorithm is widely used due to its simplicity and robustness, there is no proof of convergence and counter examples are known. As oppose to some other attempted optimization methods, including the Steepest Descent and Quasi-Newton methods which both failed, we found the Nelder-Mead method to be appropriate for optimizing the ISHIC parameters.

The in silico model used here is not appropriate for simulating hepatic intrinsic clearance of compounds such tolbutamide that are slowly metabolized because cell death is not accounted for in this work. In fact. Even the *in vitro* clearance of such low extraction ratio drugs is not easy to predict [14]. Sophisticated *in vitro* methods have been proposed to measure the *in vitro* intrinsic clearance of slowly metabolized drugs [14-15].

A practical limitation may be Swarm [10] itself. It is not an easy-use platform for non-computer scientists. It is designed to serve as a generic platform for modeling and simulating complex space-time dynamics. It provides a set of classes for defining an agent's properties and behaviors using computer language objective C.

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