UNIVERSIDAD SAN PABLO CEU
FACULTAD DE FARMACIA
Dpto CIENCIAS FARMACÉUTICAS Y DE LA SALUD

TESIS DOCTORAL

A SIGNAL PROCESSING APPROACH TO PHARMACOKINETICS, PHARMACODYNAMICS AND BIOPHARMACEUTICS DATA ANALYSIS

CARLOS ÓSCAR SORZANO SÁNCHEZ

Madrid, 2014

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Memoria que presenta

Carlos Óscar Sorzano Sánchez

Para optar al grado de Doctor por la Universidad CEU San Pablo

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INFORMAN:

Que la memoria para optar al grado de Doctor en Farmacia realizada por D. Carlos Óscar Sorzano Sánchez, doctor ingeniero de Telecomunicación, cuyo título es:

A SIGNAL PROCESSING APPROACH TO PHARMACOKINETICS, PHARMACODYNAMICS AND BIOPHARMACEUTICS DATA ANALYSIS

Ha sido desarrollada bajo su dirección y reúne todos los requisitos necesarios para su juicio y calificación.

Madrid, a 1 de Septiembre de 2014

Fdo. Dr. Antonio Aguilar Ros

Fdo. Dra. Consuelo Montejo Rubio

Mi más sincero agradecimiento
a todos los que de alguna forma
han contribuido al desarrollo de
este trabajo.

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Resumen

La farmacocinética es aquella parte de la materia farmacéutica que estudia la evolución temporal de la cantidad total en el cuerpo de un determinado fármaco y sus metabolitos así como su concentración en diferentes tejidos y plasma. Esta evolución es de vital importancia ya que para muchos fármacos existe una ventana terapéutica dentro de la cual son efectivos (por debajo de una determinada concentración el fármaco no tiene ningún efecto; y por encima de otra concentración tiene efectos tóxicos). El conocimiento de la concentración, por sí mismo, puede ser útil para evitar efectos nocivos; sin embargo, muy probablemente, estemos también interesados en comprender los efectos fisiológicos del fármaco sobre un determinado parámetro (por ejemplo, la presión sanguínea, la temperatura corporal, o el ritmo cardiaco). Éste es el dominio de la farmacodinamia, construir un modelo del efecto fisiológico que dependa de la concentración del fármaco. Por su parte, la biofarmacia propone modelos precisos de la liberación del fármaco, su disolución y absorción. Los tres juntos (biofarmacia, farmacocinética y farmacodinamia) intentan caracterizar las propiedades LADME (Liberación, Absorción, Distribución, Metabolismo y Excreción) de un fármaco concreto así como sus efectos toxicológicos y efectos terapéuticos.

La herramienta matemática más utilizada es el modelado mediante ecuaciones diferenciales, ya que esto no sólo permite ajustar las observaciones de concentración a lo largo del tiempo, sino que además permite predecir la misma para dosis diferentes a la empleada para la determinación de los parámetros del modelo. Una vez fijado el conjunto de modelos matemáticos, el interés de esta tesis se centra sobre los aspectos técnicos del conjunto así como su generalización para incluir un mayor número de efectos. Esto contrasta con un enfoque más farmacológico que estaría orientado a los parámetros concretos de un fármaco, su distribución dentro de una población, su variación con diferentes estados fisiológicos y la interacción entre fármacos, y su comparación con otros fármacos.

La aproximación clásica a todos estos esfuerzos de modelado ha tratado cada efecto por separado, de forma que los modelos farmacocinéticos o no incluyen o incluyen modelos biofarmacéuticos muy simples. El motivo es que se han centrado fundamentalmente en el uso de fórmulas analíticas cuyos parámetros deben ser estimados a partir de muestras experimentales obtenidas en el laboratorio. Cada modelo debe ser resuelto explícitamente y debido a la complejidad de la matemática subyacente, en muchas ocasiones únicamente hay soluciones integrales para situaciones relativamente simples. Los métodos de cálculo de parámetros, además, suelen estar únicamente resueltos como casos particulares y con una formulación poco general. En oposición a esta perspectiva, en esta tesis se defiende que el modelado biofarmacéutico, farmacocinético y farmaco-

dinámico puede ser unificado desde una perspectiva de ingeniería en un marco común de señales (dosis, concentraciones y cantidad total de fármaco) y sistemas (el paciente y el sistema de medida). Las señales son simplemente funciones y distribuciones matemáticas, y se pueden utilizar para representar cualquier variación de uno o varios parámetros fisiológicos a lo largo del tiempo. Por su parte, los sistemas son todas aquellas operaciones que transforman una señal en otra. Por último, debemos tener en cuenta que normalmente no tenemos acceso a la verdadera señal subyacente sino tan sólo a una versión deteriorada de aquella. Se dice que la señal observada es ruidosa entendiendo por ruido cualquier interferencia determinista o aleatoria que modifique el valor observado de la señal que deseamos medir. El modelo unificado consistiría en un conjunto de ecuaciones diferenciales que relacionan las entradas y las salidas del sistema. El sistema continuo así construido debe ser discretizado de forma que, una vez se conozcan los parámetros del sistema, se puedan realizar simulaciones numéricas muy precisas por medios computacionales. De hecho, se propone el modelado del ruido de las medidas como el mecanismo de alcanzar la identificación de los parámetros del sistema por medio de una aproximación de máxima verosimilitud. Esta identificación se puede realizar para cualquier régimen de administración y se propone determinar los intervalos de confianza asociados a estos parámetros mediante un muestreo por *bootstrapping*.

El objetivo de esta tesis ha sido el de dotar de un escenario de sistemas discretos a los problemas encontrados en biofarmacia, farmacocinética y farmacodinamia. En este contexto, todos los problemas de estimación de parámetros se reducen a un único problema de identificación de sistemas. El problema a resolver es el mismo para cualquier tipo de sistema y se debe poder utilizar de forma conjunta con procedimientos de estadística no paramétrica que estimen la varianza y la covarianza de los paramétros del sistema, la evaluación de su bondad de ajuste, y la comparación entre sistemas alternativos. Esta aproximación también es válida para resolver problemas con parámetros que varían con el tiempo así como para analizar la distribución de estos parámetros en una población. El escenario debe ser lo suficientemente general como para poder manejar posologías a intervalos de tiempo irregulares así como para realizar el diseño de la propia posología. El marco matemático debe ser capaz de manejar dosis intra- y extra-vasculares con cualquier tipo de medidas de concentración (concentración de fármaco en plasma, orina, tejido, ...), medidas simultáneas o alternas. Además, la implementación de un nuevo modelo farmacológico en el nuevo marco debe ser lo suficientemente sencillo como para permitir que el usuario se concentre en la tarea de modelado sin necesidad de ocuparse, además, de las tareas más "administrativas" de la programación.

Desde esta perspectiva de señales, sistemas y ruido identificamos varias de las limitaciones de las aproximaciones que habitualmente se utilizan en Farmacia como son el abuso del concepto de deconvolución y el no aprovechamiento del régimen transitorio de la señal de concentración de fármaco hasta que no se alcanza el estado de equilibrio (*steady state*).

A lo largo de la tesis proporcionamos el marco de sistemas discretos (normalmente no lineales) buscado. Relacionamos este marco con conceptos normalmente utilizados en farmacocinética y farmacodinámica como los mínimos cuadrados, los mínimos cuadrados ponderados y la estimación Bayesiana, justificando teóricamente todas estas aproximaciones y explicitando sus hipótesis constitutivas. Proponemos en la tesis el empleo de algoritmos de optimización

global como pueden ser los algoritmos genéticos de forma que se superen los problemas asociados a la convergencia local de algoritmos basados en gradiente conjugado o gradiente descendiente y la consiguiente dependencia de la solución final con la primera estimación de los parámetros. Mostramos cómo estimar los intervalos de confianza de cada parámetro utilizando estadística no paramétrica (muestreo por *Bootstrapping*), cómo utilizar esta distribución para determinar posibles respuestas farmacocinéticas y farmacodinámicas por medio de la simulación de Monte Carlo, y demostramos, a través de la Matriz de Información de Fisher, que existe un límite inferior (la cota de Crámer-Rao) por debajo de la cual ningún método de ajuste, por sofisticado que sea, puede reducir la varianza de un determinado valor. De hecho, mostramos cómo la Matriz de Información de Fisher puede ser utilizada para diseñar de forma óptima los instantes de recogida de muestra de forma que se minimice la incertidumbre sobre los parámetros del sistema. En la tesis estudiamos la estabilidad, sensibilidad de primer y segundo orden de los modelos propuestos. Proponemos nuevas formas de normalizar estas sensibilidades de forma que éstas sean comparables entre parámetros con órdenes de magnitud muy diferentes. También mostramos cómo se puede calcular la sensibilidad en el caso de no disponer de una fórmula analítica de la respuesta farmacocinética a partir del análisis de la propia ecuación diferencial que define el modelo. Durante el proceso de discretización, debemos prestar especial atención al periodo de muestreo de la señal analógica. Éste es un concepto normalmente no contemplado en los textos farmacéuticos y que, sin embargo, puede comprometer seriamente la precisión y la estabilidad de las predicciones de los modelos.

También mostramos cómo plantear todos estos conceptos en los problemas concernientes a la farmacocinética (farmacocinética lineal, modelos compartimentales, diferentes modelos de aclaramiento y regeneración de moléculas, así como el escalado alométrico, farmacocinética no lineal, saturación enzimática, inducción e inhibición enzimática, efectos sobre el flujo sanguíneo, unión a proteínas, modelos de metabolitos y velocidad de reacción), a la farmacodinamia (efecto inducido y unión a receptores, modelos genéricos, modelos indirectos, transducción y compartimentos de tránsito, modelos de desarrollo de tolerancia y rebote, y modelos de efectos fisiológicos discretos), y la biofarmacia (difusión, disolución y absorción). En aquellos puntos donde es posible hemos generalizado diferentes modelos, habitualmente presentados como modelos independientes, y demostramos que todos son casos particulares de un modelo más general. Adicionalmente, hemos provisto de un modelo dinámico basado en ecuaciones diferenciales a muchos efectos que en el ámbito farmacéutico se tratan únicamente desde un punto de vista estático (una vez que se alcanza el equilibrio). Nuestra formulación permite el análisis del transitorio de dichas variables. En este proceso hemos descubierto algunas ecuaciones que definen sistemas variables en el tiempo definidos por ecuaciones diferenciales no autónomas (algo no muy habitual en el mundo de la ingeniería).

En la Sección de Resultados mostramos la aplicación de todos estos principios a ejemplos concretos de modelado y estimación de parámetros en diferentes aplicaciones de farmacocinética, farmacocinética clínica, toxicocinética, farmacodinámica y biofarmacia. Estos resultados muestran cómo el nuevo marco de análisis abre la puerta al estudio de situaciones anteriormente vedadas a las herramientas tradicionalmente utilizadas en la práctica farmacéutica como pueden ser el ajuste simultaneo a dos vías de administración, el análisis con parámetros

variables con el tiempo, y el análisis de sistemas fuertemente no lineales. De forma destacada, en la Sección 4.7 se desarrolla un modelo muy complejo que unifica estas tres disciplinas en un único marco teórico. Por último, se muestran posibles errores de modelado que normalmente no son comentados en los textos farmacéuticos.

En el Apéndice se muestra un artículo actualmente en revisión sobre el uso de la Matriz de Información de Fisher para la determinación de los instantes óptimos de muestreo en un fármaco del que se conoce la distribución poblacional de sus parámetros farmacocinéticos y en los que no se tienen acceso a otros parámetros que también afectan a la respuesta (*nuisance parameters*).

Chapter 1

Introduction

1.1 The pharmaceutical problem

Pharmacokinetics refers to the study of the time evolution of the amount of a certain drug in the body as well as its concentration at different tissues and plasma. This evolution is of crucial importance because for many drugs there is a therapeutic window within which the drug is effective (below a certain concentration, the drug has no effect; and above a certain concentration, the drug may become toxic). Concentration alone may be useful to avoid toxicologic effects; however, we are, most likely, also interested in understanding the physiological effect of the drug on a certain parameter (e.g., blood pressure, temperature, or heart rate). This is the domain of **pharmacodynamics**, which constructs a model of the physiological effect that depends on the drug concentration. Accurate models for the drug release, dissolution and absorption can be given from **biopharmaceutics**. Altogether the field aims to characterize the ADME (Absorption, Distribution, Metabolization, and Excretion) features of a particular drug as well as its toxicological and therapeutic effects.

The classical approach to all these modelling efforts has addressed each effect separately. It has also focused on giving closed-form formulas whose parameters can be estimated from experimental samples obtained at the laboratory (see Fig. 1.1). Each case has to be explicitly solved, and due to the complexity of the underlying mathematics, many times there are only formulas for relatively simple situations. In contrast to this view we defend that biopharmaceutical, pharmacokinetical and pharmacodynamical modelling can be jointly unified from an engineering perspective into a common signals and systems framework. The unified model consists of a set of differential equations relating inputs and outputs to the system. The continuous system constructed above is then discretized so that accurate numerical simulations can be performed once the system parameters are known. In fact, to identify the system parameters, a system identification is proposed in which measurement noise is modelled, and according to this model the most likely system parameters can be determined. This identification can be performed for any arbitrary dosing regime and empirical confidence intervals can be determined.

In the following pages we discuss certain aspects not specifically covered in the thesis but that can be easily derived from the framework proposed or that

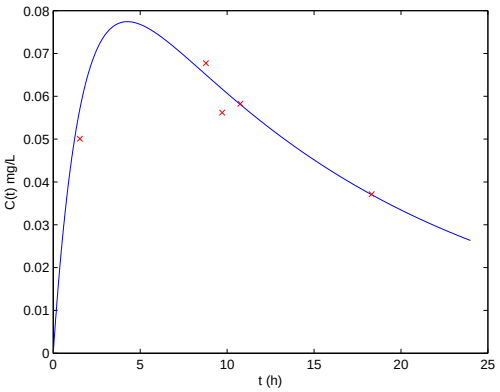

Figure 1.1: Example of drug concentration in plasma and its samples for an oral dosis.

are superseded by the framework.

Non-compartmental pharmacokinetics

Non-compartmental pharmacokinetics has currently found its application niche for evaluating drug exposure. This approach does not need any model for the concentration and it exclusively relies on the experimental samples to estimate parameters such as the Mean Residence Time (MRT; the mean time that a drug molecule is inside the body), the Mean Absorption Time (MAT; the mean time that takes the drug to pass into the body), the Clearance (the amount of plasma whose drug is eliminated by unit time), etc. In all these analysis the Area-under-the-curve (AUC) plays a central role (see Fig. 1.2). However, accurate measurement of this area requires a fine sampling of the curve (many measurements) and require extrapolation techniques for the area after the last experimental sample. Extrapolation is normally based on the assumption of an exponential decay whose constant has to be estimated from the final part of the curve. The main criticisms to this approach are that it is only valid for linear systems (nonlinear effects cannot be handled), the AUC has no physiological meaning and it confounds clearance and dose. Additionally, the formulas to calculate MRT are only valid in specific administration regimes (single bolus, constant rate infusion, etc.) and they are normally given as recipes.

Regression modelling

Alternatively, we may fit a suitable curve with few parameters and estimate the AUC from this curve. In the example of Fig. 1.2, an appropriate function is

$$C(t) = C_0 \left(e^{-Kt} - e^{-K_a t}\right) \tag{1.1}$$

By standard nonlinear regression we may estimate C_0, K and K_a. As in the previous case, the function does not have any physiological meaning and all its merit is that it has a low error when approximating the experimental samples. In nonlinear regression it is very important to have reasonable initial values for the model parameters and there are "magic" formulas to estimate them.

Linear compartmental modelling

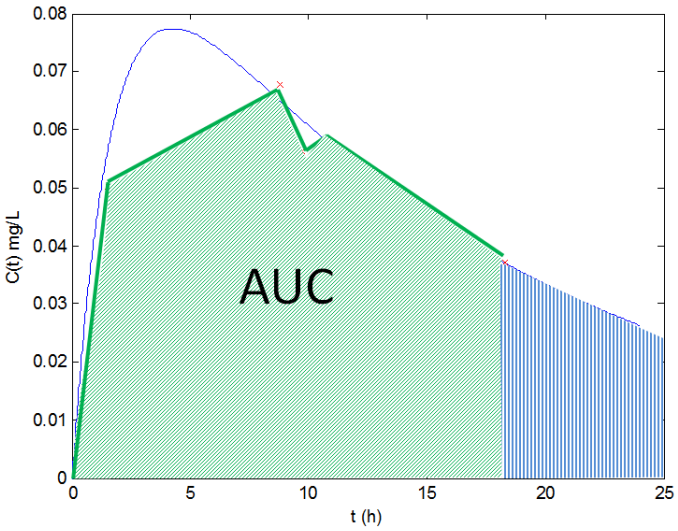

Figure 1.2: Noncompartmental analysis of the concentration profile in Fig. 1.1. AUC represents an estimate of the Area-under-the-curve from the experimental samples. Note that the area after the last sample has to be extrapolated.

Compartmental modelling assumes that the body can be divided in different compartments within which the drug concentration is constant. For instance, all the circulatory system may be a compartment (normally referred to as the central compartment). Some organs with large blood flows are also normally considered to be included in the central compartment. Other tissues with less blood flow are normally included into one or several peripheral compartments. Then, we must write a differential equation describing the evolution over time of the concentration within each compartment. Actually, Eq. (1.1) is the solution of the differential equation

$$\frac{dC(t)}{dt} = \frac{K_a F D_{po}}{V} e^{-K_a t} - KC(t) \tag{1.2}$$

This equation models the absorption of an oral dose, e.g. at the intestine. K_a is the absorption rate, F is the fraction of drug that actually gets into the circulatory system, V is the volume of the compartment (which does not necessarily coincide with the volume of blood due to plasma protein binding and other effects discussed along the thesis), and K is the elimination rate. All these parameters are further discussed in the thesis chapters. In Eq. (1.1), C_0 represents $C_0 = \frac{K_a F D_{po}}{V(K_a - K)}$. The differential equation above is linear and that is why the methodology is called linear compartmental modelling.

Compartmental modelling is based on a physiological modelling of the drug kinetics which is a clear advantange over other models since the model parameters intuitively represent physiological mechanisms. However, in its traditional treatment, only those cases for which there exists a closed-form solution of the differential equation are studied. This strongly narrows the possibilities of ex-

ploring irregular doses regimes and limits the analysis of physiological processes such as enzyme mediated reactions at different levels. As in the previous cases, "magic formulas" are given to provide initial estimates of the model parameters. The amount of formulas needed to deal with these models can dauntingly grow, and traditionally, compartmental modelling analysis is based on a huge list of particular cases.

In fact, this was the motivation of the present thesis: to unify all these particular cases into a few general formulas that can handle multiple physiological effects and any dosing regime. At the same time, all initial value formulas have been substituted by intervals of feasibility and powerful global optimization algorithms are employed to find the model parameters within these intervals. As a result of the identification methodology, we may even empirically provide confidence intervals for the modelling parameters.

Nonlinear compartmental modelling

Nonlinear compartmental modelling aims at faithfully modelling physiological effects by incorporating different aspects that make the differential equation to become nonlinear. Linearity or nonlinearity is a technical, mathematical aspect. At this point it suffices to point out that nonlinear differential equations are more difficult to solve and they require more accurate numerical approximations to guarantee their stability. One of the most important nonlinear effects being modelled is the fact that many biochemical reactions are mediated by enzymes, whose maximum processivity rate is normally bounded by its concentration and that the reaction speed normally depends on the concentration of the ligands involved. This directly translates into a concentration dependence of the absorption and elimination rates, which are no longer constants but varying over time. The framework developed in this thesis is capable of handling any nonlinear effect that we may desire to model.

Population studies

Note that all the modelling above refers to estimating the model parameters for a particular concentration profile from samples drawn from a single individual (see Fig. 1.1). It is expected that different individuals may respond differently to the same dosis, in concentration as well as on physiological effects. This can be easily modelled by giving the statistical distribution of the model parameters for a given population. For doing so, we would need to find the parameters for different individuals and gather all those parameter estimates into a single statistical distribution. Interestingly, we may even classify the population into responders and non-responders to the therapy and use the model parameters to perform this classification.

Exposure

Body exposure to a particular drug is an important parameter when trying to determine the drug toxicological and therapeutical effects. There have recently been several definitions of exposure all of them reasonable and acceptable. It has been defined as: 1) the stedy state concentration of the drug after regular intakes; 2) the maximum concentration after a single intake; 3) the AUC after a single intake; 4) area under the concentration profile and above the minimum therapeutic concentration. We may apply all these definitions to the study of the total drug concentration or the free drug concentration (those drug molecules that are not bound and sequestered, consequently without any therapeutic or toxic effect). In any case, the methodology proposed in these cases allows calculating any of the definitions above. Since the drug concentra-

tion (total or unbound) can be readily by simulated after system identification, we simply need to measure the desired quantity to determine exposure.

Inter-species scaling

Many drugs are first tested in animals, in fact the FDA regulations require testing in two roedents and one non-roedent before testing in humans, to characterize their therapeutic and toxic effects. The effective doses used for animals have to be scaled to humans so that in the first experiments with humans we never induce any adverse effect (although we may not induce any beneficial effect either). Then, the dose is progressively increased. The more accurate we predict the effective doses needed for humans, the less experiments we will need in clinical trials and the more cost effective will be our process. Inter-species scaling deals with the problem of predicting several pharmacokinetical parameters from one species to another. This scaling is mostly based on the body weight of the individuals.

1.2 Numerical solution of differential equations

The approach adopted in this thesis is tightly related to the numerical solution of differential equations. Conceptually, discrete systems (the ones proposed in this thesis to solve pharmacokinetics and pharmacodynamics problems) can be viewed as a simple change of notation in the traditional field of numerical solution of differential equations. Although this is true, it is only partially true since discrete systems have given raise to a whole theory of its own, and not only as numerical solutions of differential equations. In fact, this theory is what makes our current world to be so "digital". In the following paragraphs let us dive deeper in the concepts traditionally involved in the numerical approximation of differential equations.

Ordinary differential equations date back to the XVIIth century and they were independently invented by the English physicist Isaac Newton and the German mathematician Gottfried Leibniz about 1680 (Archibald et al., 2004). Except in very limited occasions, differential equations do not have a closed-form solution as in Eqs. (1.2) and (1.1). Alternatively, the field of applied mathematics have developed approximate tools to handle the rest of cases. In general, they approximate the solution $C(t)$ as a Taylor series around a given point (in the following example around $t = 0$):

$$C(t) = \sum_{n=0}^{\infty} a_n t^n \tag{1.3}$$

whose differential is

$$\frac{dC(t)}{dt} = \sum_{n=0}^{\infty} n a_n t^{n-1} \tag{1.4}$$

Then, the homogeneous differential equation (Eq. (1.2)) becomes

$$\begin{gathered} \sum_{n=0}^{\infty} n a_n t^{n-1} = -K \sum_{n=0}^{\infty} a_n t^n \\ 0a_0 t^{-1} + 1a_1 t^0 + 2a_2 t^1 + 3a_3 t^2 + ... = -K(a_0 t^0 + a_1 t^1 + a_2 t^2 + ...) \end{gathered} \tag{1.5}$$

or what is the same, a_0 can be any arbitrary constant because from the term at t^{-1} we learn that $0a_0 = 0$, and for $n \geq 1$

$$(n+1)a_{n+1} = -Ka_n \Rightarrow a_{n+1} = -\frac{K}{n+1}a_n \tag{1.6}$$

However, our differential equation (Eq. (1.2)) is non-homogeneous. We could use the technique of dominant balance. However, note that this technique is rather inconvenient because:

- This solution is only valid within the convergence radius of the Taylor expansion. Beyond that time point, we need to re-expand around a point within the convergence radius. In this way we can progress along the time axis.
- The convergence of the Taylor sum may be rather slow, especially for points far from the expansion time point.
- The recurrence equation (Eq. 1.6) may not be easily to solve, particularly for non-linear equations.

An alternative approach, and the one followed in this thesis, proposes to discretize the differential equation. Discretization is the process by which we transform a continuous function into a discrete function. A continuous function depends on a continuous variable $t \in \mathbb{R}$:

$$f(t) : \mathbb{R} \to \mathbb{R} \tag{1.7}$$

A discrete function depends on a discrete variable $n \in \mathbb{Z}$

$$f[n] : \mathbb{Z} \to \mathbb{R} \tag{1.8}$$

We normally make coincide the discrete function $f[n]$ with values of the continuous one

$$f[n] = f(nT_s) \tag{1.9}$$

where T_s is called the sampling rate and is measured in time units. The discretization is based on the Taylor expansion of the function $f(t)$

$$f(t-h) = f(t) + f'(t)(-h) + O(h^2) \tag{1.10}$$

where $O(h^2)$ are terms that depend on $h^2, h^3, h^4, ...$ and they are called second order term, third order term, etc. If h is sufficiently small (for instance if h is much smaller than 1), these terms tend to be much smaller than the first order term and can be disregarded. Solving for $f'(t)$ we would have a simple way of approximating the derivative

$$f'(t) = \frac{f(t) - f(t-h)}{h} + \frac{O(h^2)}{h} = \frac{f(t) - f(t-h)}{h} + O(h) \tag{1.11}$$

If we make $T_s = h$ and take into account Eq. (1.9) we would have

$$f'(t) = \frac{f[n] - f[n-1]}{T_s} + O(T_s) \tag{1.12}$$

We may increase the accuracy of this approximation by choosing more elements in the Taylor expansion and more sample points. For instance:

$$\begin{array}{l} f(t-h) = f(t) + f'(t)(-h) + \frac{f''(t)}{2}(-h)^2 + O(h^3) \\ f(t-2h) = f(t) + f'(t)(-2h) + \frac{f''(t)}{2}(-2h)^2 + O(h^3) \end{array} \tag{1.13}$$

Let us now multiply the first equation by a_1 and the second by a_2

$$\begin{array}{l} a_1 f(t-h) = a_1 f(t) + a_1 f'(t)(-h) + a_1 \frac{f''(t)}{2}(-h)^2 + a_1 O(h^3) \\ a_2 f(t-2h) = a_2 f(t) + a_2 f'(t)(-2h) + a_2 \frac{f''(t)}{2}(-2h)^2 + a_2 O(h^3) \end{array} \tag{1.14}$$

and now let us sum both equations

$$a_1 f(t-h) + a_2 f(t-2h) = (a_1+a_2) f(t) - h(a_1+2a_2) f'(t) + \frac{h^2}{2}(a_1+4a_2) f''(t) + O(h^3) \tag{1.15}$$

We may set the coefficient going with $f'(t)$ to 1 and the one going with $f''(t)$ to 0

$$\begin{array}{rcl} a_1 + 2a_2 & = & 1 \\ a_1 + 4a_2 & = & 0 \end{array} \tag{1.16}$$

whose solution is $a_1 = 2$ and $a_2 = -\frac{1}{2}$. the we have

$$\begin{array}{l} 2f(t-h) - \frac{1}{2} f(t-2h) = \frac{3}{2} f(t) - h f'(t) + O(h^3) \Rightarrow \\ f'(t) = \frac{3f(t) - 4f(t-h) + f(t-2h)}{2h} + O(h^2) \end{array} \tag{1.17}$$

As we did before, a more accurate approximation is obtained by the discretization

$$f'(t) = \frac{3f[n] - 4f[n-1] + f[n-2]}{T_s} + O(T_s^2) \tag{1.18}$$

The differential equation Eq. (1.2) becomes

$$\frac{3C[n] - 4C[n-1] + C[n-2]}{T_s} = \frac{K_a F D_{po}}{V} e^{-K_a n T_s} - K C[n] \tag{1.19}$$

At this point we may solve for $C[n]$

$$C[n] = \frac{1}{3 + K T_s} \left(4C[n-1] - C[n-2] + T_s \frac{K_a F D_{po}}{V} e^{-K_a n T_s} \right) \tag{1.20}$$

This recursion is valid for $n \geq 0$ since we are assuming that the extravascular dose is given at $t = 0$. Consequently, $C(t) = 0$ for $t < 0$, which is referred to as the rest condition. Consequently, $C[n] = 0$ for $n < 0$. The rest condition allows us to calculate $C[0]$ using $C[-1] = C[-2] = 0$.

At Eq. (1.19) we inadvertedly have already taken a decision that results in what is called an implicit formula. It is implicit because to have a recursion formula we have needed to solve for $C[n]$ in Eq. (1.19). In this case, it was relatively easy, but it might be rather difficult in certain nonlinear cases. Explicit and implicit schemes come after the following reasoning. Many of the equations we deal with in pharmacokinetics and pharmacodynamics can be written in the form

$$y'(t) = F(t, y(t)) \tag{1.21}$$

For the moment, let us consider the first order, backward approximation of the derivative $y'(t) \approx \frac{y(t)-y(t-h)}{h}$. If we discretize the differential equation as

$$\frac{y(t)-y(t-h)}{h} = F(t, y(t)) \Rightarrow y(t) = y(t-h) + hF(t, y(t)) \tag{1.22}$$

To have a recursion formula we need to solve for $y(t)$ in the equation above. This may not be an easy task if F is a non-linear function in y. Let us now consider the first order, forward approximation of the derivative $y'(t) \approx \frac{y(t+h)-y(t)}{h}$. The discretized differential equation becomes

$$\frac{y(t+h)-y(t)}{h} = F(t, y(t)) \Rightarrow y(t+h) = y(t) + hF(t, y(t)) \tag{1.23}$$

The recursion is now much more straightforward.

We may get an explicit discretization of the differential equation at Eq. (1.2) using the centered second order first derivative approximation $y'(t) = \frac{y(t+h)-y(t-h)}{2h} + O(h^2)$. In this particular case, we would have

$$\frac{C[n+1]-C[n-1]}{2T_s} = \frac{K_a F D_{po}}{V} e^{-K_a n T_s} - KC[n] \tag{1.24}$$

which gives the recursion

$$C[n+1] = C[n-1] + 2T_s \frac{K_a F D_{po}}{V} e^{-K_a n T_s} - 2KT_s C[n] \tag{1.25}$$

If we change n by $n-1$ in the equation above we get

$$C[n] = C[n-2] + 2T_s \frac{K_a F D_{po}}{V} e^{-K_a (n-1) T_s} - 2KT_s C[n-1] \tag{1.26}$$

Compare Eqs. (1.20) and (1.26). They are supposed to give different approximations to the same differential equation. However, they have different numerical properties. Implicit methods are, maybe, much more stable than explicit methods (if this is the case, the differential equation is said to be *stiff*). The theory of stability of differential equations is well beyond the scope of this thesis. It suffices to know that explicit methods can be made more stable by choosing a sampling rate, T_s, sufficiently small. In this thesis, we promote the use of implicit methods. Additionally, since the computational burden is relatively low, we can afford to have very small sampling rates, T_s in the order of 1 minute, as compared to the rate at which physiological variables are supposed to change.

Chapter 2

Objectives

The objective of this thesis is to set the problems encountered in biopharmacy, pharmakokinetics and pharmacodynamics in a discrete system scenario. In this setting, all estimation problems reduce to a single system identification problem. This problem is the same for any kind of system and can be used in conjunction with non-parametric statistical procedures to estimate the variance and covariance of the system parameters, the evaluation of its goodness of fit, and the comparison among competing systems. This approach is also valid to solve problems with time varying parameters as well as population parameters. The developed framework must be general enough to deal with arbitrary dosing regimens as well as for the design of the posology itself. Also, the framework must deal simultaneously with extravascular and intravascular doses and with any kind of drug concentration measurement (drug concentration at plasma, urine, tissue, ...) including alternative measurements at different time points.

Being so general, the framework should lend itself to relatively simple computer implementations that allows the practitioner to concentrate on the modelling problem disregarding most "house-keeping" functions that should be provided by the generic framework.

Chapter 3

Materials and methods

3.1 Materials

The material required for this thesis is a computer and any programming language. For simplicity, we will make use of MATLAB (`http://www.mathworks.com/products/matlab`) for the design of the Graphical User Interfaces (GUIs) and for the availability of its Global Optimization Toolbox (`http://www.mathworks.com/products/global-optimization`) and Optimization Toolbox (`http://www.mathworks.com/products/optimization`). Heavy calculations associated to the model will be performed in C++ and compiled with any compiler (in Windows we have compiled with Microsoft Visual C++ and in Linux with the GNU gcc compiler). The C++ routines are bound to Matlab through the use of MEX files.

3.2 Methods

The discretization proposed in this thesis along with the system simulation and system identification approach proposed can be considered as the single method of the thesis. As a rude approximation we may describe the method as:

1. Step 1: Write the differential equations describing the dynamics of the system being analyzed including the doses as an external excitation of the continuous system. In general, the set will describe a non-linear, non-homogeneous, multiple-input, multiple-output, continuous system.

2. Step 2: Discretize the differential equation by a set of difference equations. In general, the set will describe a non-linear, non-homogeneous, multiple-input, multiple-output, discrete system.

3. Step 3: Identify the system parameters by minimizing with respect to the system parameters the likelihood of the observed concentrations (the system output) given knowledge about the system input (doses). This system identification step is independent of the type of system and can be employed to estimate the variance and covariance of the system parameters as well as empirical distributions of these parameters in a given population of individuals.

Once the system is identified (estimates for its parameters are given), the system can be simulated in order to distinguish among different models or to design a specific posology.

An important consequence of this method is that the three fields (biopharmaceutics, pharmacokinetics, and pharmacodynamics) collapse into a single discipline and most models normally studied in the literature become variations of a single theme. The modelling process becomes a highly modular task in which all our biological knowledge or assumptions go into Step 1. Step 2 is normally largely disregarded in the standard pharmaceutical approach which prefers the closed-form solution of the model at Step 1. However, this second approach severely limits the complexity of the systems to analyze as well as the dose regimens utilized. These are not limitations any longer in the approach defended in the thesis. Finally, Step 3 finds the parameters of the model by finding a suitable optimization approach that maximizes some goal function normally derived under fixed statistical assumptions on the measurement errors (another aspect that is normally omitted in the standard pharmaceutical literature). In this thesis we prefer a global+local optimizer approach, while in the standard pharmaceutical literature it is preferred a local approach with *ad hoc* initial values for the parameters. Although equivalent, again this second approach limits its applicability to relatively simple cases.

Being in summary relatively simple, most of the thesis is devoted to slowly present this methodology and to show how to incorporate different physiological, biochemical and biophysical effects by the selection of the appropriate differential equation. We may consider the different thesis sections as the building blocks that have to be combined in order to construct a complex PKPD model. In many cases, especially at the beginning, while the discretization methodology is not well-established yet, we show how to discretize the differential equations. As the text progresses, we assume that the discretization methodology is already known and many times we omit this step. In the Results section, we show several examples in which this methodology is applied. Particularly interesting is a complete example in which building blocks from biopharmaceutics, pharmacokinetics and pharmacodynamics are combined in a single model. However, the defended methodology does not need to model the whole system, it may be used only to model a particular part of the system (biopharmaceutics or pharmacokinetics or pharmacodynamics, or any combination of them).

3.3 Linear compartmental pharmacokinetics

3.3.1 One-compartment intravenous bolus

Maybe, this is the simplest model we can address. The drug is administered directly into the blood stream at time $t = 0$, it is assumed to immediately distribute in the whole **distribution volume** and its disappearance is related to the elimination by some (unspecified) mechanisms. The model is fully explained in (Gabrielsson and Weiner, 2007)[Section 2.2].

Drug concentration at time 0 can be calculated as the ratio between the intravenous bolus and the distribution volume

$$C(0) = \frac{Dose_{iv}}{V} \tag{3.1}$$

This initial concentration decays over time as the drug is cleared. The clearance model is assumed to follow a first order differential equation that states that the variation of the amount of drug in the body ($A_b(t)$) over time decreases since a small volume of blood (denoted as Cl, **Clearance**) is totally cleared. Note that the amount of drug in this small volume is $ClC(t)$ where $C(t)$ is the concentration at a given time point:

$$\frac{dA_b(t)}{dt} = -ClC(t) \tag{3.2}$$

Since the concentration in the body is the total amount of drug in the body over the distribution volume, we have

$$\frac{dC(t)}{dt} = \frac{1}{V}\frac{dA_b(t)}{dt} = -\frac{Cl}{V}C(t) \tag{3.3}$$

Reorganizing the terms we have

$$\frac{dC(t)}{C(t)} = -\frac{Cl}{V}dt \tag{3.4}$$

So the clearance have dimensions of [Volume/time] (normally mL/min or L/min).

The solution of this differential equation is (see Fig. 3.1)

$$C(t) = C(0)e^{-\frac{Cl}{V}t} = \frac{Dose_{iv}}{V}e^{-\frac{Cl}{V}t} \tag{3.5}$$

The ratio $\frac{Cl}{V}$ is called the **elimination rate constant** (K_e) and has dimensions [1/time]. Note that the model is fully described by the distribution volume V and the clearance Cl. Note also that this concentration profile only makes sense for $t \geq 0$; otherwise, the drug concentration before giving the dose must be 0. We may write this mathematically using the so-called step function, $u(t)$. It is defined as

$$u(t) = \begin{cases} 0 & t < 0 \\ 1 & t \geq 1 \end{cases} \tag{3.6}$$

In this way, the concentration over time is

$$C(t) = \frac{Dose_{iv}}{V}e^{-\frac{Cl}{V}t}u(t) \tag{3.7}$$

From these parameters, we may derive some other interesting parameters. For instance, the drug **half-time** is the time it takes to reduce concentration by 1/2.

$$\begin{aligned} C(t_{\frac{1}{2}}) = \frac{C(0)}{2} &= C(0)e^{-\frac{Cl}{V}t_{\frac{1}{2}}} \\ \frac{1}{2} &= e^{-\frac{Cl}{V}t_{\frac{1}{2}}} \\ -\log 2 &= -\frac{Cl}{V}t_{\frac{1}{2}} \\ t_{\frac{1}{2}} &= \log 2\frac{V}{Cl} = 0.693\frac{V}{Cl} \end{aligned} \tag{3.8}$$

The **Mean Residence Time** tries to estimate the mean time that a given molecule stays in the body (Rosenbaum, 2011)[Section 10.2]. For a first order elimination model, the amount of drug eliminated in a differential period of time is (see Eq. (3.2))

$$dA_e(t) = -dA_b(t) = ClC(t)dt \tag{3.9}$$

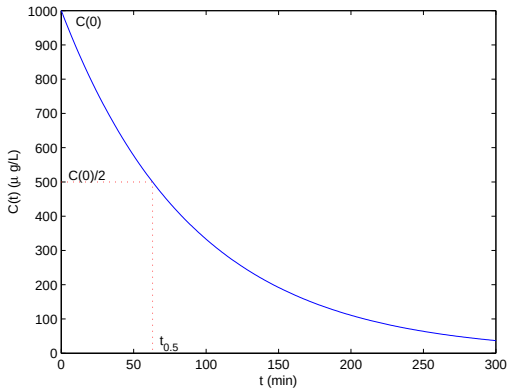

Figure 3.1: Typical response of the concentration over time for a single intravenous bolus applied at time $t = 0$.

This amount of drug has been in the body a time t (this is its residence time). The mean residence time can be calculated as the average over all molecules as

$$MRT = \frac{\int_0^\infty t(ClC(t)dt)}{\int_0^\infty ClC(t)dt} = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} = \frac{AUMC_0^\infty}{AUC_0^\infty} \tag{3.10}$$

where AUC_0^∞ and $AUMC_0^\infty$ are referred to as the Area-Under-the-Curve and Area-Under-the-first-Moment-Curve, respectively.

In this case

$$\begin{aligned} AUC_0^\infty &= \int_0^\infty C(t)dt = \int_0^\infty C(0)e^{-K_e t}dt = \frac{C(0)}{K_e} \\ AUMC_0^\infty &= \int_0^\infty tC(t)dt = \int_0^\infty tC(0)e^{-K_e t}dt = \frac{C(0)}{K_e^2} \end{aligned} \tag{3.11}$$

The Mean-Residence-Time is calculated as

$$MRT = \frac{AUMC_0^\infty}{AUC_0^\infty} = \frac{1}{K_e} \tag{3.12}$$

Classical estimation of parameters

In a real application, we need to estimate the model parameters. As we have seen in the previous section, the model is fully determined by the volume of distribution V and the clearance Cl. However, we may also estimate many other secondary parameters that are derived from this two ($AUC0^\infty$, MRT, $t_{\frac{1}{2}}$, ...)

In this model, the data available to estimate the parameters is normally the amount of drug in the injected bolus ($Dose_{iv}$) and different measures of the drug concentration along a number of time points. For instance, after injecting 10 mg of a given substance in the blood stream, its concentration over time drops as shown in the table below:

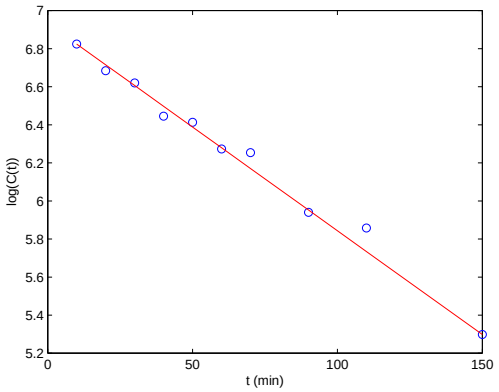

Figure 3.2: Logarithm of the concentration over time for a single intravenous bolus applied at time $t = 0$.

Time (min)	**Concentration (μg/L)**
10	920
20	800
30	750
40	630
50	610
60	530
70	520
90	380
110	350
150	200

At this point, the traditional pharmacological approach provides all kinds of different formulas to estimate the different parameters as a function of the data available. We have to admit that in the case of a single intravenous injection, this may be an easy approach due to the simplicity of the concentration response. However, this is not the general case and pharmacological applications currently addresses more complex situations in which non-linear differential equations are involved. At this point, it is illustrative to show how the traditional approach proceeds.

It is normally exploited the fact that the logarithm of the concentration (Eq. (3.5)) follows a straight line (see Fig. 3.2)

$$\log C(t) = \log C(0) - \frac{Cl}{V}t = \log C(0) - K_e t \tag{3.13}$$

The elimination rate K_e can be easily identified as the slope of the line. Let t_0 and t_F the first and last time points for which we have experimental measurements. In this particular example, $t_0 = 10$ and $t_F = 150$

$$K_e = -\frac{\log(C(t_F)) - \log(C(t_0))}{t_F - t_0} = -\frac{\log(200) - \log(920)}{150 - 10} = 0.0109\text{min}^{-1} \tag{3.14}$$

$C(0)$ can be calculated as the intercept of the straight line with the vertical axis in the logarithmic plot (see Fig. 3.2):

$$\begin{array}{rcl} \log(C(t_0)) & = & \log(C(0)) - K_e t_0 \Rightarrow \\ \log(C(0)) & = & \log(C(t_0)) + K_e t_0 \\ & = & \log(920) + 0.011 \cdot 10 \Rightarrow C(0) = 1.03 \cdot 10^3 \end{array} \tag{3.15}$$

These estimates are normally refined using a least-squares fitting, normally through a local minimization starting from these values as starting point. In this case, these two values are refined to:

$$\begin{array}{rcl} K_e & = & 0.0104\text{min}^{-1} \\ C(0) & = & 1.011 \cdot 10^3 \mu\text{g/L} \end{array} \tag{3.16}$$

We may recover the V and Cl parameters through the definition of K_e and $C(0)$:

$$\begin{array}{rcccl} C(0) & = & \frac{Dose_{iv}}{V} & \Rightarrow & V = \frac{Dose_{iv}}{C(0)} = \frac{10 \cdot 10^3}{1.011 \cdot 10^3} = 9.889\text{L} \\ K_e & = & \frac{Cl}{V} & \Rightarrow & Cl = K_e V = 0.104 \cdot 9.889 = 0.1033\text{L/min} \end{array} \tag{3.17}$$

Finally, we can calculate parameters like the drug half-time, the MRT and the AUC_0^∞:

$$\begin{array}{rcl} t_{\frac{1}{2}} & = & \frac{\log 2}{K_e} = 66.39\text{min} \\ MRT & = & \frac{1}{K_e} = 95.78\text{min} \\ AUC_0^\infty & = & \frac{C(0)}{K_e} = \frac{1.011 \cdot 10^3}{0.0104} = 96.85\text{mg} \cdot \text{ min / L} \end{array} \tag{3.18}$$

It has to be noted that the formulas shown in this section strictly applies to the case of the application of one intravenous bolus, and cannot be extrapolated to any other situation. In fact, traditional pharmacokinetics books now enter into a wide profusion of formulas to handle different cases. Instead, in this thesis we promote the adoption of a holistic approach to pharmacokinetics and pharmacodynamics through the use of the systems theory used in engineering. This theory unifies all kinds of compartimental and non-compartimental models by considering the different concentrations as output signals of a given system. From an engineering point of view a system is any device that transforms one or several input signals into one or several output signals. Mathematically, they are often described as differential equations that are discretized so that they can be implemented in a computer by a difference equation. From a pharmacokinetics point of view, a system is a tool that transforms the input to the system (the administered dose, orally, intravenously or through any other means) into different outputs (drug concentrations at different organs or locations). In the following section we briefly introduce systems and set the context in which they can be used to solve pharmacokinetics problems.

3.3.2 A signal processing approach to pharmakokinetics

Signals, ...

The concentration of a drug in the blood stream over time is a signal, the amount of cleared drug over time is a signal, the different administered drug doses are

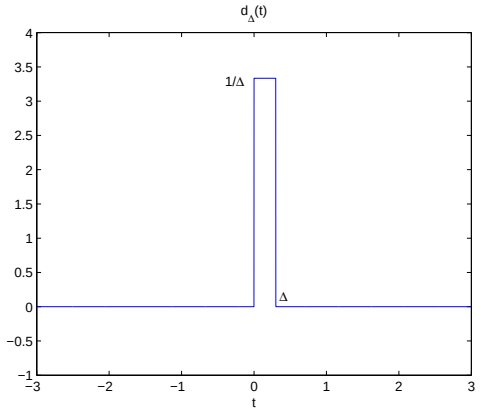

Figure 3.3: Dirac's delta is often regarded as the limit of the function $d_\Delta(t)$ when Δ goes to 0.

signals, ... In fact, a signal is any function that goes from a domain onto another domain:

$$s : U \to V \tag{3.19}$$

Of special interest in pharmacokinetics are those functions that express the variation of a physical quantity over time:

$$\begin{array}{rcl} s : \mathbb{R} & \to & \mathbb{R} \\ t & \to & s(t) \end{array} \tag{3.20}$$

for instance, the concentration of a drug in plasma $C(t)$. From a signal processing point of view, these signals are called continuous signals because the time domain varies in a continuous way (t can take any value). This implies that systems (see below) are normally described as differential equations, in which the concentration is the unknown.

Signals found as the solution of these differential equations need not be continuous (for instance, the concentration profile in Fig. 3.1 is not continuous). Instead, they are normally found in a Sobolev space (Leoni, 2009), which informally can be defined as a vector space of functions equipped with a norm that combines the L^p-norm of the function itself and its derivatives up to a given order. The functions in this space have sufficient derivatives to solve the differential equation.

Particularly important in the context of pharmacokinetics is the Dirac's delta ($\delta(t)$). This delta is not a function but a distribution (a generalized function), although it can be manipulated as if it were a function. Although incorrect, it is customary to think of it as the limit of the pulse function below when Δ goes to 0 (see Fig. 3.3):

$$d_\Delta(t) = \begin{cases} \frac{1}{\Delta} & 0 \leq t \leq \Delta \\ 0 & \text{otherwise} \end{cases} \tag{3.21}$$

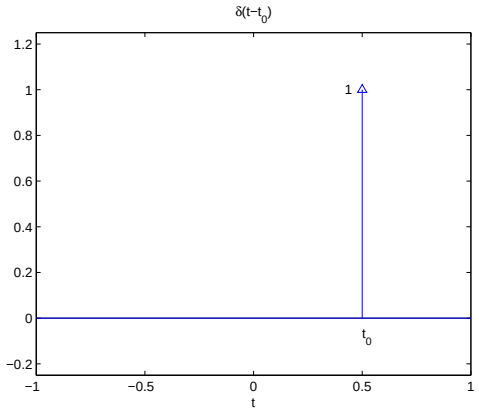

Figure 3.4: Dirac's delta shifted to the time point t_0

Two important properties of this generalized function are

$$\begin{aligned} \int_{-\infty}^{\infty} \delta(\tau)d\tau &= 1 \\ \int_{-\infty}^{\infty} x(\tau)\delta(\tau)d\tau &= x(t) \end{aligned} \tag{3.22}$$

This is one of the reasons why Dirac's delta is not a function. A function that is zero everywhere except at a single point should have a zero integral, but Dirac's delta has integral 1.

Dirac's delta is often represented with an arrow at the location it occurs. For instance, we may shift its location by simply changing its argument $\delta(t-t_0)$ (see Fig. 3.4)

The delta function is important because bolus can be described as delta functions whose amplitude is the size of the bolus and that they are shifted to the time instant at which the bolus is administered. Similarly, repeated doses can be represented as a collection of delta functions, each one of the amplitude corresponding to the bolus size. These bolus deltas will be input to the system that will translate them into different concentration profiles. For instance, Fig. 3.5 shows a fancy dosing plan. A signal processing approach has no problem in dealing with this kind of dosing plans. However, it would be impossible to derive a single equation as was performed in Section 3.3.1.

..., systems, ...

A system is any physical set that transforms one or several input signals into one or several output signals. For continuous signals, they are normally specified by a differential equation. In the case of pharmacokinetics, the system is the patient's body that transforms the drug intake (a signal as we saw in the previous section) into a drug concentration at the different tissues and blood stream (see Fig. 3.6).

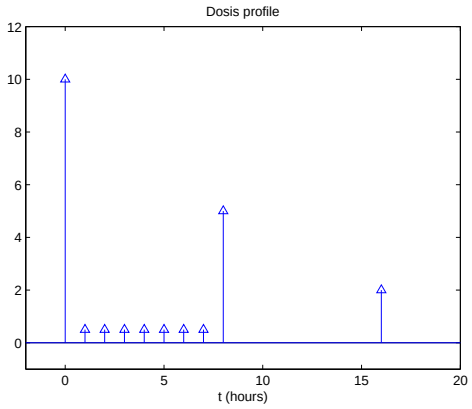

Figure 3.5: Dose plan in which 10 mg of a substance was administered at time $t = 0$; 8 hours later, 5 mg; and 8 hours laters, 2 mg. Between the first and second doses, the patient was administered 0.5 mg every hour.

Figure 3.6: A patient can be regarded as a system that transform the signal corresponding to the drug doses into a plasma concentration of that drug.

For instance, the system represented in Eq. (3.4) can be modelled as

$$\frac{dC(t)}{dt} = -\frac{Cl}{V}C(t) + \frac{dose_{iv}(t)}{V} \tag{3.23}$$

We see that Eq. (3.4) and Eq. (3.23) are rather similar. The difference is that the latter includes an input signal (the drug dose over time). In fact, a single dose at time 0 can be modelled as

$$dose_{iv}(t) = Dose_{iv}\delta(t) \tag{3.24}$$

Then, the output of the system is

$$C(t) = \frac{Dose_{iv}}{V}e^{-\frac{Cl}{V}t}u(t) \tag{3.25}$$

Exactly, the same concentration profile as in Eq. (3.5). The advantage of the model in Eq. (3.23) is that it can admit any kind of dose plan, not only a single bolus at time 0. The concentration profile is no longer computed analytically with closed forms as in Eq. (3.5) but numerically with the help of a computer.

To be able to use a computer program to solve the problem, we need to discretize the problem and go from a continuous signal (with values for any possible value of t) to a discrete approximation to this signal. This is normally done by sampling the input signal every T_s minutes. If the sampling period is small enough and all the signals in the system are bandlimited, Nyquist theorem (Oppenheim and Schafer, 2010)[Chapter 4] guarantees that the original continuous signal can be recovered exactly. For any continuous signal, $x_c(t)$, its sampled discrete version is defined as

$$x[n] = x_c(nT_s) \tag{3.26}$$

Continuous systems can be approximated by discrete systems (they transform input discrete signals into discrete output signals). For instance, the system of Eq. (3.23) can be easily approximated as

$$\frac{C[n] - C[n-1]}{Ts} = -\frac{Cl}{V}C[n-1] + \frac{dose_{iv}[n]}{V} \tag{3.27}$$

Note that $\frac{Cl}{V}C(t)$ has been approximated by $\frac{Cl}{V}C[n-1]$ because its meaning is the concentration decrease due to the drug clearing. Obviously, the drug cleared is a function of the previous concentration, and not of the current concentration. Interestingly, we see that the derivative in Eq. (3.23) is approximated by a first difference

$$\frac{dC(t)}{dt} = \lim_{\Delta t \to 0}\frac{C(t) - C(t-\Delta t)}{\Delta t} \approx \frac{C[n] - C[n-1]}{T_s} \tag{3.28}$$

Reorganizing the terms in Eq. (3.27) we arrive at

$$C[n] = (1 - K_eT_s)C[n-1] + \frac{dose_{iv}[n]}{V}T_s \tag{3.29}$$

where we have used the fact that $K_e = \frac{Cl}{V}$. In this way, we have defined a recursive equation that we may use to calculate the current concentration

based on the knowledge of the previous concentration. Obviously, before the application of the bolus, the concentration is 0 (that is, $C[n] = 0$ for $n < 0$).

If we use this formula to reproduce Fig. 3.2, the error is in the order of 0.1% of the true values when $T_s = 1$min. We may achieve more accurate results by employing numerical techniques to solve differential equations. Given a differential equation of the form

$$\frac{dC(t)}{dt} = f(t, C(t)) \tag{3.30}$$

Runge-Kutta's explicit method of order 4 states that we may follow the recursion (Chapra and Canale, 2010)[Chapter 25]

$$C[n] = C[n-1] + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4) \tag{3.31}$$

with

$$\begin{array}{l} k_1 = T_s f((n-1)T_s, C[n-1]) \\ k_2 = T_s f((n-1)T_s + \frac{T_s}{2}, C[n-1] + \frac{k_1}{2}) \\ k_3 = T_s f((n-1)T_s + \frac{T_s}{2}, C[n-1] + \frac{k_2}{2}) \\ k_4 = T_s f((n-1)T_s + T_s, C[n-1] + k_3) \end{array} \tag{3.32}$$

In our case,

$$f(t, C(t)) = -K_e C(t) \tag{3.33}$$

this gives

$$\begin{array}{l} k_1 = -K_e C[n-1] T_s \\ k_2 = -\left(K_e - \frac{T_s}{2} K_e^2\right) C[n-1] T_s \\ k_3 = -\left(K_e - \frac{T_s}{2} K_e^2 + \frac{T_s^2}{4} K_e^3\right) C[n-1] T_s \\ k_4 = -\left(K_e - T_s K_e^2 + \frac{T_s^2}{2} K_e^3 - \frac{T_s^3}{4} K_e^4\right) C[n-1] T_s \end{array} \tag{3.34}$$

Combining all together, we have the recursion (compare to Eq. (3.29))

$$C[n] = \left(1 - K_e T_s + \frac{1}{2} K_e^2 T_s^2 - \frac{1}{6} K_e^3 T_s^3 + \frac{1}{24} K_e^4 T_s^4\right) C[n-1] + \frac{dose_{iv}[n]}{V} T_s \tag{3.35}$$

This time the numerical error is in the order of 10^{-9}%.

Unfortunately, explicit Runge-Kutta recursions are not A-stable (Butcher, 2008)[Chapter 35]. Implicit Runge-Kutta recursions are much better behaved in this regard. The implicit equivalent of Eq. (3.31) for order 4 is

$$C[n] = C[n-1] + b_1 k_1 + b_2 k_2 \tag{3.36}$$

with

$$\begin{array}{l} k_1 = T_s f((n-1)T_s + c_1 T_s, C[n-1] + a_{11} k_1 + a_{12} k_2) \\ k_2 = T_s f((n-1)T_s + c_2 T_s, C[n-1] + a_{21} k_1 + a_{22} k_2) \end{array} \tag{3.37}$$

and

$$\left(\begin{array}{c|cc} c_1 & a_{11} & a_{12} \\ c_2 & a_{21} & a_{22} \\ \hline & b_1 & b_2 \end{array}\right) = \left(\begin{array}{c|cc} \frac{1}{2} - \frac{\sqrt{3}}{6} & \frac{1}{4} & \frac{1}{4} - \frac{\sqrt{3}}{6} \\ \frac{1}{2} + \frac{\sqrt{3}}{6} & \frac{1}{4} + \frac{\sqrt{3}}{6} & \frac{1}{4} \\ \hline & \frac{1}{2} & \frac{1}{2} \end{array}\right) \tag{3.38}$$

This latter table is called the Butcher tableau of the Gauss-Legendre method (Butcher, 2008)[Chapter 34].

In this case in which the function f is given in Eq. (3.33), we have to solve the following equation system for k_1 and k_2:

$$\begin{aligned} k_1 &= -K_e T_s (C[n-1] + a_{11}k_1 + a_{12}k_2) \\ k_2 &= -K_e T_s (C[n-1] + a_{21}k_1 + a_{22}k_2) \end{aligned} \tag{3.39}$$

The solution is

$$\begin{aligned} k_1 &= -\frac{1+K_e T_s (a_{22}-a_{12})}{1+K_e T_s (a_{11}+a_{22})+K_e^2 T_s^2 (a_{11}a_{22}-a_{12}a_{21})} K_e T_s C[n-1] \\ k_2 &= -\frac{1+K_e T_s (a_{11}-a_{21})}{1+K_e T_s (a_{11}+a_{22})+K_e^2 T_s^2 (a_{11}a_{22}-a_{12}a_{21})} K_e T_s C[n-1] \end{aligned} \tag{3.40}$$

Let us define

$$\begin{aligned} k_1' &= \frac{1+K_e T_s (a_{22}-a_{12})}{1+K_e T_s (a_{11}+a_{22})+K_e^2 T_s^2 (a_{11}a_{22}-a_{12}a_{21})} \\ k_2' &= \frac{1+K_e T_s (a_{11}-a_{21})}{1+K_e T_s (a_{11}+a_{22})+K_e^2 T_s^2 (a_{11}a_{22}-a_{12}a_{21})} \end{aligned} \tag{3.41}$$

Then, the recursion becomes

$$C[n] = \left(1 - K_e T_s \frac{k_1' + k_2'}{2}\right) C[n-1] + \frac{dose_{iv}[n]}{V} T_s \tag{3.42}$$

The error drops down by a factor of magnitude, $10^{-10}\%$ and the recursion is guaranteed to be A-stable (Butcher, 2008)[Chapter 34].

In any case, the system is a first order system with slightly different coefficients for the recursion. If we call a_1 the coefficient multiplying the term $C[n-1]$, we have

$a_1 = 0.989600000000000$ for Eq. (3.29)
$a_1 = 0.989654080000000$ for Eq. (3.35)
$a_1 = 0.989653893009263$ for Eq. (3.42)

However, in all cases the model is of the form:

$$C[n] = a_1 C[n-1] + b_0 dose_{iv}[n] \tag{3.43}$$

Additionally, we may introduce a lag to account for the delay between the dose application and the arrival to blood. In the case of an intravenous bolus, this delay is negligible. Notwithstanding, the more general model is

$$C[n] = a_1 C[n-1] + b_0 dose_{iv}[n - n_{lag}] \tag{3.44}$$

Note that this model is a generalization of the intravenous constant rate infusion, which in traditional pharmacokinetics is treated as a separate case with its own equations for the evolution of the concentration in blood over time and its own methodology to estimate parameters. In our case, the constant rate infusion is simply achieved by setting

$$dose_{iv}[n] = R_{in} T_s u[n] \tag{3.45}$$

where R_{in} is the infusion rate in mg/min.

..., and noise

There can be a few sources of noise or imperfections in our system modelling:

- Noise related to the prescription: If the drug prescription is *"take 10 mg every 8 hours"* the patient will hardly take the dose with an exact interval of 8 hours. Most likely, there will be a time deviation of plus/minus 30 minutes, for example. In general, we may model this noise as a random number uniformly distributed with a maximum value ΔT_{max}. Therefore

$$\Delta t_{intake} \sim U(-\Delta T_{i,max}, \Delta T_{i,max}) \tag{3.46}$$

  Similarly, the dose is hardly made exactly of 10 mg of the compound. Instead, it will have a slightly different amount that can be modelled as percentage deviation that is uniformly distributed between $-A_{max}\%$ and $A_{max}\%$. In this way, the real amount is actually:

$$Dose_{actual} = Dose_{ideal}(1 + \frac{\Delta A}{100}) \tag{3.47}$$

  where

$$\Delta A \sim U(-\Delta A_{max}, \Delta A_{max}) \tag{3.48}$$

- Noise related to measurements: In the same way, blood or urine samples will hardly be taken at the specified time. If the nurse is instructed to take a blood sample every 30 minutes, it is likely that there is a small deviation of 1 or 2 minutes, while the annotated time is still 30 minutes. We can similarly model this noise in time as a normally distributed variable whose standard deviation is σ_t:

$$\Delta t_m \sim N(0, \sigma_t^2) \tag{3.49}$$

  Finally, all physical measurements have an associated uncertainty that is normally modelled as a Gaussianly distributed random variable with zero mean and standard deviation σ_m. The units of σ_m are the same as the ones for the measurement. In this case, we will consider it to be [mg/L] or ppm (parts per million).

$$\epsilon_m \sim N(0, \sigma_m^2) \tag{3.50}$$

  This noise is said to be additive because the observed concentration is presumed to be the true concentration plus some measurement error:

$$C_{obs} = C_{true} + \epsilon_m \tag{3.51}$$

  This is normally the most accepted noise and it is the model used in Least Squares (or, what is the same, Ordinary Least Squares; see next section). However, there are analytical techniques whose error is within a percentage of the drug concentration. That means that there is more measurement noise when the concentration we are trying to estimate is larger. A correct model for this measurement process would be

$$C_{obs} = C_{true}(1 + \epsilon_m) \tag{3.52}$$

where ϵ_m is a normally distributed error. In this case, the noise model is said to be multiplicative. To deal with this measurement process, we should use Weighted Least Squares (see next section). Instead of assuming that ϵ_m is normally distributed, we could assume that the distribution of $1+\epsilon_m$ is log-normal. That implies that if we take the logarithm

$$\log C_{obs} = \log C_{true} + \log(1+\epsilon_m) \tag{3.53}$$

we are back to the additive case (and consequently to the Ordinary Least Squares).

System identification

Related to the measurement noise model is system identification. The goal of this problem is to identify the parameters that define the system. In the previous example the clearance, Cl, and the distribution volume, V. Since we do not have regular samples of the concentration, but irregularly distributed samples, the problem is similar to that of curve fitting. Let us, at this point, reformulate the problem in order to have a comprenhensive overview. Given a dose regimen (input signal to the system) and the system parameters, that in general we will refer to them as Θ, the system responds with a measurable concentration $C_\Theta(t)$ where we have used Θ as subscript to emphasize the dependence of $C(t)$ with the system parameters. Let us presume that the true underlying measurement is given by the pair $(t_i^u, C_\Theta(t_i^u))$, that we will refer to as $\mathbf{X}_i^u$. However, the actual observation corresponds to a perturbed version of this truly underlying measurement

$$\mathbf{X}_i = \mathbf{X}_i^u + \boldsymbol{\epsilon}_i \tag{3.54}$$

System identification using **Maximum Likelihood** tries to identify the system parameters that maximize the likelihood of observing the ensemble of measurements (presume we have N of such measurements). If these observations are statistically independent, then the identification problem becomes

$$\hat{\Theta} = \max_{\Theta} \prod_{i=1}^{N} f(\mathbf{X}_i|\Theta) \tag{3.55}$$

Since the logarithm is a monotonic function, optimizing a function or its logarithm does not change the location of the optimum. However, in this case, the log-likelihood is normally optimized because it simplifies the mathematical expressions

$$\hat{\Theta} = \max_{\Theta} \log\left(\prod_{i=1}^{N} f(\mathbf{X}_i|\Theta)\right) = \max_{\Theta} \sum_{i=1}^{N} \log(f(\mathbf{X}_i|\Theta)) \tag{3.56}$$

The likelihood of observing a given vector $\mathbf{X}_i$ depends on its distance to the actual measurement $\mathbf{X}_i^u$ (see Fig. 3.7)

$$\boldsymbol{\epsilon}_i = \mathbf{X}_i - \mathbf{X}_i^u \tag{3.57}$$

and its probability density function $f_{\boldsymbol{\epsilon}}(\boldsymbol{\epsilon})$. However, the ideal measurement is not accessible, and we have to evaluate all possibilities

$$f(\mathbf{X}_i|\Theta) = \int_{-\infty}^{\infty} f_{\boldsymbol{\epsilon}}(\mathbf{X}_i - \mathbf{X}_i^u) d\mathbf{X}_i^u \tag{3.58}$$

Note that because the possible $\mathbf{X}_i^u$ points lie in a curve, the previous integral is a line integral and can be expressed as an integral on a single parameter

$$f(\mathbf{X}_i|\Theta) = \int_{-\infty}^{\infty} f_{\boldsymbol{\epsilon}}((t_i, Ci) - (t_i^u, C_\Theta(t_i^u)))dt_i^u \tag{3.59}$$

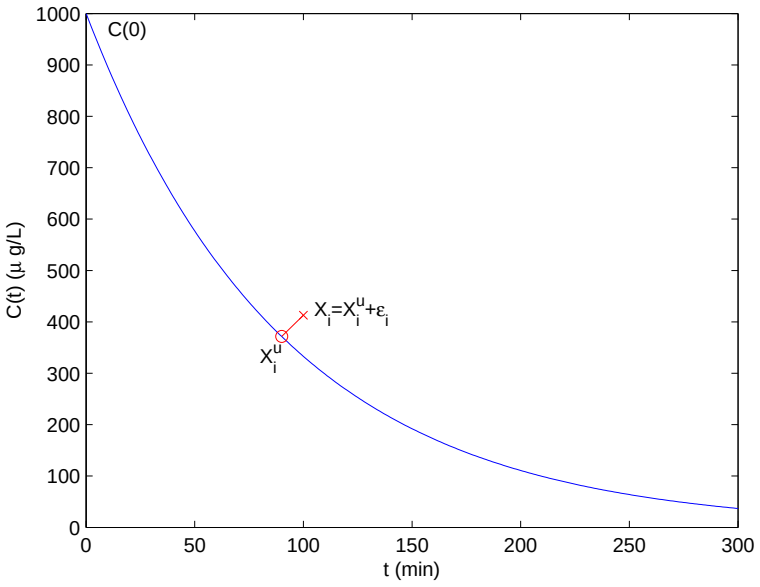

Figure 3.7: Example of concentration profile. The true measurement should have been $\mathbf{X}_i^u$. However, the observed value was $\mathbf{X}_i$

Depending on the distribution we presume for the errors, we have different optimization problems:

- Least squares (LS): If we presume that there is no error in the time variable and the measurement error is normally distributed, the statistical distribution of the error is defined by the following probability density function,

$$f_{\boldsymbol{\epsilon}}(\epsilon_t, \epsilon_m) = \delta(\epsilon_t)\frac{1}{\sqrt{2\pi\sigma_m^2}}e^{-\frac{\epsilon_m^2}{2\sigma_m^2}} \tag{3.60}$$

and the likelihood in Eq. (3.59) becomes (see Dirac's delta properties in Eq. (3.22)):

$$\begin{aligned} f(\mathbf{X}_i|\Theta) &= \int_{-\infty}^{\infty} \delta(t_i - t_i^u)\frac{1}{\sqrt{2\pi\sigma_m^2}}e^{-\frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2}}dt_i^u \\ &= \frac{1}{\sqrt{2\pi\sigma_m^2}}e^{-\frac{(C_i - C_\Theta(t_i))^2}{2\sigma_m^2}} \end{aligned} \tag{3.61}$$

Its logarithm is

$$\log f(\mathbf{X}_i|\Theta) = -\frac{1}{2}\log(2\pi\sigma_m^2) - \frac{(C_i - C_\Theta(t_i))^2}{2\sigma_m^2} \tag{3.62}$$

The optimization problem in Eq. (3.56) becomes:

$$\begin{aligned}
\hat{\Theta} &= \max_\Theta \sum_{i=1}^{N} \log(f(\mathbf{X}_i|\Theta)) \\
&= \max_\Theta \sum_{i=1}^{N} \left(-\tfrac{1}{2}\log(2\pi\sigma_m^2) - \tfrac{(C_i - C_\Theta(t_i))^2}{2\sigma_m^2}\right) \\
&= \max_\Theta \left\{-\tfrac{N}{2}\log(2\pi\sigma_m^2) - \tfrac{1}{2\sigma_m^2}\sum_{i=1}^{N}(C_i - C_\Theta(t_i))^2\right\} \\
&= \max_\Theta \left\{-\tfrac{1}{2\sigma_m^2}\sum_{i=1}^{N}(C_i - C_\Theta(t_i))^2\right\} \\
&= \max_\Theta \left\{-\sum_{i=1}^{N}(C_i - C_\Theta(t_i))^2\right\} \\
&= \min_\Theta \left\{\sum_{i=1}^{N}(C_i - C_\Theta(t_i))^2\right\}
\end{aligned} \tag{3.63}$$

This is the famous Least Squares (LS) criterion, that is, by far the most widely used optimization criterion in pharmacokinetics. In the derivation provided in this thesis we have highlighted the fact that it is the maximum likelihood estimate when we assume no errors in the time measurements and a gaussian error in the concentration. Least Squares can be extended to the case in which the variance of the measurements change over time (Banks et al., 2009).

- Total least squares (TLS): If we, now, presume a gaussian error for both measurements (time and concentration) we have

$$f_{\boldsymbol{\epsilon}}(\epsilon_t, \epsilon_m) = \frac{1}{\sqrt{2\pi\sigma_t^2}} e^{-\frac{\epsilon_t^2}{2\sigma_t^2}} \frac{1}{\sqrt{2\pi\sigma_m^2}} e^{-\frac{\epsilon_m^2}{2\sigma_m^2}} = \frac{1}{2\pi\sigma_m\sigma_t} e^{-\left(\frac{\epsilon_t^2}{2\sigma_t^2} + \frac{\epsilon_m^2}{2\sigma_m^2}\right)} \tag{3.64}$$

The likelihood of observing a given vector is

$$\begin{aligned}
f(\mathbf{X}_i|\Theta) &= \int_{-\infty}^{\infty} \tfrac{1}{2\pi\sigma_m\sigma_t} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2}\right)} dt_i^u \\
&= \tfrac{1}{2\pi\sigma_m\sigma_t} \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2}\right)} dt_i^u
\end{aligned} \tag{3.65}$$

Its logarithm is

$$\log f(\mathbf{X}_i|\Theta) = -\log(2\pi\sigma_m\sigma_t) + \log \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2}\right)} dt_i^u \tag{3.66}$$

The optimization problem in Eq. (3.56) becomes:

$$\begin{aligned}
\hat{\Theta} &= \max_{\Theta} \sum_{i=1}^{N} \log(f(\mathbf{X}_i|\Theta)) \\
&= \max_{\Theta} \left\{ \sum_{i=1}^{N} \left(-\log(2\pi\sigma_m\sigma_t) + \log \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2} \right)} dt_i^u \right) \right\} \\
&= \max_{\Theta} \left\{ -N\log(2\pi\sigma_m\sigma_t) + \sum_{i=1}^{N} \log \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2} \right)} dt_i^u \right\} \\
&= \max_{\Theta} \left\{ \sum_{i=1}^{N} \log \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2} \right)} dt_i^u \right\} \\
&= \min_{\Theta} \left\{ -\sum_{i=1}^{N} \log \int_{-\infty}^{\infty} e^{-\left(\frac{(t_i - t_i^u)^2}{2\sigma_t^2} + \frac{(C_i - C_\Theta(t_i^u))^2}{2\sigma_m^2} \right)} dt_i^u \right\}
\end{aligned} \tag{3.67}$$

Note that the parameters σ_t and σ_m are part of the model parameters and are, generally, unknown. In this way, they have to be estimated from the data itself. To do so, we can adopt an iterative approach (in fact, it is an Expectation-Maximization approach):

1. Start with some initial values of $\sigma_t^{(0)}$ and $\sigma_m^{(0)}$.
2. Construct the covariance matrix $\Sigma^{(0)} = \begin{pmatrix} \sigma_t^{(0)} & 0 \\ 0 & \sigma_m^{(0)} \end{pmatrix}$
3. Estimate the rest of parameters of Θ by the optimization in Eq. (3.67).
4. For each experimental observation $\mathbf{X}_i = (t_i, C_i)$ find the point in the curve $\mathbf{X}_i^u = (t, C_\Theta(t))$ that minimizes the Mahalanobis distance $(\mathbf{X}_i - \mathbf{X}_i^u)^T (\Sigma^{(k)})^{-1} (\mathbf{X}_i - \mathbf{X}_i^u)$
5. Reestimate the covariance matrix as $\Sigma^{(k+1)} = \sum_{i=1}^{N} (\mathbf{X}_i - \mathbf{X}_i^u)(\mathbf{X}_i - \mathbf{X}_i^u)^T$ and set the off-diagonal terms to 0.
6. Go back to Step 3 till convergence.

- Weighted Least Squares: Let us now assume the multiplicative noise model in which ϵ_m is normally distributed, and there is no noise in the time measurements. This case is much more difficult because the true values are unknown and the observed values have a distribution given by

$$C_i \sim N(C_i^u, (C_i^u \sigma_m)^2) \tag{3.68}$$

If we approximate C_i^u by our estimate $C_\Theta(t_i)$, which is our best estimate of the unknown true concentration value, then The probability density function of our measurements would become

$$f(\mathbf{X}_i|\Theta) = \frac{1}{\sqrt{2\pi(C_\Theta(t_i)\sigma_m)^2}} e^{-\frac{1}{2}\left(\frac{C_i - C_\Theta(t_i)}{C_\Theta(t_i)\sigma_m}\right)^2} \tag{3.69}$$

As we already did in the case of the Least Squares, we would have

$$\begin{aligned}
\hat{\Theta} &= \max_{\Theta} \sum_{i=1}^{N} \log(f(\mathbf{X}_i|\Theta)) \\
&= \max_{\Theta} \sum_{i=1}^{N} \left(-\tfrac{1}{2}\log(2\pi(C_\Theta(t_i)\sigma_m)^2) - \tfrac{1}{2}\left(\frac{C_i - C_\Theta(t_i)}{C_\Theta(t_i)\sigma_m}\right)^2 \right)
\end{aligned} \tag{3.70}$$

which does not fall under any standard regression framework. If we approximate C_i^u by the measurement, a noisier estimate of the unknown true concentration, then

$$\begin{aligned}\hat{\Theta} &= \max_{\Theta} \sum_{i=1}^{N} \left(-\tfrac{1}{2}\log(2\pi(C_i\sigma_m)^2) - \tfrac{1}{2}\left(\tfrac{C_i - C_\Theta(t_i)}{C_i\sigma_m}\right)^2 \right) \\ &= \min_{\Theta} \left\{ \sum_{i=1}^{N} \tfrac{(C_i - C_\Theta(t_i))^2}{C_i^2} \right\}\end{aligned} \tag{3.71}$$

that is a Weighted Least Squares (WLS) problem and it is the approach already taken in (Banks et al., 2009). If we now collect all measurements and predicted values in vectors we could write the previous objective function as

$$\hat{\Theta} = \min_{\Theta} (\mathbf{C} - \mathbf{C}_\Theta)^T W^{-1} (\mathbf{C} - \mathbf{C}_\Theta) \tag{3.72}$$

where W is a diagonal matrix whose ii-th entry is C_i^2. If W were a full matrix, then this technique would be called Generalized Least Squares and we would be explicitly accounting for correlations among different samples. There are efficient algorithms for all these problems (Bjork, 1996).

- Bayesian regression: If we have *a priori* knowledge about the distribution of the system parameters, Θ, that is, we know the probability density function $f_\Theta(\Theta)$, we would simply have to substitute the *a priori* likelihood at Eq. 3.55 by its *a posteriori* counterpart

$$\hat{\Theta} = \arg\max_{\Theta} \prod_{i=1}^{N} f(\mathbf{X}_i|\Theta) f_\Theta(\Theta) \tag{3.73}$$

If, as is generally the case, $f_\Theta(\Theta)$ is an empirical distribution represented by a sum of Gaussians or a tabulated distribution, the problem above does not have a closed-form solution as has to be numerically solved using any optimization algorithm. However, this software makes a simplification that we explain below (Lacarelle et al., 1994). As we have seen, maximizing a function or its logarithm does not change the parameters estimate since the logarithm is a monotonic function. Consequently, we may also find the parameters by maximizing

$$\begin{aligned}\hat{\Theta} &= \arg\max_{\Theta} \log \prod_{i=1}^{N} f(\mathbf{X}_i|\Theta) f_\Theta(\Theta) \\ &= \arg\max_{\Theta} \sum_{i=1}^{N} \log f(\mathbf{X}_i|\Theta) + \sum_{i=1}^{N} \log f_\Theta(\Theta)\end{aligned} \tag{3.74}$$

As we did in the case of Least Squares, above, let us assume a Gaussian distribution for the measurement noise (Eq. (3.62)) and let us assume a multivariate Gaussian distribution as the *a priori* for the parameters

$$\begin{aligned}f_\Theta(\Theta) &= (2\pi)^{-\frac{p}{2}} |\Sigma|^{-\frac{1}{2}} \exp\left(-\tfrac{1}{2}(\Theta - \boldsymbol{\mu})^T \Sigma^{-1} (\Theta - \boldsymbol{\mu})\right) \Rightarrow \\ \log f_\Theta(\Theta) &= -\tfrac{p}{2}\log(2\pi) - \tfrac{1}{2}\log|\Sigma| - \tfrac{1}{2}(\Theta - \boldsymbol{\mu})^T \Sigma^{-1} (\Theta - \boldsymbol{\mu})\end{aligned} \tag{3.75}$$

where p is the number of parameters, $\boldsymbol{\mu}$ is the expected mean of the set of parameters and Σ is its covariance matrix. Maximizing Eq. (3.74) is the

same as minimizing

$$\hat{\Theta} = \arg\min_{\Theta} \sum_{i=1}^{N} \left(\frac{C_i - C_\Theta(t_i)}{\sigma_m} \right)^2 + N(\Theta - \boldsymbol{\mu})^T \Sigma^{-1} (\Theta - \boldsymbol{\mu}) \quad (3.76)$$

If the parameters are independent, then the covariance matrix is diagonal and the minimization problem above becomes

$$\hat{\Theta} = \arg\min_{\Theta} \sum_{i=1}^{N} \left(\frac{C_i - C_\Theta(t_i)}{\sigma_m} \right)^2 + N \sum_{k=1}^{p} \left(\frac{\Theta_k - \boldsymbol{\mu}_k}{\sigma_k} \right)^2 \quad (3.77)$$

where σ_m is the standard deviation of the measurement noise and σ_p the standard deviation of the p-th parameter. Except for the N in front of the second summatory, which is normally dropped in pharmacokinetics books, this is the expression normally referred to as Bayesian estimation in the standard pharmaceutical literature (Shargel et al., 2012)[p.601], (Gabrielsson and Weiner, 2007)[p.772]. This is in fact the approach followed by Abbott's PKS pharmacokinetics software (Lacarelle et al., 1994). However, it is important noting that the formula spread in the pharmaceutical literature does not weight correctly the prior probability according to a Bayesian framework, and that it has been obtained under the simplification that the parameter distribution is a multivariate Gaussian with independent parameters. With the current computing power, these assumptions are not needed anymore and more realistic modelling can be used in which the joint distribution of parameters can be explicitly considered, or at least, it can be accurately approximated by a Gaussian mixture. The drawback of this approach is that the landscape of solutions of the optimization problem becomes much more complex and globally convergent optimization algorithms are needed.

To measure the impact of noise in the time measurements we performed the following experiment. We simulated 1,000 times the process of taking concentration samples from a one-compartment intravenous bolus (see Section 3.3.1) whose true parameters are $Cl = 0.1$ (L/min) and $V = 10$ (L). We assumed that the measurement error is $\sigma_m = 0.001$ (ppm or, equivalently, mg/L; see Eq. (3.50)). We performed three sets of experiments: in one of them we assumed that there was no time measurement noise ($\sigma_t = 0$ (min)); in the second one, we assumed that $\sigma_t = 0.5$ (min); in the third one, we assumed that $\sigma_t = 2$ (min). Note that for a Gaussian distribution the typical measurement error is between $-3\sigma_t$ and $3\sigma_t$. In Fig. 3.8 we show the 1,000 estimates of the model parameters in the three cases. We first see that the joint distribution of parameters is not Gaussian. The marginal distributions of the clearance parameter is not Gaussian. However, the hypothesis that the marginal distribution of the elimination rate constant is Gaussian is rejected in the case of $\sigma_t = 0$ (p-value=0.0455) and cannot be rejected (with a Lilliefors test of normality) in the case of $\sigma_t = 0.5$ and $\sigma_t = 2$. In any case, we see that the average parameter values are unbiased in the three cases (with a precision between 0.02% and 0.05% of the true value), and the increase of measurement time noise is translated into a larger variance of the estimates (especially, for the distribution volume). However, the standard deviation of the two parameters is still rather low (for the clearance, it grows from 1.09% ($\sigma_t = 0$) to 1.33% ($\sigma_t = 2$); while for the distribution volume, it

grows from 0.93% ($\sigma_t = 0$) to 1.86% ($\sigma_t = 2$)). Additionally, a Kolmogorov-Smirnov test for the equality of the two marginal distributions could be rejected only for the case of $\sigma_t = 2$ and not for $\sigma_t = 0.5$. We see that the effect of an increase of measurement time noise does not translate, in this experiment, into a biased estimate of the underlying model parameters but in an increase of the estimation variance (only significant for a large time measurement noise), which is still under acceptable conditions. Consequently, we do not explore the TLS alternative any further.

Confidence intervals and correlation for model parameters

An important issue when identifying a system is to estimate the uncertainty associated to each of its parameters. In this way we determine their identifiability. Bootstrapping is a common resampling technique aiming at estimating the variance associated to each of the model parameters (Efron and Tibshirani, 1993). An interesting observation from Fig. 3.8 is that the distribution of the estimated parameters is centered with respect to the true parameters. This allows using the Bootstrap Percentile method for the estimation of confidence intervals (Efron and Tibshirani, 1993). This method goes as follows:

- Given a set of N measurements (t_i, C_i) from a response profile, we construct N_B bootstrap samples by resampling with replacement N samples from the original samples. Note that since the resampling is performed with replacement, an original sample may be repeated several times in the bootstrap sample. This is called Monte Carlo bootstrap resampling.
- We estimate the model parameters for each of the bootstrap samples using any system identification method (see Section 3.3.2). For each bootstrap sample, we will get a model parameters estimate, Θ_b, that is, we get a total of N_B model parameters estimates.
- For each model parameter, i, we construct a confidence interval of confidence level $1-\alpha$ as $[\Theta^{(i)}_{(\frac{\alpha}{2})}, \Theta^{(i)}_{(1-\frac{\alpha}{2})}]$.

Additionally, we may estimate the parameter covariance matrix from the N_B model parameters estimates as

$$\begin{aligned} \overline{\Theta} &= \tfrac{1}{N_B}\sum_{b=1}^{N_B}\Theta_b \\ C_\Theta &= \tfrac{1}{N_B-1}\sum_{b=1}^{N_B}(\Theta_b-\overline{\Theta})(\Theta_b-\overline{\Theta})^T \end{aligned} \tag{3.78}$$

and detect significant associations through the Spearman's rank correlation coefficient (Kendall, 1970), that is a non-parametric equivalent of Pearson's correlation coefficient for which we can also test its significance (Best and Roberts, 1975).

To illustrate this approach we performed the following experiment. We generated 16 samples in 8 hours from a one-compartment model with 0.1 (L/min) as cleareance and 10 (L) as distribution volume. We set the measurement error to 0.001 (mg/L) and assumed no time measurement error. Then, we estimated with 1,000 bootstrap resamplings the confidence interval for each one of the model parameters. The 95% confidence interval for the cleareance is [0.09792,0.10244]

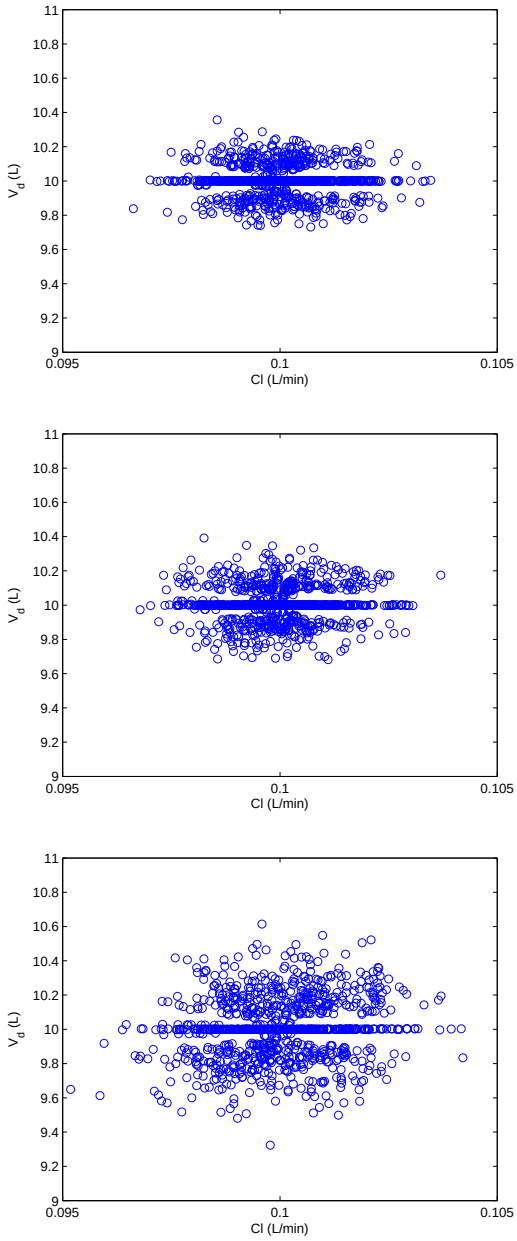

Figure 3.8: Estimates of the parameters of the one-compartment intravenous model for $\sigma_m = 0.001$ (mg/L) and $\sigma_t = 0$ (min; top), $\sigma_t = 0.5$ (min; middle) and $\sigma_t = 2$ (min; bottom).

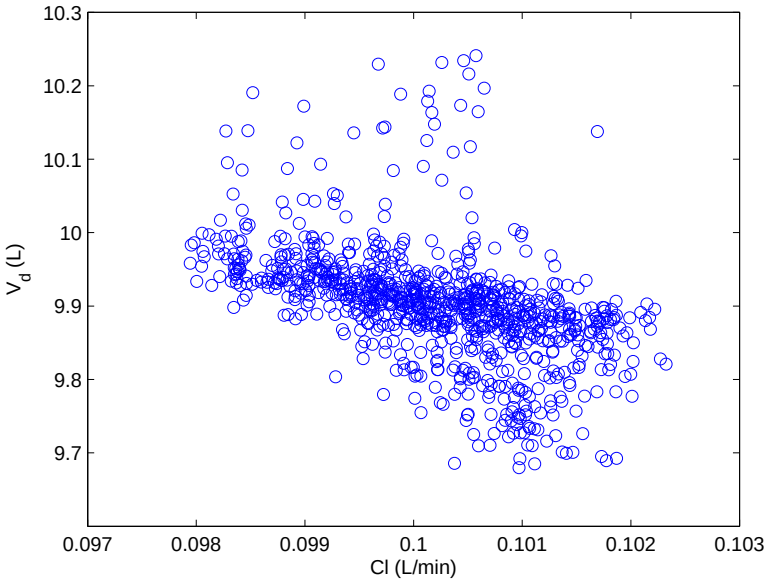

Figure 3.9: Bootstrap estimates of the parameters of the one-compartment intravenous model for $\sigma_m = 0.001$ (mg/L) and $\sigma_t = 0$. The true underlying parameters are a clearance of 0.1 (L/min) and a distribution volume of 10 (L).

and for the distribution volume [9.6730,10.2415]. Interestingly, there is a negative association between these two parameters as shown in Fig. 3.9. The Spearman's rank correlation coefficient is -0.6361 and its p-value is in the order of 10^{-105} (that is the correlation is significantly different from 0). This means that if the clearance parameter is incorrectly overestimated, the estimation method will tend to underestimate the distribution volume to compensate for this error and viceversa. In Fig. 3.9 we can see how for a particular realization of a fitting (a single dataset with only 16 measurements), the bootstrap estimation process may be biased. However, the method is not biased if the expectation of the process is considered (repeating infinite times the process of taking 16 samples from the same distribution and performing a bootstrap estimate of the confidence intervals).

Goodness of fit

In regression, there are a number of accepted measures of goodness of fit. All of them make use of the residuals of the fitting, that are defined as the difference between the observed concentrations and the predicted concentrations. In particular, at any time t_i with observed concentration C_i and predicted concentration $C_\Theta(t_i)$, the residual is

$$\epsilon_i = C_i - C_\Theta(t_i) \tag{3.79}$$

Let us assume that there are N pairs of observations (t_i, C_i) and p parameters in the model (in the example of the one-compartment, $p = 2$, clearance and

distribution volume). Below we list the most widely used measures of goodness of fit:

- Residual variance: $\hat{\sigma}_\epsilon^2 = \frac{1}{N-1}\sum_{i=1}^{N}(\epsilon_i - \bar{\epsilon})^2$.
- Coefficient of determination: $R^2 = 1 - \frac{\hat{\sigma}_\epsilon^2}{\hat{\sigma}_C^2}$, where $\hat{\sigma}_C^2$ is the variance of the concentration measurements. This represents the fraction of unexplained variance.
- Adjusted coefficient of determination: $R^2_{\text{adj}} = 1 - (1 - R^2)\frac{N-1}{N-p-1}$. The adjusted coefficient of determination is not a measure of fit but a relative comparison of the suitability of different nested models.
- Akaike's Information Criterion: $AIC = \log \hat{\sigma}_\epsilon^2 + 2\frac{p}{N}$.
- Bayesian Information Criterion: $BIC = \log \hat{\sigma}_\epsilon^2 + (\log N)\frac{p}{N}$.
- Final Prediction Error: $FPE = \frac{N+p}{N-p}\hat{\sigma}_\epsilon^2$, whose logarithm (a monotonic function of the FPE) can be written as $LFPE = \log \hat{\sigma}_\epsilon^2 + \log \frac{N+p}{N-p}$.

The goal is to minimize the residual variance (or optimize the coefficient of determination) without overfitting the data, that is not adding too many parameters to the model so that we compromise the capability of the model to generalize the underlying behaviour. In general none of them are "correct" since every measure has been derived under some assumptions that may not hold in real data. In practice, one should check all of them and choose a model that is in a sensible range provided by the different measures (for instance, AIC may favour a model with 5 parameters and BIC with 3 parameters; the true model must be somewhere between 3, 4 or 5 parameters). Generally speaking, AIC and BIC can be regarded as equivalent to a likelihood ratio test with different significance thresholds(Burnham and Anderson, 2004). AIC becomes like a significance test with $\alpha = 0.16$ while BIC has a decreasing α as the number of samples increases ($\alpha = 0.13$ for $N = 10$, $\alpha = 0.032$ for $N = 100$, $\alpha = 0.0086$ for $N = 1000$). In this way, as the number of samples increases AIC tends to prefer models with more parameters than BIC.

3.3.3 One-compartment extravascular administration

1st order absorption

Let us assume now that we administer the drug extravascularly, e.g., with an oral tablet. The amount of drug in the gut will be absorbed into the body and the amount of drug in the body will consequently increase. The absorption process involves dissolving the tablet and absorbing the drug across the gastrointestinal membranes. Additionally, part of the drug may be degraded in the intestine and more importantly at the liver (first pass effect). This gives raise to the concept of bioavailability, that is the fraction of drug that actually reaches the systemic circulation. If we look at the rate at which the amount of drug disappears from the intestine, we can write a differential equation, assuming a first order dissapearance, as

$$\frac{dA_g(t)}{dt} = -(K_a + K_d)A_g(t) \tag{3.80}$$

where K_a is the absorption coefficient and K_d is the degradation coefficient. The solution of this equation is

$$A_g(t) = D_{po}e^{-(K_a+K_d)t}u(t) \tag{3.81}$$

where D_{po} is the amount of drug in the oral (*per os*) dose (assumed to be given at time $t = 0$). If we now look at the rate of input of the drug into the body, we see that (for $t \geq 0$)

$$\frac{dA_b(t)}{dt} = K_a A_g(t) = K_a D_{po} e^{-(K_a+K_d)t} \tag{3.82}$$

We may rewrite the previous equation as

$$\frac{dA_b(t)}{dt} = (K_a + K_d)F D_{po} e^{-(K_a+K_d)t} \tag{3.83}$$

where we have defined

$$F = \frac{K_a}{K_a + K_d} \tag{3.84}$$

F is called the **bioavailability** and has no units. Ideally, we would like F to be 1, that is, there is no degradation in the administration route. However, in practice, F is normally a number between 0 and 1. With the definition of the bioavailability, we may define the concept of apparent absorption that integrates both absorption and degradation into a single constant

$$K_{app} = K_a + K_d \tag{3.85}$$

and rewrite the rate of drug input into the body as

$$\frac{dA_b(t)}{dt} = K_{app} F D_{po} e^{-K_{app}t} \tag{3.86}$$

which is a more standard way of writing the first order input system. However, we prefer its original formulation given in Eq. (3.82). Now, considering that the amount of drug into the body can be written as $A_b(t) = VC(t)$ where V is the volume of distribution and $C(t)$ the concentration. We may rewrite the previous differential equation as

$$V\frac{dC(t)}{dt} = K_a D_{po} e^{-(K_a+K_d)t} \tag{3.87}$$

To be complete, we have to add the rate at which the drug dissappears from the body, that as in the case of the intravascular administration is given by a clearance parameter

$$V\frac{dC(t)}{dt} = K_a D_{po} e^{-(K_a+K_d)t} - ClC(t) \tag{3.88}$$

This is the differential equation governing the concentration of drug in the body assuming a first order absorption and degradation in the administration route, and a first order elimination in the body (see Fig. 3.10 for a graphical representation of the model).

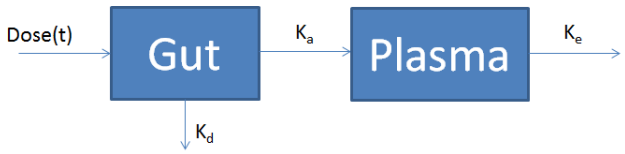

Figure 3.10: Model of extravascular administration with absorption in the gut.

The closed form solution of this equation is given in classical textbooks (see Section 2.2.4 of Gabrielsson and Weiner (2007)) as

$$C(t) = \frac{K_a D_{po}}{V(K_a + K_d) - Cl} \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) u(t) \tag{3.89}$$

In this model, we assume that the absorption process starts at a single anatomical compartment (in this example, the gut). However, the tablet needs some time to reach the gut which is normally modelled through a time lag as

$$C(t) = \frac{K_a D_{po}}{V(K_a + K_d) - Cl} \left(e^{-\frac{Cl}{V}(t-t_{transit})} - e^{-(K_a+K_d)(t-t_{transit})} \right) u(t-t_{transit}) \tag{3.90}$$

The identification of the model parameters (V, Cl, K_a, K_d) in classical pharmacokinetics books involves a pletorah of equations and particular cases (for instance, distinguishing the case in which $K_a + K_d > \frac{Cl}{V}$ and the case in which $K_a + K_d < \frac{Cl}{V}$, this is known in the pharmacokinetics literature as the flip-flop effect). Additionally, Eq. (3.90) is only valid for a single intake at time $t = 0$ and it has to be modified for repeated intakes and there is no closed form solution for repeated intakes of different amounts of drugs or at irregular time intervals. Moreover, the model assumes that drug degradation starts at the same time as absorption, but this may not be true. For instance, degradation may start at the stomach much earlier and with a different degradation constant with respect to the degradation constant in the gut. If we adopt a fitting approach based on differential equations, as defended in this thesis, we may overcome all these difficulties and even generalize the previous model to deal with more general situations. This will be addressed in Section 3.3.3.

0th order absorption

Although absorption is normally a complex, relatively slow process (and that is why it is considered to be first order), there are certain molecules that are rather fast to absorb. In this case, the absorption can be considered a zero-th order process, that is, the absorption occurs at a constant rate independently of the amount of drug in the intestine. The equivalent to Eq. (3.82) in this case would be

$$\frac{dA_b(t)}{dt} = R_{in} \tag{3.91}$$

where R_{in} is the rate (in mg/min) at which the drug passes from the gut to the plasma. At this rate, a dose of D_{po} mg will take $T_{abs} = \frac{D_{po}}{R_{in}}$ to get into the plasma. The classical solution of the zero-th order absorption for $t > T_{abs}$ is

$$C(t) = \frac{R_{in}}{Cl} \left(1 - e^{-\frac{Cl}{V}f(t)} \right) e^{-\frac{Cl}{V}(t-f(t))} \tag{3.92}$$

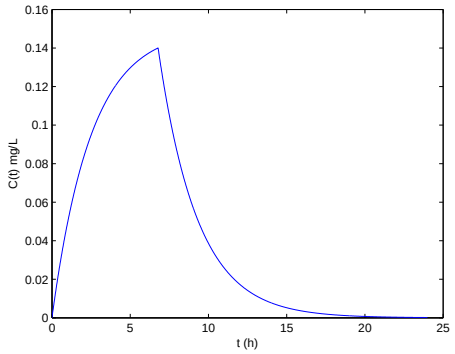

Figure 3.11: Typical response of a zero-th order absorption model.

with

$$f(t) = \begin{cases} t & 0 \leq t < T_{abs} \\ T_{abs} & t \geq T_{abs} \end{cases} \tag{3.93}$$

Note that Eq. (3.91) is valid only for $t < T_{abs}$, since for $t > T_{abs}$ there is no drug in the gut to pass into blood, and $\frac{dA_b(t)}{dt} = 0$. Note also that this model is exactly the same as the constant rate infusion discussed at the end of Section 3.3.2. A typical concentration profile of this kind of systems is shown in Fig. 3.11.

It could also be that both processes (zero-th and first order) coexist. In this case, the increase of drug in plasma is said to be of mixed order and it would be given by

$$\frac{dA_b(t)}{dt} = R_{in} + K_a A_g(t) \tag{3.94}$$

In this equation we clearly see that the word "order" refers to the exponent of the different terms of $A_g(t)$. This equation is valid until $A_g(t) = 0$ because all drug in the intestine has disappeared.

Absorption from multiple sites

In the previous model, we have assumed that absorption occurs in a single organ (in our example, intestine). However, it might well be that it occurs at multiple sites. To illustrate how to modify the model in this case, let us assume that we are only interested in the bucal cavity and the intestine. An easy way to model this situation is to divide the drug amount in two fractions: the fraction of drug that is absorbed in the bucal cavity (with absorption rate K_{a1}) and the fraction of drug that is absorbed in the intestine (with absorption rate K_{a2}). Let's call α the first fraction (with $0 \leq \alpha \leq 1$) and $1-\alpha$ the second fraction. The increase of drug amount in plasma due to the two mechanisms of absorption would be for $t > t_{transit}$ (Gabrielsson and Weiner, 2007)[Section 2.2.11]

$$\frac{dA_b(t)}{dt} = \alpha K_{a1} D_{po} e^{-K_{a1}t} + (1-\alpha) D_{po} K_{a2} e^{-K_{a2}(t-t_{transit})} \tag{3.95}$$

However, note that this model is not so well suited to the actual situation since it predicts that for $t > t_{transit}$ (that is, once the drug has reached the intestine)

there is still absorption in the bucal cavity (which is rather unlikely). In practice this is not a problem because $K_{a1} > K_{a2}$ (there is a quicker absorption in the bucal cavity than in the intestine) and in practical terms the first term can be assumed to be 0 when $t > t_{transit}$. Moreover, degradation in the bucal cavity and the intestine is neglected. A more appropriate model would have been to distinguish between amount of drug in blood ($A_b(t)$) and in the extravascular organs ($A_{ev}(t)$). Then, we simply need to say that the absorption rate varies over time (first it is absorbed at the bucal cavity, then at the stomach, then partly at the stomach and partly in the intestine, then completely in the intestine, and even inside the intestine we may assign different absorption rates at its different segments)

$$\frac{dA_b(t)}{dt} = K_a(t)A_{ev}(t) \tag{3.96}$$

In the model above, we have assumed a first order absorption (although verying over time) and neglected degradation. In next section, we propose a generalized model that takes into account all the effects described in Sections 3.3.3, 3.3.3, and 3.3.3.

Generalized model

As already introduced above, let us distinguish between the amount of drug in blood ($A_b(t)$) and the amount of drug in the extravascular organs ($A_{ev}(t)$). Let us adhere to the symbols introduced in this section. Then, the following system of differential equations fully describe absorption and degradation at multiple sites as well as mixed order absorption. Moreover, it can be used with a single or multiple intakes (even at irregular intervals and with different drug doses).

$$\begin{array}{l} \frac{dA_{ev}(t)}{dt} = -R_{in}(t) - (K_a(t) + K_d(t))A_{ev}(t) + D_{po}(t) \\ V\frac{dC(t)}{dt} = R_{in}(t) + K_a(t)A_{ev}(t) - ClC(t) \end{array} \tag{3.97}$$

Obviously, the identification of this model is not an easy task because we need to determine the functions $R_{in}(t)$, $K_a(t)$ and $K_d(t)$. These functions have contributions from all the extravascular organs through which the extravascular doses are traversing. However, these functions may be discretized as accurately as desired. Following the idea of defining $t_{transit}$ as the transit time from the bucal cavity to the intestine, we may discretize these functions in two parts before and after $t_{transit}$. It is also convenient to separate the extravascular organs in two functions (one for the bucal cavity and another one for the intestine). Let us consider first the effect on a single intake taken at time $t = 0$.

- For $\underline{0 \leq t < t_{transit}}$:

$$\begin{array}{l} \frac{dA_{ev1}(t)}{dt} = -R_{in1} - (K_{a1} + K_{d1})A_{ev1}(t) + D_{po}\delta(t) \\ V\frac{dC(t)}{dt} = R_{in1} + K_{a1}A_{ev1}(t) - ClC(t) \end{array} \tag{3.98}$$

- For $\underline{t \geq t_{transit}}$:

$$\begin{array}{l} \frac{dA_{ev2}(t)}{dt} = -R_{in2} - (K_{a2} + K_{d2})A_{ev2}(t) + A_{ev1}(t_{transit})\delta(t - t_{transit}) \\ V\frac{dC(t)}{dt} = R_{in2} + K_{a2}A_{ev2}(t) - ClC(t) \end{array} \tag{3.99}$$

The model considers a single intake of D_{po} (mg) at $t = 0$, 1st order absorption and degradation as well as zero-th order absorption at the first extravascular organ and it is valid till $t = t_{transit}$. At this time, the remaining amount of drug in the extravascular organ 1 is passed to the extravascular organ 2, and the absorption and degradation continues from this organ as it was done in the first organ although with different constants. Note that the models in Sections 3.3.3 and 3.3.3 are particular cases of this generalized model with $t_{transit} = 0$, and $R_{in2} = 0$ (Section 3.3.3) or $K_{a2} = K_{d2} = 0$ (Section 3.3.3). The model in Section 3.3.3 is superseded by this one.

Since the model above is non-linear (due to its disruptive behavior at $t = t_{transit}$), for multiple doses, we cannot simply compute the blood concentrations due to each of the dosis and then add the results (application of the superposition principle). Instead, we need to track each individual dosis. For each one of them, consider whether it contributes to the first or the second extravascular organ and calculate its particular contribution to absorption and degradation in that organ.

In this thesis, we will only fully explain the discretization of one of the branches (since the other one is totally analogous) which for simplicity will be rewritten as

$$\begin{array}{l} \frac{dA_{ev}(t)}{dt} = -R_{in} - (K_a + K_d)A_{ev}(t) + D_{po}(t) \\ V\frac{dC(t)}{dt} = R_{in} + K_a A_{ev}(t) - ClC(t) \end{array} \tag{3.100}$$

To solve this equation system we will make use of an explicit Runge-Kutta's method of order 4 (see Eq. (3.31)). We will reformulate this method for equation systems. Let $\mathbf{y}(t)$ be a vector function with n components (in our case $n = 2$) meeting the equation system

$$\frac{d\mathbf{y}(t)}{dt} = \mathbf{f}(t, \mathbf{y}(t)) \tag{3.101}$$

Then, we may iterate as

$$\mathbf{y}[n] = \mathbf{y}[n-1] + \frac{1}{6}(\mathbf{k}_1 + 2\mathbf{k}_2 + 2\mathbf{k}_3 + \mathbf{k}_4) \tag{3.102}$$

with

$$\begin{array}{l} \mathbf{k}_1 = T_s\mathbf{f}((n-1)T_s, \mathbf{y}[n-1]) \\ \mathbf{k}_2 = T_s\mathbf{f}((n-1)T_s + \frac{T_s}{2}, \mathbf{y}[n-1] + \frac{\mathbf{k}_1}{2}) \\ \mathbf{k}_3 = T_s\mathbf{f}((n-1)T_s + \frac{T_s}{2}, \mathbf{y}[n-1] + \frac{\mathbf{k}_2}{2}) \\ \mathbf{k}_4 = T_s\mathbf{f}((n-1)T_s + T_s, \mathbf{y}[n-1] + \mathbf{k}_3) \end{array} \tag{3.103}$$

In our case,

$$\mathbf{f}(t, \mathbf{y}(t)) = \begin{pmatrix} -R_{in} - (K_a + K_d)A_{ev}(t) \\ \frac{R_{in}}{V} + \frac{K_a}{V}A_{ev}(t) - \frac{Cl}{V}C(t) \end{pmatrix} \tag{3.104}$$

The final recursion is

$$\begin{array}{rcl} A_{ev}[n] & = & c_{aa}A_{ev}[n-1] - c_{0a}R_{in} + D_{po}[n]T_s \\ C[n] & = & c_{cc}C[n-1] + \frac{1}{V}\left(c_{ac}K_a A_{ev}[n-1] + c_{0c}R_{in}\right) \end{array} \tag{3.105}$$

where we have defined

$$\begin{aligned}
K_{app} &= K_a + K_d \\
K_e &= \frac{Cl}{V} \\
c_{aa} &= 1 - K_{app}T_s + \frac{1}{2}K_{app}^2T_s^2 - \frac{1}{6}K_{app}^3T_s^3 + \frac{1}{24}K_{app}^4T_s^4 \\
c_{0a} &= T_s - \frac{1}{2}K_{app}T_s^2 + \frac{1}{6}K_{app}^2T_s^3 - \frac{1}{24}K_{app}^3T_s^4 \\
c_{cc} &= 1 - K_eT_s + \frac{1}{2}K_e^2T_s^2 - \frac{1}{6}K_e^3T_s^3 + \frac{1}{24}K_e^4T_s^4 \\
c_{0c} &= T_s - \frac{1}{2}(K_a + K_e)T_s^2 + \frac{1}{6}(K_a(K_{app} + K_e) + K_e^2)T_s^3 \\
&\quad -\frac{1}{24}(K_a(K_{app}^2 + K_{app}K_e + K_e^2) + K_e^3)T_s^4 \\
c_{ac} &= T_s - \frac{1}{2}(K_{app} + K_e)T_s^2 + \frac{1}{6}(K_{app}(K_{app} + K_e) + K_e^2)T_s^3 \\
&\quad -\frac{1}{24}(K_{app}(K_{app}^2 + K_{app}K_e + K_e^2) + K_e^3)T_s^4
\end{aligned} \tag{3.106}$$

Fig. 3.12 shows the blood concentration over time for a number of systems with varying absorption, clearance and constant infusion parameters. All experiments were performed for a single dose at time $t = 0$ of 1 mg, a distribution volume $V = 10$ (L) and a degradation constant $K_d = 0.005$ (min^{-1}). For those cases with $R_{in} = 0$ we have compared the results from this model from those of Eq. 3.90. The mean error is in the order of $10^{-7}\%$. Note that the model behaves as expected for the first order extravascular model and generalizes it as it includes a zero-th order absorption. Additionally, the generalized model can be easily put into the system identification framework proposed in this thesis and there is no need for closed or approximated formulas to estimate its parameters. The framework can handle any dosing regime.

3.3.4 Multicompartment models

The one-compartment model is not appropriate for drugs with a rapid administration and when concentration measures are taken frequently. The reason is that it takes time for the drug to distribute into tissues and reach equilibrium (maybe due to perfusion or diffusion rate limited processes). In these cases, a multicompartment model is better suited to the data modelling. A central compartment represents blood and all those organs that are rapidly equilibrated while peripheral compartments represent more slowly equilibrated organs. The different compartments may be all connected to the central compartment (mammillary model) or be connected in sequence (catenary model) (see Fig. 3.13).

To illustrate the methodology introduced in this thesis, let us consider one of the most used multicompartmental models, the two compartment model. This model considers a central compartment composed by blood and all tissues that achieve a quick equilibrium with blood, and a peripheral compartment formed by those tissues with a slow equilibirum with blood. Let us consider simultaneously extravascular and intravenous administration. As done so far, we will focus on the amount of drug that remains extravascularly and the concentration at the different compartments. Let us also assume that the central compartment has a distribution volume V_c and the peripheral compartment V_p. The diagram in Fig. 3.14 represents the drug flow among the different compartments. Note that this model allows for first and zero-th order absorption as well as for any arbitrary dosing regimen including oral and intravenous administration.

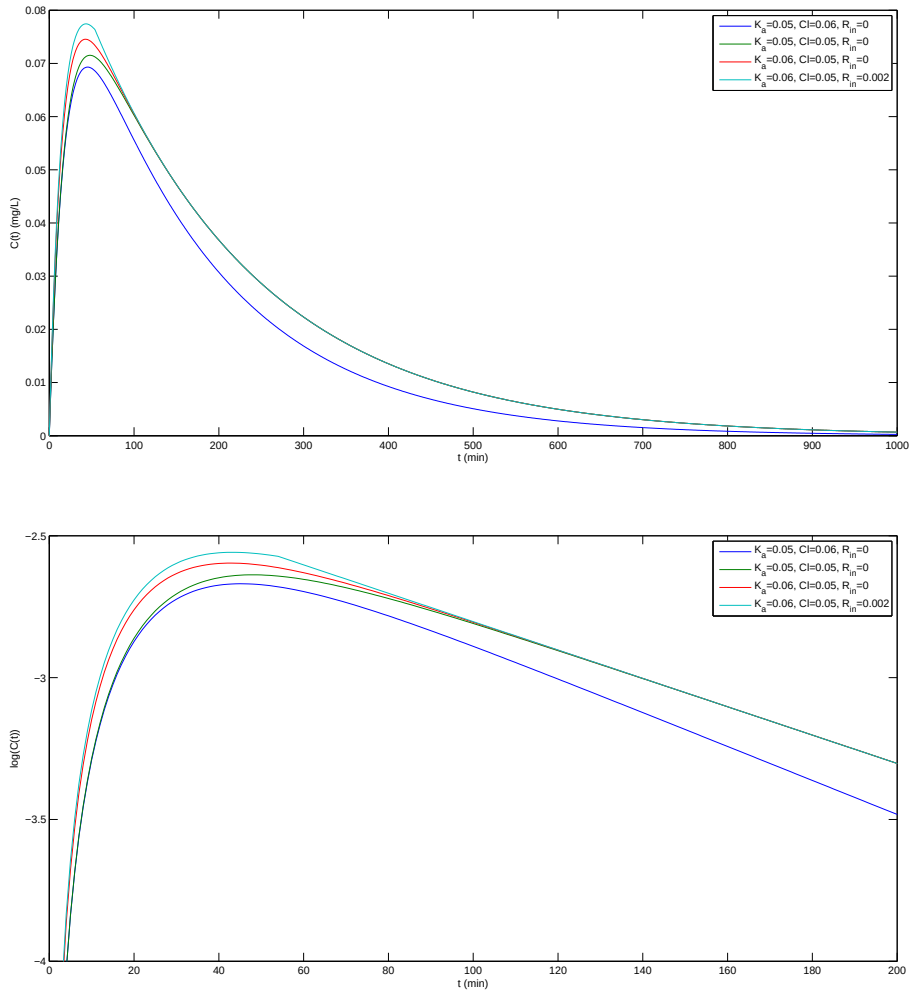

Figure 3.12: Drug concentration profiles in the blood stream for different parameters (top) and its logarithm (bottom)

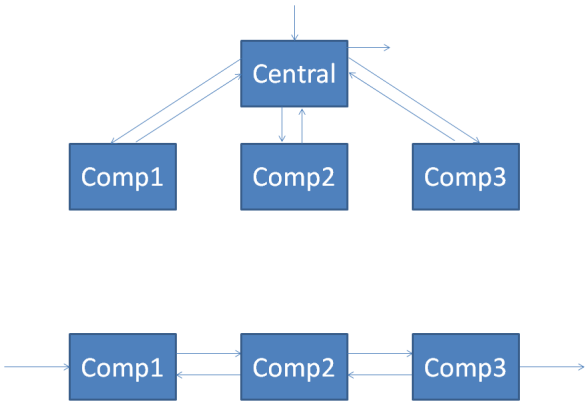

Figure 3.13: Mamillary multicompartment model (top) and catenary multicompartment model (bottom).

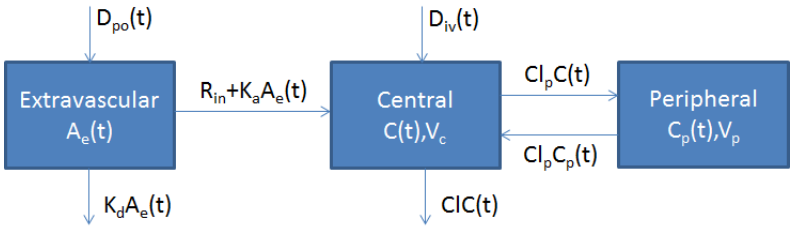

Figure 3.14: Generalized two-compartment model

The model can be described with the differential equation system

$$\begin{array}{rcl} \frac{dA_e(t)}{dt} & = & -(R_{in} + (K_a + K_d)A_{ev}(t)) + D_{po}(t) \\ V_c\frac{dC(t)}{dt} & = & -(Cl + Cl_p)C(t) + Cl_pC_p(t) + R_{in} + K_aA_{ev}(t) + D_{iv}(t) \\ V_p\frac{dC_p(t)}{dt} & = & -Cl_pC_p(t) + Cl_pC(t) \end{array} \tag{3.107}$$

Note that the generalized one-compartment model in Eq. (3.100) is a particularization of this model with $Cl_p = 0$. In fact, a good starting point for Cl_p is given by solving for Cl_p in the following equation

$$QV_c = (Cl_p + Cl)V_{blood} \tag{3.108}$$

where Q is the blood flow into the organs contained in the peripheral compartment and V_{blood} is the volume of blood of the individual. The equation is simply assuming that $Q \approx Cl_p + Cl$ and $V_c \approx V_{blood}$.

Now, following the methodology explained in Eq. (3.101) and subsequent equations applied to the function

$$\mathbf{f}(t, \mathbf{y}(t)) = \begin{pmatrix} -(R_{in} + (K_a + K_d)A_{ev}(t)) \\ -\frac{Cl+Cl_p}{V_c}C(t) + \frac{Cl_p}{V_c}C_p(t) + \frac{R_{in}}{V_c} + \frac{K_a}{V_c}A_{ev}(t) \\ -\frac{Cl_p}{V_p}C_p(t) + \frac{Cl_p}{V_p}C(t) \end{pmatrix} \tag{3.109}$$

The resulting recursion is

$$\begin{array}{rcl} A_{ev}[n] & = & c_{aa}A_{ev}[n-1] - c_{0a}R_{in} + D_{po}[n]T_s \\ C[n] & = & c_{cc}C[n-1] + \frac{1}{V_c}\left(c_{ac}K_aA_{ev}[n-1] + c_{0c}R_{in}\right) + c_{pc}K_{pc}C_p[n-1] + \frac{1}{V_c}D_{iv}[n]T_s \\ C_p[n] & = & c_{cp}K_{pp}C[n-1] + \frac{1}{V_c}K_{pp}\left(c_{ap}K_aA_{ev}[n-1] + c_{0p}R_{in}\right) + c_{pp}C_p[n-1] \end{array} \tag{3.110}$$

where we have defined

$$
\begin{aligned}
K_{app} &= K_a + K_d \\
K_e &= \frac{Cl}{V_c} \\
K_{pc} &= \frac{Cl_p}{V_c} \\
K_{pp} &= \frac{Cl_p}{V_p} \\
c_{aa} &= 1 - K_{app}T_s + \tfrac{1}{2}K_{app}^2T_s^2 - \tfrac{1}{6}K_{app}^3T_s^3 + \tfrac{1}{24}K_{app}^4T_s^4 \\
c_{0a} &= T_s - \tfrac{1}{2}K_{app}T_s^2 + \tfrac{1}{6}K_{app}^2T_s^3 - \tfrac{1}{24}K_{app}^3T_s^4 \\
c_{cc} &= 1 - K_eT_s + \tfrac{1}{2}K_e^2T_s^2 - \tfrac{1}{6}K_e^3T_s^3 + \tfrac{1}{24}K_e^4T_s^4 \\
&\quad -K_{pc}T_s + \tfrac{1}{2}K_{pc}^2T_s^2 - \tfrac{1}{6}K_{pc}^3T_s^3 + \tfrac{1}{24}K_{pc}^4T_s^4 \\
&\quad +K_{pc}K_e\left(T_s^2 - \tfrac{1}{2}(K_e + K_{pc})T_s^3 + \tfrac{1}{6}(K_e^2 + \tfrac{3}{2}K_eK_{pc} + K_{pc}^2)T_s^4\right) \\
&\quad +K_{pc}K_{pp}\left(\tfrac{1}{2}T_s^2 - \tfrac{1}{6}(K_{pp} + 2K_{pc})T_s^3 + \tfrac{1}{24}(K_{pp}^2 + 3K_{pc}K_{pp} + 3K_{pc}^2)T_s^4\right) \\
&\quad +K_eK_{pc}K_{pp}\left(-\tfrac{1}{3}T_s^3 + \tfrac{1}{24}(2K_{pp} + 6K_{pc} + 3K_e)T_s^4\right) \\
c_{ac} &= T_s - \tfrac{1}{2}K_aT_s^2 + \tfrac{1}{6}K_a^2T_s^3 - \tfrac{1}{24}K_a^3T_s^4 \\
&\quad +K_d\left(-\tfrac{1}{2}T_s^2 + \tfrac{1}{6}(K_d + 2K_a)T_s^3 - \tfrac{1}{24}(K_d^2 + 3K_aK_d + 3K_a^2)T_s^4\right) \\
&\quad +K_e\left(-\tfrac{1}{2}T_s^2 + \tfrac{1}{6}(K_e + K_a)T_s^3 - \tfrac{1}{24}(K_e^2 + K_aK_e + K_a^2)T_s^4\right) \\
&\quad +K_{pc}\left(-\tfrac{1}{2}T_s^2 + \tfrac{1}{6}(K_{pc} + K_a)T_s^3 - \tfrac{1}{24}(K_{pc}^2 + K_aK_{pc} + K_a^2)T_s^4\right) \\
&\quad +\tfrac{1}{6}(K_dK_e + K_dK_{pc} + K_{pc}K_{pp} + 2K_eK_{pc})T_s^3 \\
&\quad -\tfrac{1}{24}(K_dK_e^2 + K_d^2K_e + K_{pc}K_{pp}^2 + K_dK_{pc}^2 + K_d^2K_{pc} + K_dK_{pc}K_{pp})T_s^4 \\
&\quad -\tfrac{1}{24}(2K_dK_eK_{pc} + 2K_eK_{pc}K_{pp} + 2K_{pc}^2K_{pp} + 3K_eK_{pc}^2 + 3K_e^2K_{pc})T_s^4 \\
&\quad -K_a\tfrac{1}{24}(2K_dK_e + 2K_dK_{pc} + 2K_eK_{pc} + K_{pc}K_{pp})T_s^4 \\
c_{0c} &= T_s - \tfrac{1}{2}K_aT_s^2 + \tfrac{1}{6}K_a^2T_s^3 - \tfrac{1}{24}K_a^3T_s^4 \\
&\quad -\tfrac{1}{2}(K_e + K_{pc})T_s^2 + \tfrac{1}{6}(K_e^2 + K_{pc}^2 + 2K_eK_{pc} + K_{pc}K_{pp})T_s^3 \\
&\quad -\tfrac{1}{24}(K_e^3 + K_{pc}^3 + 3K_eK_{pc}^2 + 3K_e^2K_{pc} + K_{pc}K_{pp}^2 + 2K_{pc}^2K_{pp} + 2K_eK_{pc}K_{pp})T_s^4 \\
&\quad +K_a\tfrac{1}{6}(K_d + K_e + K_{pc})T_s^3 \\
&\quad -K_a\tfrac{1}{24}(K_d^2 + K_e^2 + K_{pc}^2 + K_aK_e + K_dK_e + K_aK_{pc} + K_dK_{pc} + K_{pc}K_{pp})T_s^4 \\
&\quad -K_a\tfrac{1}{24}(2K_aK_d + 2K_eK_{pc})T_s^4 \\
c_{pc} &= T_s - \tfrac{1}{2}K_{pc}T_s^2 + \tfrac{1}{6}K_{pc}^2T_s^3 - \tfrac{1}{24}K_{pc}^3T_s^4 \\
&\quad -\tfrac{1}{2}(K_e + K_{pp})T_s^2 \\
&\quad +\tfrac{1}{6}(2K_eK_{pc} + K_e^2 + K_{pp}^2 + 2K_{pc}K_{pp} + K_eK_{pp})T_s^3 \\
&\quad -\tfrac{1}{24}(K_e^3 + K_{pp}^3 + 3K_eK_{pc}^2 + 3K_{pc}^2K_{pp} + 3K_e^2K_{pc} + 3K_{pc}K_{pp}^2)T_s^4 \\
&\quad -\tfrac{1}{24}(K_eK_{pp}^2 + K_e^2K_{pp} + 4K_eK_{pc}K_{pp})T_s^4
\end{aligned}
\tag{3.111}
$$

$$
\begin{aligned}
c_{cp} &= T_s - \tfrac{1}{2}K_{pp}T_s^2 + \tfrac{1}{6}K_{pp}^2T_s^3 - \tfrac{1}{24}K_{pp}^3T_s^4 \\
&\quad -\tfrac{1}{2}(K_e + K_{pc})T_s^2 + \tfrac{1}{6}(K_e^2 + K_{pc}^2 + K_eK_{pp} + 2K_{pc}K_{pp} + 2K_eK_{pc})T_s^3 \\
&\quad -\tfrac{1}{24}(K_e^3 + K_{pc}^3 + K_eK_{pp}^2 + K_e^2K_{pp} + 3K_{pc}K_{pp}^2 + 3K_{pc}^2K_{pp})T_s^4 \\
&\quad -\tfrac{1}{24}(3K_eK_{pc}^2 + 3K_e^2K_{pc} + 4K_eK_{pc}K_{pp})T_s^4 \\
c_{ap} &= \tfrac{1}{2}T_s^2 - \tfrac{1}{6}K_{pp}T_s^3 + \tfrac{1}{24}K_{pp}^2T_s^4 \\
&\quad -\tfrac{1}{6}(K_a + K_d + K_e + K_{pc})T_s^3 \\
&\quad +\tfrac{1}{24}(K_a^2 + K_d^2 + K_e^2 + K_{pc}^2 + K_aK_{pp} + K_aK_{pc} + K_dK_{pp} + K_dK_{pc})T_s^4 \\
&\quad +\tfrac{1}{24}(K_eK_{pp} + K_aK_e + K_dK_e + 2K_eK_{pc} + 2K_{pc}K_{pp} + 2K_aK_d)T_s^4 \\
c_{0p} &= \tfrac{1}{2}T_s^2 - \tfrac{1}{6}(K_a + K_{pp} + K_e + K_{pc})T_s^3 \\
&\quad +\tfrac{1}{24}(K_a^2 + K_{pp}^2 + K_e^2 + K_{pc}^2 + K_aK_{pp} + K_aK_d + K_aK_e + K_aK_{pc} + K_eK_{pp})T_s^4 \\
&\quad +\tfrac{1}{24}(2K_{pc}K_{pp} + 2K_eK_{pc})T_s^4 \\
c_{pp} &= 1 - K_{pp}T_s + \tfrac{1}{2}K_{pp}^2T_s^2 - \tfrac{1}{6}K_{pp}^3T_s^3 + \tfrac{1}{24}K_{pp}^4T_s^4 \\
&\quad +\tfrac{1}{2}K_{pc}K_{pp}T_s^2 - \tfrac{1}{6}K_{pp}(K_{pp}^2 + K_{pc}^2 + 2K_{pc}K_{pp} + K_eK_{pc})T_s^3 \\
&\quad +\tfrac{1}{24}K_{pc}K_{pp}(K_{pc}^2 + K_e^2 + 3K_{pp}^2 + 2K_eK_{pc} + 2K_eK_{pp} + 3K_{pc}K_{pp})T_s^4
\end{aligned}
\tag{3.112}
$$

Note that it seems counterintuitive that the recursion defined in Eq. (3.110)

uses $A_{ev}[n-1]$ and R_{in} to compute $C_p[n]$ when in the model these two magnitudes are not directly connected. However, we have to realize that this lack of direct connection is correctly shown in the continuous model (Eq. (3.107), but they become connected in the discrete recursion simply by the numerical method used to solve the differential equation system. In fact, the coefficients c_{ap} and c_{0p} do not contain first order terms, and consequently they would not appear in a first order approximation of the derivative, as expected. It is also remarkable the simplicity of Eq. (3.110) for a two-compartment model that considers extravascular and intravenous administration in any dosing regimen, first and zero-th order absorption, and degradation in the extravascular domain.

Additionally, this model allows the calculation of important pharmacological parameters as the Mean Residence Time (MRT) of a molecule in the system and the Mean Transit Time (MTT) of a molecule in the central compartment:

$$\begin{array}{rcl} MRT & = & \frac{V_c+V_p}{Cl} \\ MTT & = & \frac{V_c}{Cl+Cl_p} \end{array} \tag{3.113}$$

In general, the mean time that a molecule stays in a combination of compartments is the total volume associated to that combination divided by the clearance of the combination. The MRT and MTT formulas above respond to this general principle.

As an example, Fig. 3.15 shows the evolution of the extravascular amount of drug and the concentration in the two compartments for a single intake of 1 mg of drug in the case of oral and intravenous administration. The model parameters are $K_a = 0.0167$ (1/min), $K_d = 0.0033$ (1/min), $Cl = 0.1167$ (L/min), $Cl_p = 0.8333$ (L/min), $R_{in} = 0$ (mg/min), $V_c = 50$ (L), $V_p = 60$ (L).

3.3.5 Clearance

The methodology proposed in this thesis can be extended to physiological modelling. In this section, we develop the details for the physiological modelling of clearance. Let us concentrate first on hepatic clearance as an example, in fact one of the main mechanisms for drug elimination. Although the theory exposed here is not limited to this organ. We understand as drug clearance any mechanism by which a drug ceases to perform its therapeutic task either because it has been biochemically inactivated (for instance by hepatocytes in the liver) or it has been eliminated from the body (for instance by kidneys). Actually, the first mechanism has proved to be much more important than the second one for many drugs, and both together are responsible for the elimination of 90% of the drugs (Rosenbaum, 2011)[Section 5.1] (the other important elimination route is through bile; minor routes involve sweat and exhalation). In general terms, one could say that molecules whose molecular weight is below 500 Da are primarily cleared by extracellular hydrolisis, between 500 Da and 1 kDa by carrier mediated uptake into hepatocytes, between 1 and 50 kDa by glomerular filtration, between 50 and 200 kDa by receptor mediated endocytosis, and beyond this weight by opsonization and phagocytosis.

Hepatic clearance

For a drug to be inactivated, it has to be biochemically accessible to an enzyme that modifies it producing a metabolite that is further metabolized or finally

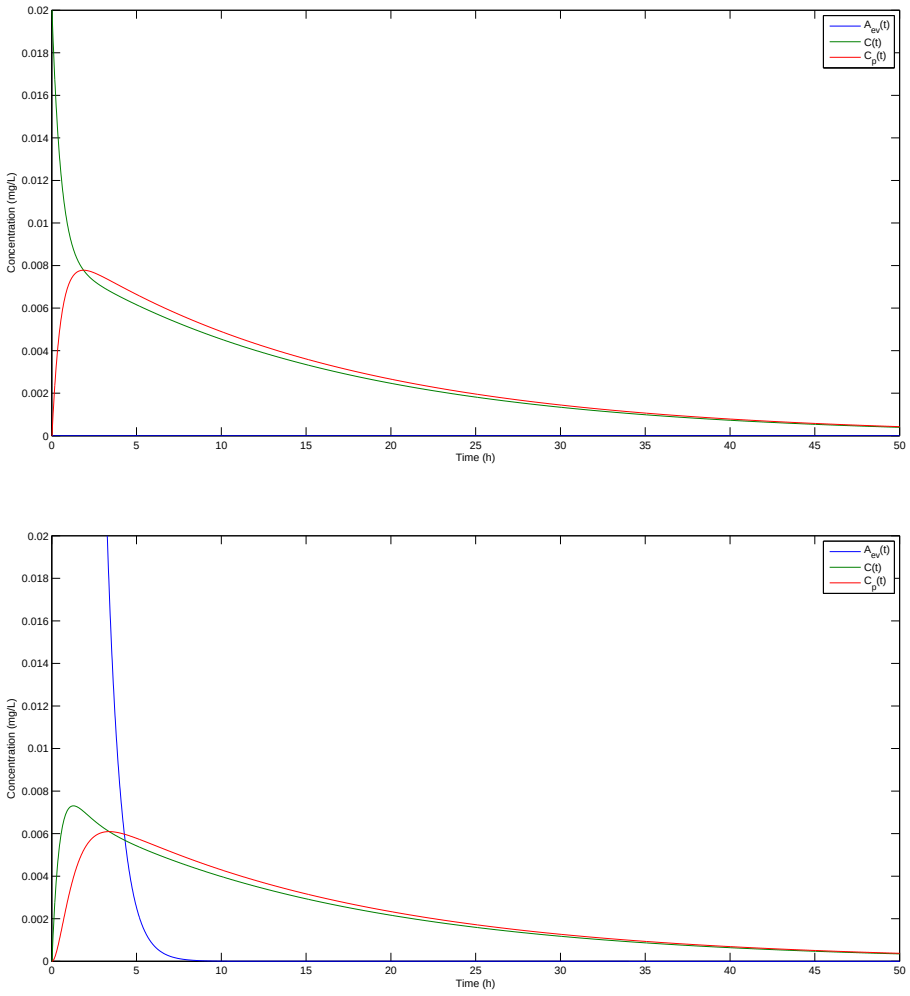

Figure 3.15: Generalized two-compartment model response to 1 mg intraveously (top) and orally (bottom).

excreted in urine or bile. From the pharmacokinetics point of view, the drug molecule has been eliminated. If the metabolite has some therapeutic or toxic activity, it should be included in the pharmacokinetics study. This will be performed in Section 3.4.5.

Enzymatic modification is not possible for those drug molecules that has been bound to plasma proteins at specific sites (there might be binding sites that still leave the inactivation site accessible). In the literature, f_u is defined as the fraction of unbound drug molecules in plasma (or in some cases, in tissues). We will stick to this nomenclature although f_u would be better defined as the fraction of drug molecules that cannot be enzymatically modified because they are bound.

Hepatic metabolism is normally classified in two phases: Phase I and Phase II (Rosenbaum, 2011)[Section 5.4]. Phase I metabolism is responsible for about 70% of hepatic clearance and it results in small chemical modifications, most of the times oxidations (normally by adding hydroxil group or removing a methyl group) although some reductions can occur. The Cytochrome P450 (CYP) enzyme system is by far the most important family of enzymes acting at this phase. At Phase II, the drug itself or its Phase I product is conjugated with a polar function such as a glucoronide, sulfate or glutathione. The UDP-glucoronosyltransferases (UGTs) is the most important family of enzymes, responsible for about 10% of the drugs cleared by metabolic elimination.

There are several hepatic models, mostly based on fluid dynamics and blood flow. In this quick summary, we will give the details for the **well-stirred model** and the results for the rest. The well-stirred model considers the liver to be an homogeneous organ whose drug concentration is uniform. It considers the drug concentration at its input C_{in} and at its output C_{out}, and relates both magnitudes by an extraction factor E_H, a number between 0 and 1 that represents the fraction of drug concentration that is eliminated in the liver:

$$C_{out} = C_{in}(1 - E_H) \tag{3.114}$$

It is said that a drug is highly cleared if $E_H > 0.7$ and it is poorly cleared if $E_H < 0.3$.

Let us consider the following differential equation for the dynamics of the amount of drug in the liver:

$$V_H \frac{dC_H}{dt} = Q_H C_{in} - Q_H C_{out} - f_u Cl_{int} C_{out} \tag{3.115}$$

where V_H is the volume of the liver, C_H is its drug concentration, Q_H is the blood flow into the liver and Cl_{int} is the **intrinsic clearance**, that is, the maximal clearance capacity if the process would not be limited by blood flow. Note that the amount of drug eliminated is $Q_H C_{in}$ and that C_{in} is inversely proportional to the distribution volume. Drugs with a large distribution volume will also be poorly eliminated.

In steady state, there is no change of the drug concentration in liver, that is, $\frac{dC_H}{dt} = 0$ and consequently

$$Q_H C_{in} - Q_H C_{out} - f_u Cl_{int} C_{out} = 0 \Rightarrow C_{out} = C_{in} \frac{Q_H}{Q_H + f_u Cl_{int}} \tag{3.116}$$

Comparing this last equation with Eq. (3.114) we see that

$$1 - E_H = \frac{Q_H}{Q_H + f_u Cl_{int}} \Rightarrow E_H = \frac{f_u Cl_{int}}{Q_H + f_u Cl_{int}} \tag{3.117}$$

The hepatic clearance is defined as the blood flow into the liver times the extraction factor

$$Cl_H = Q_H E_H = \frac{Q_H f_u Cl_{int}}{Q_H + f_u Cl_{int}} \tag{3.118}$$

The units of clearance are obviously the same units as for the blood flow (e.g., L/min), and we see that the blood flow into the liver is an upper bound for the drug clearance. In fact for highly cleared drugs, the clearance process is said to be limited by blood flow (if blood flow increases, clearance also increases), while for poorly cleared drugs, clearance is limited by extraction (increases in blood flow do not translate into increases of clearance because the process is limited by the biochemical reaction taking place).

Note that we may substitute this expression in any of the models presented so far (see Eqs. (3.42), (3.105), and (3.110)). The system dynamics would not change at all and we simply would have to add the different physiological parameters to the system parameters to be identified. Note that f_u and Cl_{int} are not uniquely identifiable (assume that the true parameters are f_u and Cl_{int}, then $f'_u = Kf_u$, as long as $0 \leq f'_u \leq 1$, and $Cl'_{int} = \frac{1}{K} Cl_{int}$ provide the same product, $f_u Cl_{int} = f'_u Cl'_{int}$). Although, less obvious the same happens with Q_H and $f_u Cl_{int}$. Let's say that Q_H and $f_u Cl_{int}$ are the true parameters that result in a clearance Cl_H. It can be verified that

$$Q_H = \frac{Cl_H f_u Cl_{int}}{f_u Cl_{int} - Cl_H} \tag{3.119}$$

If we incorrectly estimate the product $f_u Cl_{int}$ to be $Kf_u Cl_{int}$, then we can compensate and still produce a clearance Cl_H by finding a different Q'_H

$$Q'_H = \frac{Cl_H K f_u Cl_{int}}{K f_u Cl_{int} - Cl_H} \tag{3.120}$$

For this reason it is important to provide tight constraints to the physiological parameters, if they are known. For instance, the average blood flow into the liver is estimated to be about 1.45 (L/min). We may performed a constrained least-squares minimization forcing Q_H to be between 1.4 and 1.5. The same would apply for f_u. f_u may be estimated *in vitro* or *in vivo* by any other means and we may perform a constrained minimization. Note that physiological parameters may greatly change among species. For instance, the liver blood flow of a mouse is about $1.8{\cdot}10^{-3}$ (L/min). Similarly, different species may have different plasma proteins, most of them will be evolutionary analogs of each other, but their affinity by a given drug compound may be different and this translates into different f_u.

Interestingly, our identification framework allows for a more accurate, probabilistic approach. In the paragraph above, we simply constrained Q_H to be between 1.4 and 1.5. However, let's say that we know the likelihood of Q_H taking any of the values in the interval. This is given by its probability density function $f_{Q_H}(q_H)$. Similarly, let's say we know the probability density function for f_u ($f_{F_u}(f_u)$). This is not unreasonable since we may estimate these functions

in our experiments to estimate the mean values of those quantities. In these circumstances we may estimate the probability density function of Cl_{int} in the following way:

- Take regular or random samples of Q_H and f_u within the specified interval. Let's refer to a specific realization of this pair as $\left(Q_H^{(s)}, f_u^{(s)}\right)$.
- We know that the likelihood of observing this pair is

$$f_{Q_H F_u}\left(Q_H^{(s)}, f_u^{(s)}\right) = f_{Q_H}\left(Q_H^{(s)}\right) f_{F_u}\left(f_u^{(s)}\right) \tag{3.121}$$

- We then fit our pharmacokinetic model by setting Q_H and f_u to fixed values $\left(Q_H^{(s)}, f_u^{(s)}\right)$ and get an estimate of Cl_{int}, that we will refer to as $Cl_{int}^{(s)}$
- Repeat this process to obtain S tuples $(Q_H^{(s)}, f_u^{(s)}, Cl_{int}^{(s)})$.

At this point we may calculate the marginal distribution of Cl_{int} with a kernel estimator

$$\begin{aligned} f_{Cl_{int}}(cl_{int}) &= \int\int f_{Cl_{int}}(cl_{int}|q_h, f_u) f_{Q_H F_u}(q_h, f_u) dq_h df_u \\ &\approx \sum_{s=1}^{S} K_\sigma\left(cl_{int} - Cl_{int}^{(s)}\right) f_{Q_H F_u}\left(Q_H^{(s)}, f_u^{(s)}\right) \end{aligned} \tag{3.122}$$

where $f_{Cl_{int}}(cl_{int}|q_h, f_u)$ is the conditional probability of Cl_{int} given q_h and f_u and $K_\sigma(x)$ is a kernel whose width is parameterized by σ with the property that $\int_{-\infty}^{\infty} K_\sigma(x)dx = 1$. The reader interested in kernel estimators may read Tsybakov (2008).

We may substitute the well-stirred liver model by more realistic models (Gabrielsson and Weiner, 2007)[Section 2.5]. For instance, the **parallel tube model** explictly accounts for the larger drug uptake of hepatocytes at the portal venous entry of the liver than at the hepatic vein (the liver output). In this case, the extraction factor is modelled as

$$E_H = 1 - \exp\left(-\frac{f_u Cl_{int}}{Q_H}\right) \tag{3.123}$$

The **distributed model** further refines this concept by considering the liver to be composed of parallel small tubes. In each of them, the clearance process takes place. Since not all the small tubes are exactly equal, there is a variance associated to the different clearing capability of the tubes modelled by a constant ϵ^2. In this case,

$$E_H = 1 - \exp\left(-\frac{f_u Cl_{int}}{Q_H} - \frac{1}{2}\epsilon^2\left(\frac{f_u Cl_{int}}{Q_H}\right)^2\right) \tag{3.124}$$

Finally, the **dispersion model** considers the blood mixing that takes place within the hepatic sinusoids. It is parameterized by the dispersion number D_N and the efficiency number R_N so that

$$\begin{aligned} a &= \sqrt{1 + 4R_N D_N} \\ E_H &= 1 - \frac{4a}{(1+a)^2 \exp(\frac{a-1}{2D_N}) - (1-a)^2 \exp(\frac{a+1}{2D_N})} \end{aligned} \tag{3.125}$$

Additionally, this latter model is connected to the previous ones because $R_N = \frac{f_u Cl_{int}}{Q_H}$.

All clearance models above can be used within our system identification framework and the probabilistic methodology developed in this section, taking into account the identifiability considerations already discussed.

Let us now concentrate on the intrinsic clearance. This parameter is basically determined by the different metabolic routes through which the drug molecule is metabolized. Let's assume that each enzyme that degrades our drug responds to the Michaelis-Menten enzyme kinetics equation and that we know its maximum metabolic rate V_{max} (mg/min) and its Michaelis-Menten constant K_m (mg/L). Then, the intrinsic clearance can be calculated as

$$Cl_{int} = \sum \frac{V_{max,i}}{K_{m,i} + C} \tag{3.126}$$

being C the drug concentration in the liver, which for the moment will be considered to be a constant (in a subsequent section on nonlinear pharmacokinetics we will drop this assumption). As expected from its equation, Cl_{int} can also be quite different among different species since analog enzymes may exhibit quite different affinities and efficiencies with respect to a particular molecule.

Renal clearance

Clearance of a drug can occur through multiple mechanisms. So far, we have only considered metabolic clearance (normally performed, although not exclusively, in the liver). However, renal clearance is another common route to dispose a drug. The kidney daily filters about 200 L of plasma and produces between 1 and 2 L of urine. Part of the drug may be excreted this way. An advantage of such excretion path is that we may measure the concentration in plasma and/or urine. Obviously, having both measurements results in a more robust identification of the system. In this section we will illustrate how to model urine data in the proposed framework by assuming that we have a one-compartment model with intravenous administration. As we already know from Eq. (3.4), the differential equation governing plasma concentration is

$$\frac{dC(t)}{dt} = -\frac{Cl}{V} C(t) \tag{3.127}$$

However, clearance now has two components: one coming from metabolic clearance (Cl_H) and another one from renal clearance (Cl_R):

$$Cl = Cl_H + Cl_R \tag{3.128}$$

On the other side, the total amount of excreted drug, $A_e(t)$, responds to the following differential equation:

$$\frac{dA_e(t)}{dt} = Cl_R C(t) \tag{3.129}$$

that states that the increase in the excreted amount of drug is proportional to the plasma concentration.

At this point we apply the methodology developed in Eq. (3.101) (and subsequent equations) applied to the function

$$\mathbf{f}(t, \mathbf{y}(t)) = \begin{pmatrix} -\frac{Cl}{V}C(t) \\ Cl_R C(t) \end{pmatrix} \tag{3.130}$$

The recursion for the plasma concentration, $C(t)$, is exactly the same as the one in Eq. (3.35). For the excreted amount, $A_e[n]$, it is

$$A_e[n] = A_e[n-1] + Cl_R \left(T_s - \frac{1}{2}K_e T_s^2 + \frac{1}{6}K_e^2 T_s^3 - \frac{1}{24}K_e^3 T_s^4 \right) C[n-1] \tag{3.131}$$

The regression can now be performed simultaneously on the plasma and the urine data. Let us assume that we have N measurements of both magnitudes together (t_i, C_i, A_{ei}). We can estimate the model parameters that minimize the Least-Squares error (see Eq. (3.63))

$$\hat{\Theta} = \min_{\Theta} \left\{ \sum_{i=1}^{N} (C_i - C_\Theta(t_i))^2 + (A_{ei} - A_{e\Theta}(t_i))^2 \right\} \tag{3.132}$$

Renal clearance can also be explained in physiological terms. For instance, we may decompose Cl_R as

$$Cl_R = f_u GFR + Cl_{TS} - TR \tag{3.133}$$

The first term models the glomerular filtration rate (GFR) that takes place at the glomerulus. Water and many small molecules, including drugs, are forced to go into the renal tubule by passive diffusion caused by hydrostatic pressure. This mechanism is not so effective for negatively charged molecules since the glomerular wall is negatively charged and repels anions and for molecules whose diameter is larger than 8 nm (in fact, between 4 nm and 8 nm the efficiency of this mechanism is inversely proportional to the molecule diameter). The normal GFR for a human adult is 125 mL/min and corrections to this value can easily be calculated through Cockcroft-Gault formula as a function of the age, gender body weight and creatinine concentration in serum (Cockcroft and Gault, 1976). However, this filtration cannot occur if the molecule is bound to a plasma protein and its effective diameter is larger than 8 nm, that is why we need to multiply GFR by the fraction of unbound molecule, f_u. After the glomerulus, blood goes into the peritubular capillaries that surround the renal tubule. Further secretion of drug molecules can occur at this site, normally mediated by active membrane transporters. The effect of this second mechanism (tubular secretion) is summarized into the parameter Cl_{TS}. Finally, as the filtrate moves through the proximal tubule and the loop of Henle, water is reabsorbed into blood along with some small molecules. This effect is represented by the third term TR (tubular reabsorption). The reabsorption of a drug depends on its lipophilicity (the more lipophilic it is, the easier it is to be reabsorbed), the filtrate pH (non-ionized drug molecules can be reabsorbed), and filtrate flow through the tubule (the higher the flow, the less chances there are to reabsorb any molecule). As we discussed for the physiological modelling of hepatic clearance, the terms in Eq. (3.133) are not uniquely identifiable (we can arbitrarily modify one of the parameters and compensate through the other two) and tight optimization constraints are needed to successfully identify the physiological parameters.

3.3.6 Turnover

Some therapies include the administration of proteins, peptides, or antibodies. These are large molecules sometimes already constructed and destroyed by the own patient (for instance, diabetes patients are administered human insulin that adds on top of their own generated insulin, whose generation rate is rather low and that is why they need external supplements). In these cases, clearance is not performed only at the liver or the kidneys, but there are specific mechanisms to digest these macromolecules and reuse its components. The methodology proposed in this thesis is also compatible with this situation. We only need to add the internal generation rate to the differential equation defining the system. For instance, for the two-compartments model we simply need to add the internal generation to the second equation

$$\begin{array}{rcl} \frac{dA_e(t)}{dt} & = & -(R_{in} + (K_a + K_d)A_{ev}(t)) + D_{po}(t) \\ V_c\frac{dC(t)}{dt} & = & -(Cl + Cl_p)C(t) + Cl_pC_p(t) + R_{in} + R_{internal} + K_aA_{ev}(t) + D_{iv}(t) \\ V_p\frac{dC_p(t)}{dt} & = & -Cl_pC_p(t) + Cl_pC(t) \end{array} \tag{3.134}$$

The clearance parameter gathers the contribution from the hepatic clearance, renal clearance and any other degradation mechanism acting on the macromolecule. Note that $R_{internal}$ is identifiable and cannot be confounded with R_{in} because it does not appear in the first equation while R_{in} does. Now, we simply need to apply the same methodology as the one described in Section 3.3.4 for numerically solving the differential equation system.

Turnover rate refers to the amount of compound that is generated and destroyed per unit time in the steady state. In the absence of external inputs, in the long term the concentrations in the central and peripheral compartments will equilibrate and there will not be any real change of the concentration in the central compartment. The second equation in the equation system above would become:

$$0 = \frac{dC(t)}{dt} = -ClC_{ss} + R_{internal} \tag{3.135}$$

where C_{ss} is the steady-state concentration

$$C_{ss} = \frac{R_{internal}}{Cl} \tag{3.136}$$

The amount of drug inside the central compartment in the steady-state is

$$A_{ss} = V_cC_{ss} \tag{3.137}$$

Knowing that the generation rate is $R_{internal}$, the time needed to achieve this amount of drug is

$$t_t = \frac{A_{ss}}{R_{internal}} \tag{3.138}$$

This time is called the **turnover time** and its inverse is called the **fractional turnover rate**. Since at the steady-state $R_{internal} = ClC_{ss}$ (see Eq. (3.135)), we have

$$t_t = \frac{V_cC_{ss}}{ClC_{ss}} = \frac{V_c}{Cl} \tag{3.139}$$

This equation is formally identical to the equation of the Mean Residence Time (Eq. (3.12)). In fact, t_t can be considered to be the MRT of any drug molecule

once the steady-state is achieved. The **half-life time** is simply

$$t_{\frac{1}{2}} = \log(2)t_t \tag{3.140}$$

3.3.7 Inter-species scaling (Allometry)

It has been found that many pharmacological parameters scale with the power of the body weight among most terrestrial mammalians. This is particularly true for parameters that depend on the body size as can be the distribution volume (assuming that protein binding does not significantly change) or hepatic or renal clearance (as long as the biochemical routes involved do not change). For a given body weight, BW, it has been proposed that many parameters (in particular, clearance, distribution volume of the different compartments, Mean Residence Time, turnover time, and half-life time) they all depend as

$$X_i = aBW_i^b \tag{3.141}$$

meaning that an species with a body weight BW_i would result in a parameter X_i (being X any of the parameters mentioned above). Parameters a and b are constants and do not depend on the species. Note that this power modelling is needed for pharmacokinetical parameters, but it is not for physiological parameters (e.g., we do not need to extrapolate the blood flow into the liver for humans, but there have been experimental studies directly aimed at estimating it). Also it is needed for parameters that depend on the organ size (for instance, the intrinsic clearance, a parameter that depends mostly on the biochemical capabilities of hepatocytes, does not change; but the hepatic clearance does since it depends on the number of hepatocytes and the blood flow into the liver).

The power dependence can be used in different ways:

1. Extrapolation from a single species: If we have measured a parameter X_{animal} for a given species whose weight is BW_{animal}, we may extrapolate this paramater to humans by simply applying

$$X_{man} = X_{animal}\left(\frac{BW_{man}}{BW_{animal}}\right)^b \tag{3.142}$$

   This extrapolation can be performed for a single point estimate (resulting in a point prediction for humans) or for the limits of a confidence interval (resulting in a predicted confidence interval).

2. Extrapolation from multiple species: If we have measured the parameter X for multiple species, we may fit paramters of Eq. (3.141) to the species at hand and then extrapolate to the body weight of humans.

3. Inter-species modelling: We may integrate the power scaling law into the modelling equations. Let us take as an example the one-compartment model with intravenous administration. Let us consider a particular animal with a body weight BW_i and clearance and distribution volume Cl_i and V_i, respectively. The differential equation governing the concentration in the compartment becomes

$$\begin{array}{rcl} V_i\frac{dC(t)}{dt} & = & -Cl_iC(t) + D_{iv}(t) \\ aBW_i^b\frac{dC(t)}{dt} & = & -cBW_i^dC(t) + D_{iv}(t) \end{array} \tag{3.143}$$

Instead of looking for the parameters Cl_i and V_i for each animal, we look for the parameters a, b, c and d. We may perform the identification of these parameters for several animal individuals simultaneously.

Extrapolation always involves a prediction error. This error is more important the further the body weights of the different animals are from the human weight. This means that extrapolating pharmacological parameters from mice (whose average body weight is about 23 g) has much more error than from a dog (whose average body weight is about 14 kg). In practice, several different species can be used: mice (23 g), rats (250 g), rabbits (1.5 kg), monkeys (4.7 kg), dogs (14 kg). Human weight is estimated to be about 70 kg.

3.4 Nonlinear compartmental pharmacokinetics

All the differential equation systems explained so far are of the form

$$\frac{d\mathbf{y}(t)}{dt} = \mathbf{b} + H\mathbf{y}(t) + \mathbf{x}(t) \tag{3.144}$$

where $\mathbf{y}(t)$ is a vector of system variables like amount of drug at a given site or concentration, H is a matrix of constant values, $\mathbf{b}$ is a vector of constant values, and $\mathbf{x}(t)$ is a vector of inputs, in our systems, input doses. If $\mathbf{b} = \mathbf{0}$, this equation defines a linear system. Linear systems are characterized because of two properties:

- Doubling the input dose results in doubling the system response. In fact, this property holds for any multiple of the input $k\mathbf{x}(t)$.
- The superposition principle: the system response to two different doses is the sum of the individual response of the system to each one of the doses independently.

The introduction of $\mathbf{b}$ breaks this linearity assumption. For instance, internal generation of a molecule (see Eq. (3.134)) or zero-th order absorption (see Eq. (3.94)) fall into this category.

However, in the standard pharmacokinetics literature, the word nonlinear is not referred to this technical detail but to physiological effects such as enzyme capacity saturation, enzyme induction, time dependent system parameters, etc. These nonlinearities are particularly important at high drug concentrations. In this section we will study how to incorporate these effects into the modelling framework introduced in this thesis.

3.4.1 Enzymatic capacity saturation

Michaelis-Menten model for enzymatic reactions states that an enzyme mediated reaction rate is related to the substrate concentration as

$$v = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]} \tag{3.145}$$

where v is the reaction rate, $[P]$ is the concentration of the reaction product, $[S]$ is the concentration of substrate, V_{max} is the maximum reaction rate and K_m

is the Michaelis-Menten constant that determines the substrate concentration at which the reaction rate is one half of the maximum rate. Using this model it has been established that it translates into an intrinsic clearance given in Eq. (3.126) and reproduced here for a single enzyme

$$Cl_{int} = \frac{V_{max}}{K_m + C} \tag{3.146}$$

Remind from Eq. (3.118) that the hepatic clearance is given by

$$Cl_H = \frac{Q_H f_u Cl_{int}}{Q_H + f_u Cl_{int}} \tag{3.147}$$

where Q_H is the blood flow into the liver and f_u is the fraction of unbound drug. Substituting the intrinsic clearance by its value we get

$$Cl_H = \frac{Q_H V_{max} f_u}{V_{max} f_u + C Q_H + K_m Q_H} \tag{3.148}$$

We need to realize that f_u and V_{max} are not uniquely identifiable, but its product is. In many textbooks (Gabrielsson and Weiner, 2007)[Section 2.7.2], this clearance is approximated by $Cl_H = \frac{V_{max}}{V_{max}+C}$ to make the mathematical resolution more tractable. In this example, we will keep its full form. Note that the hepatic clearance depends on the drug concentration in the liver, C, which is normally considered to be the same as in the central compartment. To concentrate on the nonlinear effect of this dependence, we will illustrate how this nonlinear clearance reflects in the one-compartment intravenous bolus model. The differential equation governing the behavior of drug concentration over time is (see Eq. (3.23))

$$\frac{dC(t)}{dt} = -\frac{Cl_H}{V} C(t) + \frac{dose_{iv}(t)}{V} \tag{3.149}$$

If we now substitute Cl_H by its expression we get

$$\frac{dC(t)}{dt} = -\frac{1}{V} \frac{Q_H V_{max} f_u}{V_{max} f_u + C(t) Q_H + K_m Q_H} C(t) + \frac{dose_{iv}(t)}{V} \tag{3.150}$$

which is clearly a nonlinear differential equation. However, the methodology developed at Eq. (3.101) is still valid in this case. For the sake of clarity, instead of applying the 4th order Runge-Kutta's method (which gives a rather complicated expression) we will simply use a first order Runge-Kutta's method (also known as Euler's method) given by the general recursion

$$\mathbf{y}[n] = \mathbf{y}[n-1] + \mathbf{k}_1 \tag{3.151}$$

with

$$\mathbf{k}_1 = T_s \mathbf{f}((n-1)T_s, \mathbf{y}[n-1]) \tag{3.152}$$

For this specific model, the recursion is

$$C[n] = \left(1 - \frac{T_s}{V} \frac{Q_H V_{max} f_u}{V_{max} f_u + C[n-1] Q_H + K_m Q_H}\right) C[n-1] + \frac{dose_{iv}[n] T_s}{V} \tag{3.153}$$

This numerical method is not very accurate since it is only first order. However, it already illustrates the main features of our methodology: 1) any model

can be handled by simply writing its differential equation; 2) the differential equation is transformed into a difference equation; 3) the system is responsive and valid to any dosing regimen; 4) there is no need for approximations and limiting cases; 5) model parameters can be determined by system identification; 6) the empirical probability density function of the model parameters can be easily obtained. In this particular case, we see that the recursion is nonlinear in $C[n-1]$ and, therefore, defines a nonlinear system.

3.4.2 Enzymatic induction or inhibition

Let us assume that the concentration of a given enzyme responds to a model similar to the one studied for turnover (see Section 3.3.6):

$$\frac{dC_E(t)}{dt} = R_E - Cl_E C_E(t) \tag{3.154}$$

A certain drug may increase the enzyme concentration by increasing the expression of the enzyme (making R_E larger) or by decreasing its degradation (making Cl_E smaller). Contrarily, the drug may decrease the enzyme concentration by decreasing its expression or increasing its degradation. In all cases we talk of either a stimulation or an inhibition. In both cases, the effect size can be modelled by a fractional change named S for stimulation and I for inihbition. $S = 0.3$ or $I = 0.3$ would mean a 30% increase or decrease of the magnitude affected. S and I are defined as

$$\begin{array}{rcl} S & = & \frac{S_{max}C(t)}{SC_{50}+C(t)} \\ I & = & \frac{I_{max}C(t)}{IC_{50}+C(t)} \end{array} \tag{3.155}$$

where S_{max} and I_{max} are the maximal possible fraction stimulation and inhibition that can be achieved, SC_{50} is the concentration of drug at which $S = \frac{S_{max}}{2}$ (analogously for IC_{50}, and $C(t)$ is the drug concentration. With these definitions we can model enzymes whose concentration is increased or decreased because of a drug. We can distinguish four possibilities (Rosenbaum, 2011)[Section 17.4]:

- Stimulation of enzyme expression: $\frac{dC_E(t)}{dt} = R_E(1+S) - Cl_E C_E(t)$
- Inhibition of enzyme expression: $\frac{dC_E(t)}{dt} = R_E(1-I) - Cl_E C_E(t)$
- Stimulation of enzyme degradation: $\frac{dC_E(t)}{dt} = R_E - Cl_E(1+S)C_E(t)$
- Inhibition of enzyme degradation: $\frac{dC_E(t)}{dt} = R_E - Cl_E(1-I)C_E(t)$

All these are nonlinear differential equations that can be solved using the Euler's method (see Eq. (3.151)). The concentration of the enzyme affects the maximum capacity of the enzymatic reaction (see Eq. 3.145). It is normally accepted that this effect is linear:

$$V_{max} = aC_E(t) \tag{3.156}$$

where a is simply a proportionality constant.

The case presented so far is called heteroinduction or heteroinhibition because the drug affects the concentration of an enzyme that, in its turn, has an effect on some other macromolecules or metabolites. The drug is said to have

an indirect effect (it affects an enzyme that has a direct effect). Of particular interest is the case in which the enzyme affects its own metabolism. This is called autoinduction (if it inhibits its own degradation) or autoinhibition (if it stimulates its own degradation). We will further expand this example to illustrate the autoinduction case for the one-compartment intravenous model. Autoinduction occurs when the drug inhibits its own degradation. Let us assume that a certain enzyme E mediates the degradation of a drug and that the drug promotes the degradation of the enzyme. As seen in Eq. (3.148), metabolic clearance can be calculated as

$$Cl_H = \frac{Q_H V_{max} f_u}{V_{max} f_u + C(t) Q_H + K_m Q_H} = \frac{Q_H a C_E(t) f_u}{a C_E(t) f_u + C(t) Q_H + K_m Q_H} \tag{3.157}$$

Then, we can write the dynamical equations for the concentration of the drug and the concentration of the enzyme

$$\begin{array}{rcl} \frac{dC_E(t)}{dt} & = & R_E - Cl_E(1+S)C_E(t) = R_E - Cl_E\left(1 - \frac{S_{max}C(t)}{SC_{50}+C(t)}\right)C_E(t) \\ \frac{dC(t)}{dt} & = & -\frac{Cl_H}{V}C(t) + \frac{dose_{iv}(t)}{V} = -\frac{1}{V}\frac{Q_H a C_E(t) f_u}{a C_E(t) f_u + C(t) Q_H + K_m Q_H}C(t) + \frac{dose_{iv}(t)}{V} \end{array} \tag{3.158}$$

Applying Euler's method to this equation system we get

$$\begin{array}{rcl} C_E[n] & = & C_E[n-1] + R_E T_s - Cl_E T_s\left(1 + S\frac{S_{max}C[n-1]}{SC_{50}+C[n-1]}\right)C_E[n-1] \\ C[n] & = & C[n-1] - \frac{T_s}{V}\frac{Q_H a C_E[n-1] f_u}{a C_E[n-1] f_u + C[n-1] Q_H + K_m Q_H}C[n-1] + \frac{dose_{iv}[n]T_s}{V} \end{array} \tag{3.159}$$

whose parameters to be identified are R_E, Cl_E, S_{max}, SC_{50}, V, Q_H, af_u (which are not separately identifiable), and K_m. Fig. 3.16 shows the evolution over time of the drug concentration and the hepatic clearance in the case of no autoinduction $S_{max} = 0$ and autoinduction $S_{max} = 0.3$. The rest of parameters are $R_E = 0.01$ (mg/min), $Cl_E = 0.005$ (L/min), $SC_{50} = 0.02$ (mg/L), $V = 10$ (L), $Q_H = 1.45$ (L/min), $f_u = 1$, $a = 0.01$ (1/min), $T_s = 1$ (min), $K_m = 0.1$ (mg/L).

3.4.3 Effects on blood flow

Certain drugs affect blood flow either by increasing it or decreasing it. This may be modelled with coefficients similar to the stimulation and inhibition factors in Eq. (3.155). So that the effective blood flow becomes $Q_H = Q_H^{(0)}(1+S)$ or $Q_H = Q_H^{(0)}(1-I)$. Let us develop here the case of blood flow stimulation for the one-compartment intravenous bolus. The increase of blood flow directly affect the metabolic clearance

$$\begin{array}{rcl} Cl_H & = & \frac{Q_H f_u Cl_{int}}{Q_H + f_u Cl_{int}} = \frac{Q_H^{(0)}(1+S) f_u Cl_{int}}{Q_H^{(0)}(1+S) + f_u Cl_{int}} = \frac{Q_H^{(0)} f_u Cl_{int}}{Q_H^{(0)} + f_u Cl_{int}\frac{1}{1+S}} \\ & = & \frac{Q_H^{(0)} f_u Cl_{int}}{Q_H^{(0)} + f_u Cl_{int}\frac{C+SC_{50}}{C(1+S_{max})+SC_{50}}} \end{array} \tag{3.160}$$

and this increased metabolic clearance is the one that must be used in the model for the concentration

$$\begin{array}{rcl} V\frac{dC(t)}{dt} & = & -Cl_H C(t) + dose_{iv}(t) \\ & = & -\frac{Q_H^{(0)} f_u Cl_{int}}{Q_H^{(0)} + f_u Cl_{int}\frac{C(t)+SC_{50}}{C(t)(1+S_{max})+SC_{50}}}C(t) + dose_{iv}(t) \end{array} \tag{3.161}$$

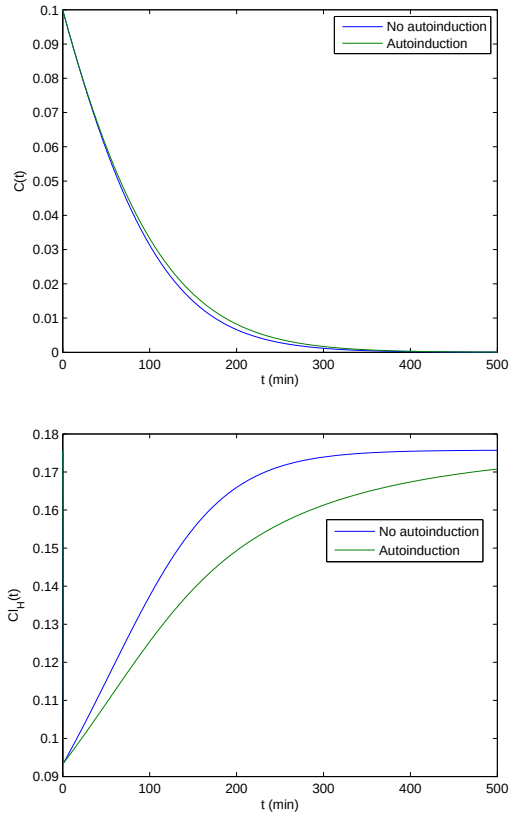

Figure 3.16: Drug concentration profile $C(t)$ for the case of no autoinduction and autoinduction after a bolus administration of 1mg (top) and the evolution of the metabolic clearance $Cl_H(t)$ (bottom).

We may apply any of the discretizing methods already seen.

3.4.4 Protein binding

Disregarding drug uptake into the blood cells, drug in plasma is circulating either bound to plasma proteins (like albumin and α_1-acid glycoprotein) or unbound. In our models so far, we considered the fraction of unbound drug to be fix, f_u. However, this may not be the case and protein binding can vary as a function of the drug concentration. It is normally accepted that the concentration of bound drug in blood follows an equation similar to the Michaelis-Menten modelling of enzymatic reaction (see Eq. (3.145)):

$$C_b = \frac{B_{max} C_u}{K_d + C_u} \tag{3.162}$$

where B_{max} is the maximum binding capacity, C_u is the unbound concentration, and K_d is the dissociation constant at equilibrium. The total drug concentration in blood is

$$C = C_b + C_u = C_u \left(1 + \frac{B_{max}}{K_d + C_u}\right) \tag{3.163}$$

If the concentration in blood of the binding protein is C_P and each protein can bind to n drug molecules, then the maximum binding capacity is $B_{max} = nC_P$. The unbound fraction can be calculated as

$$f_u = \frac{C_u}{C} = \frac{1}{1 + \frac{nC_P}{K_d + C_u}} \tag{3.164}$$

Now, we can transform a differential equation on C into a differential equation in C_u as follows. Consider, for instance, the one-compartment intravenous bolus model

$$V \frac{dC(t)}{dt} = -Cl_H C(t) + dose_{iv}(t) \tag{3.165}$$

by substituting $C(t)$ by its expression as a function of $C_u(t)$ we get

$$V \frac{d\left(C_u(t)\left(1+\frac{nC_P}{K_d+C_u(t)}\right)\right)}{dt} = -Cl_H C_u(t) \left(1 + \frac{nC_P}{K_d+C_u(t)}\right) + dose_{iv}(t) \tag{3.166}$$

We simply have now to calculate the derivative on the left

$$\begin{aligned} \frac{d\left(C_u(t)\left(1+\frac{nC_P}{K_d+C_u(t)}\right)\right)}{dt} &= \frac{dC_u(t)}{dt}\left(1 + \frac{nC_P}{K_d+C_u(t)}\right) + C_u(t)\left(-\frac{nC_P}{(K_d+C_u(t))^2}\right)\frac{dC_u(t)}{dt} \\ &= \frac{dC_u(t)}{dt}\left(1 + \frac{nC_P}{K_d+C_u(t)}\left(1 - \frac{C_u(t)}{K_d+C_u(t)}\right)\right) \end{aligned} \tag{3.167}$$

and substitute into Eq. (3.166):

$$V \frac{dC_u(t)}{dt}\left(1 + \frac{nC_P}{K_d+C_u(t)}\left(1 - \frac{C_u(t)}{K_d+C_u(t)}\right)\right) = -Cl_H C_u(t)\left(1 + \frac{nC_P}{K_d+C_u(t)}\right) + dose_{iv}(t) \tag{3.168}$$

At this point we may apply any of the discretization techinques already used in the thesis.

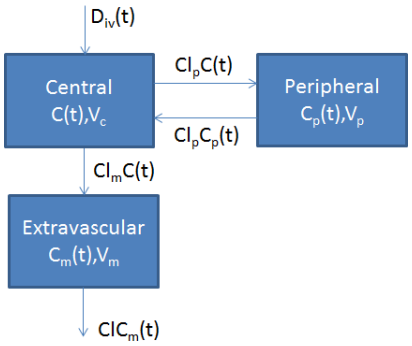

Figure 3.17: Model for a drug that is first converted into a metabolite with therapeutic effects.

3.4.5 Metabolite models

The modelling framework presented in this thesis also allows for modelling drugs that are first degraded into a metabolite with therapeutic effects and then eliminated. This model is schematically represented in Fig. 3.17. The model can be easily be represented in differential equations using the standard approach presented along the thesis

$$\begin{array}{rcl} V_c \frac{dC(t)}{dt} & = & -(Cl_m + Cl_p)C(t) + Cl_p C_p(t) + D_{iv}(t) \\ V_p \frac{dC_p(t)}{dt} & = & -Cl_p C_p(t) + Cl_p C(t) \\ V_m \frac{dC_m(t)}{dt} & = & -ClC_m(t) + Cl_m C(t) \end{array} \tag{3.169}$$

The model above is linear, but we may consider non-linear enzymatic kinetics at the metabolization of the drug. Let us consider a Michaelis-Menten enzymatic reaction as in Eq. (3.146). Then, it suffices to substitute Cl_m in the previous equation by $Cl_m = \frac{V_{max}}{K_m + C(t)}$.

3.4.6 Reaction rate

We may be particularly interested at the drug disappearance due to its binding to a particular receptor or molecule. In general, let us assume that the chemical reaction is of the form

$$dD + mM \leftrightarrow pDM \tag{3.170}$$

where D represents the drug, M the molecule it binds to, and DM the drug-molecule complex. In the general case, we assume that the chemical reaction is reversible. The case of irreversible binding is easily handled below.

The reaction rate is defined as

$$v = -\frac{1}{d}\frac{d[D]}{dt} = -\frac{1}{m}\frac{d[M]}{dt} = \frac{1}{p}\frac{d[DM]}{dt} \tag{3.171}$$

and it can be calculated as a function of the different species concentrations as

$$v = k_f [D]^{d'} [M]^{m'} - k_b [DM]^{p'} \tag{3.172}$$

where k_f is a constant (although with an important dependence on temperature) for the forward reaction, k_b is a constant for the backwards reaction, d', m' and p' are constants that have to be experimentally determined. For reactions occurring in a single step, these coefficients equal the stoichiometric coefficients $d' = d$, $m' = m$, and $p' = p$. The case of irreversible binding of the drug and the molecule simply requires setting $k_b = 0$. Note that v is normally given in $(mol \cdot L^{-1} \cdot s^{-1})$ and the units normally used along the thesis are $mg \cdot L^{-1} \cdot min^{-1}$. Consequently, we must also express concentrations in $mg \cdot L^{-1}$. At equilibrium, the net reaction rate is 0 implying

$$k_f [D]^{d'} [M]^{m'} = k_b [DM]^{p'} \Rightarrow K = \frac{k_f}{k_b} = \frac{[DM]^{p'}}{[D]^{d'} [M]^{m'}} \tag{3.173}$$

that is the standard formula for the equilibrium constant. However, note that in the methodology proposed in this thesis we are not restricted to study the equilibrium state, but that we can study the dynamics of the full system at any time.

Let us incorporate the reaction rate to the one-compartment intravenous bolus model. We simply need to add the corresponding disappearance of drug and keep track of the concentration of the other species involved in the reaction (to be consistent with our notation we will change $[D]$ by $C(t)$, $[M]$ by $C_M(t)$, $[DM]$ by $C_{DM(t)}$) :

$$\begin{array}{rcl} V\frac{dC(t)}{dt} & = & -Cl_H C(t) - dv + dose_{iv}(t) \\ & = & -Cl_H C(t) - d(k_f C^{d'}(t) C_M^{m'}(t) - k_b C_{DM}^{p'}(t)) + dose_{iv}(t) \\ \frac{dC_M(t)}{dt} & = & -mv = -m(k_f C^{d'}(t) C_M^{m'}(t) - k_b C_{DM}^{p'}(t)) \\ \frac{dC_{DM}(t)}{dt} & = & pv = p(k_f C^{d'}(t) C_M^{m'}(t) - k_b C_{DM}^{p'}(t)) \end{array} \tag{3.174}$$

In order to fully keep track of all species it is important that we know the concentration of the molecule M at $t = 0$ (when it is supposed that we administer the drug). Note also that nothing precludes this model of incorporating other nonlinear effects as turnover (see Section 3.3.6) of the molecule M.

3.5 Pharmacodynamics

Pharmacodynamics models the therapeutic effect of a certain drug at a given concentration. Administering a drug has a certain physiological target (regulating the body temperature, heart rate, blood pressure, etc.). Measuring drug concentration alone does not tell us how the target parameter, E, evolves over time. Pharmacodynamics places mathematical models to predict the effect of the drug at a particular concentration on the desired variable E. In this chapter we revise the pharmacodynamics literature under the light of the system modelling approach defended in this thesis.

3.5.1 Effect size and receptor binding

The most widely accepted model suggests that variable E is related to the occupancy of a number of receptors (may be cell receptors or extracellular receptors) that are involved in the physiological events finally resulting in a certain body

response E. A drug may act as: 1) an agonist by binding to these receptors and activating their response; 2) an inverse agonist by binding to the receptors and causing an effect opposite to that of the agonist; 3) as an antagonist by binding to the receptors and blocking their action. In any case, the drug effect is directly linked to its binding to a certain receptor. A comprehensive review of receptor binding is given by Krohn and Link (2003).

It has been proposed that the response variable E can achieve a maximum value, E_{max}, when all involved receptors are occupied. Let us assume that there is a total of N_{tot} of these receptors and that each one has an intrinsic efficacy ϵ (that is defined as the part of the response caused by a single receptor). In this case, we could compute the maximum effect as

$$E_{max} = N_{tot}\epsilon \tag{3.175}$$

In practice, it is difficult to estimate any of these two parameters and it has been alternatively proposed to compute E_{max} as a function of the concentration of receptors. Let us denote C_R as the concentration of receptors per volume unit in a certain compartment, which for the moment is assumed to be fixed. Then,

$$E_{max} = \alpha C_R \tag{3.176}$$

where α is a proportionality constant. This maximum response is presumed to be achieved when all receptors are activated. If not all of them are activated, then the actual response would be proportional to the fraction of activated receptors:

$$E(t) = E_{max}\frac{C_{R,activated}(t)}{C_R} \tag{3.177}$$

where $C_{R,activated}$ is the concentration of activated receptors. Let us study the effect of an agonist drug. The binding of this drug to the receptor results in an increase of the physiological response E because the fraction of occupied receptors increases. The variation of the bounded receptors can be modelled through the law of mass action applied to the following reaction

$$D + R_{free} \leftrightarrow DR \tag{3.178}$$

where R_{free} is a free receptor and DR is a receptor bound to a drug molecule.

The concentration of activated receptors can then be calculated as

$$C_{R,activated}(t) = C^0_{R,activated} + C_{DR}(t) \tag{3.179}$$

where $C_{DR}(t)$ is the concentration of receptors occupied by a drug molecule and $C^0_{R,activated}$ is the concentration of activated receptors when the drug has not yet been administered (the basal state). The corresponding basal level of the response variable will be denoted as E_0 (which is equal to $E_0 = E_{max}\frac{C^0_{R,activated}}{C_R}$). The concentration of receptors occupied by drug molecules can be calculated with the law of mass action:

$$\frac{dC_{DR}(t)}{dt} = k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \tag{3.180}$$

where k_1 is the rate constant of the forward reaction and k_b is the rate constant of the backwards reaction. Note that at any moment it must hold:

$$C_{R,free}(t) + C_{DR}(t) + C^0_{R,activated} = C_R \tag{3.181}$$

that is, the total concentration of receptors is the sum of those that are still free, those that are bound to a drug molecule, and those that are bound at the basal state. In particular, at $t = 0$ this makes

$$C_{R,free}(0) = C_R - C^0_{R,activated} \tag{3.182}$$

and obviously, $C_{DR}(0) = 0$. Eq. (3.180) has to be combined with the equation monitoring the amount of free receptors and an equation governing the drug concentration. For simplicity we will consider the one-compartment with intravenous doses, although nothing in the theory precludes more sophisticated models. The set of differential equations to be solved is:

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) + Dose_{iv}(t) \end{array} \tag{3.183}$$

The previous set of equations give the full dynamics of the concentration of all the species involved. However, some of its parameters may be difficult to estimate like the concentration of occupied receptors in the basal state and the total concentration of receptors. For this reason, in the following we will try to simplify this equation into a more tractable form. If the change of drug concentration over time is relatively slow with respect to the time that it takes to reaction in Eq. (3.178) to achieve equilibrium. We may consider that this reaction is at equilibrium. This gives

$$\frac{dC_{DR}(t)}{dt} = k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) = 0 \Rightarrow \frac{k_b}{k_f} = \frac{C_{R,free}(t)C(t)}{C_{DR}(t)} = K_D \tag{3.184}$$

where K_D is the dissociation constant of the receptor-drug complex. Considering that from Eq. (3.181) we have that

$$C_{R,free}(t) = C_R - C_{DR}(t) - C^0_{R,activated} \tag{3.185}$$

we can rearrange Eq. (3.184) to write

$$C_{DR}(t) = (C_R - C^0_{R,activated})\frac{C(t)}{K_D + C(t)} \tag{3.186}$$

The fraction of activated receptors would be

$$\frac{C^0_{R,activated} + C_{DR}(t)}{C_R} \tag{3.187}$$

If we refer to the fraction of occupancy as p, and we define the basal fraction of occupancy as $p_0 = \frac{C^0_{R,activated}}{C_R}$, then we have

$$\begin{array}{rcl} p & = & \frac{C^0_{R,activated} + C_{DR}(t)}{C_R} \\ & = & p_0 + \frac{C_R - C^0_{R,activated}}{C_R}\frac{C(t)}{K_D + C(t)} \\ & = & p_0 + (1 - p_0)\frac{C(t)}{K_D + C(t)} \end{array} \tag{3.188}$$

The corresponding response

$$\begin{aligned} E &= E_{max} p \\ &= E_{max}\left(p_0 + (1-p_0)\frac{C(t)}{K_D+C(t)}\right) \\ &= E_0 + (E_{max}-E_0)\frac{C(t)}{K_D+C(t)} \end{aligned} \tag{3.189}$$

We may rewrite the expression above by using the maximum effect of the drug, $E_{D,max}$, instead of the maximum physiological response:

$$E = E_0 + E_{D,max}\frac{C(t)}{K_D+C(t)} \tag{3.190}$$

In this way we may deal in the same way with drugs that increase the basal effect ($E_{D,max} > 0$) or that decrease it ($E_{D,max} < 0$).

In some textbooks (Gabrielsson and Weiner, 2007)[Sec. 3.3], E_0 is disregarded yielding the well-known dependency

$$E = E_{max}\frac{C(t)}{K_D+C(t)} \tag{3.191}$$

However, it is worth noting that this expression has been obtained after two simplifications: the basal response is negligible ($E_0 = 0$), and that the receptor-drug binding reaction is much faster than the decay of the drug concentration.

Within the framework defended in this thesis we are not restricted to these simplified effect models in which the receptor binding is assumed to be faster than the drug concentration changes. Instead, we may integrate the effect within the full dynamical description of the system as follows. Let us consider the dynamical equations in Eq. (3.183). We simply need to calculate the instantaneous proportion of activated receptors and the corresponding effect:

$$\begin{aligned} \frac{dC_{DR}(t)}{dt} &= k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\ \frac{dC_{R,free}(t)}{dt} &= -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} &= -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) + Dose_{iv}(t) \\ p(t) &= \frac{C_{DR}(t)}{C_{DR}(t)+C_{R,free}(t)} \\ E &= E_{max} p(t) \end{aligned} \tag{3.192}$$

Multiple receptor binding

It might well be that the drug binds to multiple receptors with different affinities and that each receptor has a different effect size. We may model this as two chemical reactions:

$$\begin{aligned} D + R_{free,1} &\leftrightarrow DR_1 \\ D + R_{free,2} &\leftrightarrow DR_2 \end{aligned} \tag{3.193}$$

We only need to consider the additive effect of both receptors by modifying Eq. (3.177) to

$$E(t) = E_{max,1}\frac{C_{R_1,activated}(t)}{C_{R_1}} + E_{max,2}\frac{C_{R_2,activated}(t)}{C_{R_2}} \tag{3.194}$$

and the equation system at Eq. (3.183) to track the number of free receptors of type 2:

$$\begin{array}{rcl} \frac{dC_{DR_1}(t)}{dt} & = & k_{f_1}C_{R_1,free}(t)C(t) - k_{b_1}C_{DR_1}(t) \\ \frac{dC_{R_1,free}(t)}{dt} & = & -k_{f_1}C_{R_1,free}(t)C(t) + k_{b_1}C_{DR_1}(t) \\ \frac{dC_{DR_2}(t)}{dt} & = & k_{f_2}C_{R_2,free}(t)C(t) - k_{b_2}C_{DR_2}(t) \\ \frac{dC_{R_2,free}(t)}{dt} & = & -k_{f_2}C_{R_2,free}(t)C(t) + k_{b_2}C_{DR_2}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_{f_1}C_{R_1,free}(t)C(t) + k_{b_1}C_{DR_1}(t) \\ & & -k_{f_2}C_{R_2,free}(t)C(t) + k_{b_2}C_{DR_2}(t) + Dose_{iv}(t) \end{array} \tag{3.195}$$

This equation system fully describes the dynamics of the system. As we did before, we may assume that receptor binding to both receptor types quickly achieves equilibrium so that Eq. (3.189) becomes

$$\begin{array}{rcl} E & = & E_{0,1} + (E_{max,1} - E_{0,1})\frac{C(t)}{K_{D_1}+C(t)} + \\ & & E_{0,2} + (E_{max,2} - E_{0,2})\frac{C(t)}{K_{D_2}+C(t)} \end{array} \tag{3.196}$$

Multiple site binding

Some receptors need multiple drug molecules to be activated. Let us say we need n drug molecules to activate the receptor

$$nD + R \leftrightarrow DR_n \tag{3.197}$$

The system dynamics are described by (equivalent to Eq. (3.183))

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & k_f C_{R,free}(t)C^n(t) - k_b C_{DR}(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_f C_{R,free}(t)C^n(t) + k_b C_{DR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_f C_{R,free}(t)C^n(t) + k_b C_{DR}(t) + Dose_{iv}(t) \end{array} \tag{3.198}$$

This equation assumes that the binding of n drug molecules is performed simultaneously to the receptor and receives the name of Hill model. This is rather unrealistic experimentally. However, this assumption results in a sigmoidal dependency that fits relatively well in experimental situations with a small modification. As we have done before, we assume that the receptor binding equilibrium is much faster than changes in the concentration. This makes that

$$\frac{dC_{DR}(t)}{dt} = 0 \Rightarrow C_{DR}(t) = \frac{k_f}{k_b}C_{R,free}(t)C^n(t) \tag{3.199}$$

Following a reasoning similar to the one developed at the beginning of this section, we would arrive to an effect size

$$E = E_0 + (E_{max} - E_0)\frac{C^n(t)}{K_D+C^n(t)} \tag{3.200}$$

This situation is experimentally unrealistic because it is difficult that n drug molecules simultaneously bind to the receptor. However, macroscopically we observe that only a fraction of those n actually bind per unit time. This is easily modelled by replacing n by h (the Hill coefficient, with $h \leq n$)

$$E = E_0 + (E_{max} - E_0)\frac{C^h(t)}{K_D+C^h(t)} \tag{3.201}$$

The Hill coefficient also helps to explain cooperative binding. Let us imagine that a single drug molecule binds a single receptor. However, once it is bound it helps other drug molecules to bind nearby receptors. This is referred to as positive cooperative binding and it is modelled by $h > 1$. Let us imagine the opposite situation in which once a drug molecule binds a receptor it prevents the binding in nearby receptors. This is called negative cooperative binding and it is modelled by $h < 1$.

Sequential binding

Let us now consider that drug molecules bind sequentially the receptor according to the following reactions

$$\begin{array}{c} D + R_{free} \leftrightarrow DR \\ D + DR \leftrightarrow D_2R \end{array} \tag{3.202}$$

The system dynamics is described by

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & k_{f_1} C_{R,free}(t) C(t) - k_{b_1} C_{DR}(t) + \\ & & k_{b_2} C_{D_2R}(t) - k_{f_2} C_{DR}(t) C(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_{f_1} C_{R,free}(t) C(t) + k_{b_1} C_{DR}(t) \\ \frac{dC_{D_2R}(t)}{dt} & = & k_{f_2} C_{DR}(t) C(t) - k_{b_2} C_{D_2R}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_{f_1} C_{R,free}(t) C(t) + k_{b_1} C_{DR}(t) + Dose_{iv}(t) \\ & & -k_{f_2} C_{DR}(t) C(t) + k_{b_2} C_{D_2R}(t) + Dose_{iv}(t) \end{array} \tag{3.203}$$

As we did before, we may assume that the receptor binding is much faster than the drug concentration changes. In this situation, both binding equilibria are achieved. At steady state

$$\begin{array}{rcl} 0 = \frac{dC_{R,free}(t)}{dt} & = & -k_{f_1} C_{R,free}(t) C(t) + k_{b_1} C_{DR}(t) \Rightarrow K_{D_1} = \frac{k_{b_1}}{k_{f_1}} = \frac{C_{R,free}(t) C(t)}{C_{DR}(t)} \\ 0 = \frac{dC_{D_2R}(t)}{dt} & = & k_{f_2} C_{DR}(t) C(t) - k_{b_2} C_{D_2R}(t) \Rightarrow K_{D_2} = \frac{k_{b_2}}{k_{f_2}} = \frac{C_{DR}(t) C(t)}{C_{D_2R}(t)} \end{array} \tag{3.204}$$

From these two equations we deduce

$$\begin{array}{l} C_{DR}(t) = \frac{1}{K_{D_1}} C_{R,free}(t) C(t) \\ C_{D_2R}(t) = \frac{1}{K_{D_2}} C_{DR}(t) C(t) = \frac{1}{K_{D_1} K_{D_2}} C_{R,free}(t) C^2(t) \end{array} \tag{3.205}$$

The total amount of receptors must remain constant

$$C^0_{R,activated} + C_{R,free}(t) + C_{DR}(t) + C_{D_2R}(t) = C_R \tag{3.206}$$

Substituting the concentrations above we have

$$C^0_{R,activated} + C_{R,free}(t) + \frac{1}{K_{D_1}} C_{R,free}(t) C(t) + \frac{1}{K_{D_1} K_{D_2}} C_{R,free}(t) C^2(t) = C_R \tag{3.207}$$

From which we deduce

$$C_{R,free}(t) = \frac{C_R - C^0_{R,activated}}{1 + \frac{C(t)}{K_{D_1}} + \frac{C^2(t)}{K_{D_1} K_{D_2}}} \tag{3.208}$$

and, consequently,

$$\begin{aligned} C_{D_2R}(t) &= \frac{1}{K_{D_1}K_{D_2}} C_{R,free}(t)C^2(t) = (C_R - C^0_{R,activated}) \frac{\frac{C^2(t)}{K_{D_1}K_{D_2}}}{1+\frac{C(t)}{K_{D_1}}+\frac{C^2(t)}{K_{D_1}K_{D_2}}} \\ &= (C_R - C^0_{R,activated}) \frac{C^2(t)}{K_{D_1}K_{D_2}+K_{D_2}C(t)+C^2(t)} \end{aligned} \tag{3.209}$$

The fraction of activated receptors can be calculated as

$$\begin{aligned} p &= \frac{C^0_{R,activated}+C_{D_2R}(t)}{C_R} \\ &= \frac{C^0_{R,activated}+(C_R-C^0_{R,activated})\frac{C^2(t)}{K_{D_1}K_{D_2}+K_{D_2}C(t)+C^2(t)}}{C_R} \\ &= p_0 + (1-p_0)\frac{C^2(t)}{K_{D_1}K_{D_2}+K_{D_2}C(t)+C^2(t)} \end{aligned} \tag{3.210}$$

Finally, the effect size is

$$E = E_0 + (E_{max} - E_0)\frac{C^2(t)}{K_{D_1}K_{D_2}+K_{D_2}C(t)+C^2(t)} \tag{3.211}$$

Competitive binding

Let us now consider that some exogeneous or endogenous molecules bind to the same site as the drug, but with no effect. Let us call such a molecule an antagonist A. The chemical reactions are

$$\begin{aligned} D + R_{free} &\leftrightarrow DR \\ A + R_{free} &\leftrightarrow AR \end{aligned} \tag{3.212}$$

The system dynamics is described by

$$\begin{aligned} \frac{dC_{DR}(t)}{dt} &= k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\ \frac{dC_{AR}(t)}{dt} &= k_{f_A} C_{R,free}(t)C_A(t) - k_{b_A} C_{AR}(t) \\ \frac{dC_{R,free}(t)}{dt} &= -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ &\quad -k_{f_A} C_{R,free}(t)C_A(t) + k_{b_A} C_{AR}(t) \\ \frac{dC_A(t)}{dt} &= -k_{f_A} C_{R,free}(t)C_A(t) + k_{b_A} C_{AR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} &= -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) + Dose_{iv}(t) \end{aligned} \tag{3.213}$$

As usual, we assume that receptor binding achieves equilibrium much faster than there are significant changes in the drug concentration. At this equilibrium there is no net variation of the bound concentrations

$$\begin{aligned} \frac{dC_{DR}(t)}{dt} = 0 \Rightarrow C_{DR}(t) &= \frac{1}{K_D} C_{R,free}(t)C(t) \\ \frac{dC_{AR}(t)}{dt} = 0 \Rightarrow C_{AR}(t) &= \frac{1}{K_{D_A}} C_{R,free}(t)C_A(t) \end{aligned} \tag{3.214}$$

Assuming that the total amount of receptors is kept constant, we have

$$C^0_{R,activated} + C_{R,free}(t) + C_{DR}(t) + C_{AR}(t) = C_R \tag{3.215}$$

Substituting the values calculated above we get

$$C^0_{R,activated} + C_{R,free}(t) + \frac{1}{K_D} C_{R,free}(t)C(t) + \frac{1}{K_{D_A}} C_{R,free}(t)C_A(t) = C_R \tag{3.216}$$

From where

$$C_{R,free}(t) = (C_R - C^0_{R,activated})\frac{1}{1+\frac{1}{K_D}C(t)+\frac{1}{K_{D_A}}C_A(t)} \tag{3.217}$$

The fraction of occupied receptors becomes (only those receptors with some effect on the physiological variable are accounted)

$$\begin{aligned} p &= \frac{C^0_{R,activated}+C_{DR}(t)}{C_R} \\ &= p_0 + \frac{C_R - C^0_{R,activated}}{C_R}\frac{1}{K_D}C_{R,free}(t)C(t) \\ &= p_0 + (1-p_0)\frac{\frac{1}{K_D}C(t)}{1+\frac{1}{K_D}C(t)+\frac{1}{K_{D_A}}C_A(t)} \\ &= p_0 + (1-p_0)\frac{C(t)}{K_D+C(t)+\frac{K_D}{K_{D_A}}C_A(t)} \\ &= p_0 + (1-p_0)\frac{C(t)}{\left(1+\frac{C_A(t)}{K_{D_A}}\right)K_D+C(t)} \end{aligned} \tag{3.218}$$

The corresponding effect is

$$E = E_0 + (E_{max}-E_0)\frac{C(t)}{\left(1+\frac{C_A(t)}{K_{D_A}}\right)K_D+C(t)} \tag{3.219}$$

Note that the effect of the competitor is a decrease in the effective K_D which results in an increase in the drug concentration to achieve the same effect. The competitor receives the name of a **competitive antagonist**.

The competitor instead may have the same effect as the drug (it is called a **full agonist**, full because it has the same maximum effect as the drug), then the proportion of activated receptors would become

$$\begin{aligned} p &= \frac{C^0_{R,activated}+C_{DR}(t)+C_{AR}(t)}{C_R} \\ &= p_0 + (1-p_0)\frac{\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}} \end{aligned} \tag{3.220}$$

and the corresponding effect

$$E = E_0 + (E_{max}-E_0)\frac{\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}} \tag{3.221}$$

We may also consider that the agonist molecule only causes a fraction of the full effect $E_{max,A} = \alpha E_{max}$ (with $0 \leq \alpha \leq 1$; it is then called **partial agonist**). Then, we separate the two effects. The final effect becomes

$$E = E_0 + (E_{max}-E_0)\frac{\frac{C(t)}{K_D}+\alpha\frac{C_A(t)}{K_{D_A}}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}} \tag{3.222}$$

An **inverse agonist** is a molecule that causes a contrarian effect to that of our drug. The effect equation is exactly the one for a partial agonist, only that $\alpha < 0$.

Non-competitive binding

An exogenous or endogeneous molecule may bind to an allosteric site of the receptor. It does not compete with the drug for the binding site but it modifies the structure of the site and reduces or increases the maximum effect attainable by the drug. The chemical reactions are

$$\begin{array}{l} D + R_{free} \leftrightarrow DR \\ A + R_{free} \leftrightarrow AR \\ D + AR \leftrightarrow DAR \end{array} \tag{3.223}$$

The system dynamics is described by

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\ \frac{dC_{AR}(t)}{dt} & = & k_{f_A} C_{R,free}(t)C_A(t) - k_{b_A} C_{AR}(t) \\ & & -k_{f_{AR}} C(t)C_{AR}(t) + k_{b_{AR}} C_{DAR}(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ & & -k_{f_A} C_{R,free}(t)C_A(t) + k_{b_A} C_{AR}(t) \\ \frac{dC_A(t)}{dt} & = & -k_{f_A} C_{R,free}(t)C_A(t) + k_{b_A} C_{AR}(t) \\ & & +k_{f_{AR}} C(t)C_{AR}(t) - k_{b_{AR}} C_{DAR}(t) \\ \frac{dC_{DAR}(t)}{dt} & = & k_{f_{AR}} C(t)C_{AR}(t) - k_{b_{AR}} C_{DAR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ & & -k_{f_{AR}} C(t)C_{AR}(t) + k_{b_{AR}} C_{DAR}(t) + Dose_{iv}(t) \end{array} \tag{3.224}$$

Assuming that the receptor binding is much faster than the variations of the drug concentration, we would have that the binding equations are at equilibrium:

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & 0 \Rightarrow C_{DR}(t) = \frac{1}{K_D} C_{R,free}(t)C(t) \\ \frac{dC_{DAR}(t)}{dt} & = & 0 \Rightarrow C_{DAR}(t) = \frac{1}{K_{D_{AR}}} C(t)C_{AR}(t) \\ \frac{dC_{AR}(t)}{dt} & = & 0 = k_{f_A} C_{R,free}(t)C_A(t) - (k_{b_A} + k_{f_{AR}} C(t))C_{AR}(t) + k_{b_{AR}} C_{DAR}(t) \\ & = & k_{f_A} C_{R,free}(t)C_A(t) - (k_{b_A} + k_{f_{AR}} C(t))C_{AR}(t) + k_{b_{AR}} \frac{1}{K_{D_{AR}}} C(t)C_{AR}(t) \\ & = & k_{f_A} C_{R,free}(t)C_A(t) - \left(k_{b_A} + \left(k_{f_{AR}} - k_{b_{AR}} \frac{1}{K_{D_{AR}}}\right) C(t)\right) C_{AR}(t) \\ & = & k_{f_A} C_{R,free}(t)C_A(t) - \left(k_{b_A} + \left(k_{f_{AR}} - k_{b_{AR}} \frac{k_{f_{AR}}}{k_{b_{AR}}}\right) C(t)\right) C_{AR}(t) \\ & = & k_{f_A} C_{R,free}(t)C_A(t) - \left(k_{b_A} + \left(k_{f_{AR}} - k_{f_{AR}}\right) C(t)\right) C_{AR}(t) \\ & = & k_{f_A} C_{R,free}(t)C_A(t) - k_{b_A} C_{AR}(t) \\ & = & 0 \Rightarrow C_{AR}(t) = \frac{1}{K_{D_A}} C_{R,free}(t)C_A(t) \end{array} \tag{3.225}$$

In fact, we may reexpress $C_{DAR}(t)$ at equilibrium as

$$C_{DAR}(t) = \frac{1}{K_{D_{AR}}} C(t)C_{AR}(t) = \frac{1}{K_{D_{AR}} K_{D_A}} C(t)C_{R,free}(t)C_A(t) \tag{3.226}$$

The amount of total receptors is fixed. Consequently,

$$C^0_{R,activated} + C_{R,free}(t) + C_{DR}(t) + C_{AR}(t) + C_{DAR}(t) = C_R \tag{3.227}$$

Substituting the values above

$$C^0_{R,activated} + C_{R,free}(t)\left(1 + \frac{C(t)}{K_D} + \frac{C_A(t)}{K_{D_A}} + \frac{C(t)C_A(t)}{K_{D_{AR}} K_{D_A}}\right) = C_R \tag{3.228}$$

From which

$$C_{R,free}(t) = (C_R - C^0_{R,activated})\frac{1}{1 + \frac{C(t)}{K_D} + \frac{C_A(t)}{K_{D_A}} + \frac{C(t)C_A(t)}{K_{D_{AR}}K_{D_A}}} \tag{3.229}$$

As we did in the case of competitive binding (previous paragraph), let us presume that only the DR receptors are effective for the physiological response being studied. We may calculate the proportion of effectively bounded receptors as

$$p = p_0 + (1 - p_0)\frac{\frac{C(t)}{K_D}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}+\frac{C(t)C_A(t)}{K_{D_{AR}}K_{D_A}}} \tag{3.230}$$

whose corresponding effect is

$$\begin{aligned} E &= E_0 + (E_{max} - E_0)\frac{\frac{C(t)}{K_D}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}+\frac{C(t)C_A(t)}{K_{D_{AR}}K_{D_A}}} \\ &= E_0 + (E_{max} - E_0)\frac{C(t)}{K_D+C(t)+\frac{K_DC_A(t)}{K_{D_A}}+\frac{K_DC(t)C_A(t)}{K_{D_{AR}}K_{D_A}}} \\ &= E_0 + (E_{max} - E_0)\frac{C(t)}{\left(1+\frac{C_A(t)}{K_{D_A}}\right)K_D+\left(1+\frac{K_DC_A(t)}{K_{D_{AR}}K_{D_A}}\right)C(t)} \end{aligned} \tag{3.231}$$

Note that the antagonist has modified both, the effective K_D (that is increased as in the case of the competitive antagonist) and the maximum achievable effect (that is decreased; the limit when $C(t)$ goes to infinity is $E_0 + \frac{E_{max}-E_0}{1+\frac{K_DC_A(t)}{K_{D_{AR}}K_{D_A}}}$).

If, as we did in the case of competitive binding, we presume that the DA and DAR complexes have some partial effect on the physiological response, then we have that the total effect is

$$E = E_0 + (E_{max} - E_0)\frac{\frac{C(t)}{K_D}+\alpha\frac{C_A(t)}{K_{D_A}}+\beta\frac{C(t)C_A(t)}{K_{D_{AR}}K_{D_A}}}{1+\frac{C(t)}{K_D}+\frac{C_A(t)}{K_{D_A}}+\frac{C(t)C_A(t)}{K_{D_{AR}}K_{D_A}}} \tag{3.232}$$

Activated and inhibited binding

Let us consider the case in which the receptor has to be in a relaxed form to be chemically active. Otherwise, the receptor is said to be in a taut (inactive) form. Let us refer to the relaxed, free form as R_{free} and as R_{taut} to the receptor in its taut form. The chemical reactions involved are

$$\begin{aligned} R_{free} &\leftrightarrow R_{taut} \\ D + R_{free} &\leftrightarrow DR \end{aligned} \tag{3.233}$$

System dynamics are described by

$$\begin{aligned} \frac{dC_{DR}(t)}{dt} &= k_fC_{R,free}(t)C(t) - k_bC_{DR}(t) \\ \frac{dC_{R,taut}(t)}{dt} &= k_{f,taut}C_{R,free}(t) - k_{b,taut}C_{R,taut}(t) \\ \frac{dC_{R,free}(t)}{dt} &= -k_fC_{R,free}(t)C(t) + k_bC_{DR}(t) + \\ &\quad -k_{f,taut}C_{R,free}(t) + k_{b,taut}C_{R,taut}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} &= -ClC(t) - k_fC_{R,free}(t)C(t) + k_bC_{DR}(t) + Dose_{iv}(t) \end{aligned} \tag{3.234}$$

Assuming that the drug concentration changes relatively slowly with respect to the rest of chemical reactions, we can assume that the receptor binding is at

equilibrium. Then, as was reasoned in the previous case,

$$\begin{aligned} K_D = \frac{C_{R,free}(t)C(t)}{C_{DR}(t)} \Rightarrow C_{DR}(t) = \frac{C_{R,free}(t)C(t)}{K_D} \\ K_T = \frac{C_{R,free}(t)}{C_{R,taut}(t)} \Rightarrow C_{R,taut}(t) = \frac{C_{R,free}(t)}{K_T} \end{aligned} \tag{3.235}$$

The total number of receptors must be constant, so

$$C^0_{R,activated} + C_{R,taut}(t) + C_{R,free}(t) + C_{DR}(t) = C_R \tag{3.236}$$

Substituting the concentrations above

$$C^0_{R,activated} + \frac{C_{R,free}(t)}{K_T} + C_{R,free}(t) + \frac{C_{R,free}(t)C(t)}{K_D} = C_R \tag{3.237}$$

From where

$$\frac{C_{R,free}(t)}{K_T} + C_{R,free}(t) + \frac{C_{R,free}(t)C(t)}{K_D} = C_R - C^0_{R,activated} \tag{3.238}$$

and

$$C_{R,free}(t) = (C_R - C^0_{R,activated})\frac{1}{\frac{1}{K_T} + 1 + \frac{C(t)}{K_D}} \tag{3.239}$$

$$\begin{aligned} E &= E_0 + (E_{max} - E_0)\frac{\frac{C(t)}{K_D}}{\frac{1}{K_T}+1+\frac{C(t)}{K_D}} \\ &= E_0 + (E_{max} - E_0)\frac{C(t)}{\left(1+\frac{1}{K_T}\right)K_D + C(t)} \end{aligned} \tag{3.240}$$

When there are inhibitors and activators, inhibitors can bind to the taut form (unbalancing the equilibrium towards taut) and activators to the relaxed form (unbalancing equilibrium towards relaxed). The factor $\frac{1}{K_T}$ becomes

$$\frac{1}{K_T}\left(\frac{1 + \frac{C_I(t)}{K_I}}{1 + \frac{C_A(t)}{K_A}}\right) \tag{3.241}$$

where $C_I(t)$ and $C_A(t)$ are the concentrations of the inhibitor and the activator, and K_I and K_A are the dissociation constant of the inhibitor-receptor complex and the activator-receptor complex, respectively. Finally, the effect size in presence of inhibitors and activators becomes

$$E = E_0 + (E_{max} - E_0)\frac{C(t)}{\left(1+\frac{1}{K_T}\left(\frac{1+\frac{C_I(t)}{K_I}}{1+\frac{C_A(t)}{K_A}}\right)\right)K_D + C(t)} \tag{3.242}$$

Binding of enantiomers

A drug may alternate between two enantiomeric states. Each one binding the target receptor with different affinity. Now, we will derive what is the combined effect of both conformations. The biochemical equation may be represented as

$$\begin{aligned} D_L \leftrightarrow D_R D_L + R_{free} \leftrightarrow D_L R \\ D_R + R_{free} \leftrightarrow D_R R \end{aligned} \tag{3.243}$$

Let us study first the equilibrium between the two enantiomers in the absence of any other effect. Their dynamical behaviour respond to

$$\begin{array}{rcl} \frac{dC_{D_L}(t)}{dt} & = & -k_1 C_{D_L}(t) + k_{-1} C_{D_R}(t) \\ \frac{dC_{D_R}(t)}{dt} & = & k_1 C_{D_L}(t) - k_{-1} C_{D_R}(t) \end{array} \tag{3.244}$$

At equilibrium both variations are zero, and the relationship between the two concentrations at equilibrium are

$$k_1 C_{D_L}(t) = k_{-1} C_{D_R}(t) \Rightarrow K = \frac{k_1}{k_{-1}} = \frac{C_{D_R}(t)}{C_{D_L}(t)} \tag{3.245}$$

The fraction of the left enantiomer and the right enantiomer become

$$\begin{array}{rcl} f_L & = & \frac{C_{D_L}(t)}{C_{D_L}(t)+C_{D_R}(t)} = \frac{C_{D_L}(t)}{C_{D_L}(t)+KC_{D_L}(t)} = \frac{1}{1+K} \\ f_R & = & \frac{C_{D_R}(t)}{C_{D_L}(t)+C_{D_R}(t)} = \frac{KC_{D_L}(t)}{C_{D_L}(t)+KC_{D_L}(t)} = \frac{K}{1+K} \end{array} \tag{3.246}$$

The full system dynamics is given by

$$\begin{array}{rcl} \frac{dC_{D_L R}(t)}{dt} & = & k_{f_L} C_{R,free}(t) C_{D_L}(t) - k_{b_L} C_{D_L R}(t) \\ \frac{dC_{D^R R}(t)}{dt} & = & k_{f_R} C_{R,free}(t) C_{D_R}(t) - k_{b_R} C_{D_R R}(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_{f_L} C_{R,free}(t) C_{D_L}(t) + k_{b_L} C_{D_L R}(t) \\ & & -k_{f_R} C_{R,free}(t) C_{D_R}(t) - k_{b_R} C_{D_R R}(t) \\ \frac{1}{V}\frac{dC_{D_L}(t)}{dt} & = & -ClC_{D_L}(t) - k_1 C_{D_L}(t) + k_{-1} C_{D_R}(t) + f_L Dose_{iv}(t) \\ \frac{1}{V}\frac{dC_{D_R}(t)}{dt} & = & -ClC_{D_R}(t) + k_1 C_{D_L}(t) - k_{-1} C_{D_R}(t) + f_R Dose_{iv}(t) \end{array} \tag{3.247}$$

Assuming that the receptor binding is much faster than the variations of the drug concentration, we would have that the binding equations are at equilibrium:

$$\begin{array}{rcl} \frac{dC_{D_L R}(t)}{dt} & = & 0 \Rightarrow C_{D_L R}(t) = \frac{1}{K_{D_L}} C_{R,free}(t) C_{D_L}(t) \\ \frac{dC_{D_R R}(t)}{dt} & = & 0 \Rightarrow C_{D_R R}(t) = \frac{1}{K_{D_R}} C_{R,free}(t) C_{D_R}(t) \end{array} \tag{3.248}$$

The amount of total receptors is fixed. Consequently,

$$C^0_{R,activated} + C_{R,free}(t) + C_{D_L R}(t) + C_{D_R R}(t) = C_R \tag{3.249}$$

Substituting the values above

$$C^0_{R,activated} + C_{R,free}(t)\left(1 + \frac{C_{D_L}(t)}{K_{D_L}} + \frac{C_{D_R}(t)}{K_{D_R}}\right) = C_R \tag{3.250}$$

From which

$$C_{R,free}(t) = (C_R - C^0_{R,activated}) \frac{1}{1 + \frac{C_{D_L}(t)}{K_{D_L}} + \frac{C_{D_R}(t)}{K_{D_R}}} \tag{3.251}$$

We may calculate the proportion of bounded receptors of each kind (left and right enantiomers)

$$\begin{array}{rcl} p_L & = & (1-p_0) \frac{\frac{C_{D_L}(t)}{K_{D_L}}}{1+\frac{C_{D_L}(t)}{K_{D_L}}+\frac{C_{D_R}(t)}{K_{D_R}}} \\ p_R & = & (1-p_0) \frac{\frac{C_{D_R}(t)}{K_{D_R}}}{1+\frac{C_{D_L}(t)}{K_{D_L}}+\frac{C_{D_R}(t)}{K_{D_R}}} \end{array} \tag{3.252}$$

Let us assume that the effect of the right enantiomer is a fraction, α, of that of the left enantiomer. The combined effect is

$$E = E_0 + (E_{max} - E_0)\frac{\frac{C_{D_L}(t)}{K_{D_L}} + \alpha\frac{C_{D_R}(t)}{K_{D_R}}}{1 + \frac{C_{D_L}(t)}{K_{D_L}} + \frac{C_{D_R}(t)}{K_{D_R}}} \tag{3.253}$$

Non-specific binding

We may consider the non-specific binding of the drug to other receptors with no therapeutic effect (Lauffenburger and Linderman, 1993)[Sec. 2.2]. Let us generically refer to these non-specific receptors as R_{NS}. This binding momentarily sequesters drug molecules and later releases them. Let us assume that non-specific binding occurs with a pool of receptors much larger than the number of drug molecules. If this is the case, we do not need to worry about the number of free receptors, which does not significantly change over time, and we may assume its concentration to be constant $C_{R_{NS}}$. The equation system at Eq. (3.183) becomes:

$$\begin{aligned} \frac{dC_{DR_{NS}}(t)}{dt} &= k_{f_{NS}} C_{R_{NS}} C(t) - k_{b_{NS}} C_{DR_{NS}}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} &= -ClC(t) - k_{f_{NS}} C_{R_{NS}} C(t) + k_{b_{NS}} C_{DR_{NS}}(t) + Dose_{iv}(t) \end{aligned} \tag{3.254}$$

At equilibrium, there is no variation of the concentration of non-specific bounded complexes

$$\frac{dC_{DR_{NS}}(t)}{dt} = 0 \Rightarrow C_{DR_{NS}}(t) = \frac{k_{f_{NS}}}{k_{b_{NS}}} C_{R_{NS}} C(t) \tag{3.255}$$

That is, the concentration of non-specifically bounded receptors is proportional to the drug concentration. Let us call this proportionality constant $K_{NS} = \frac{k_{f_{NS}}}{k_{b_{NS}}} C_{R_{NS}}$, that is,

$$C_{DR_{NS}}(t) = K_{NS} C(t) \tag{3.256}$$

which is a well-accepted model for non-specific binding (Gabrielsson and Weiner, 2007)[Sec. 3.4.1]. However, note that this model has been constructed under the assumption that the pool of non-specific receptors is much larger than the number of drug molecules, and that the non-specific binding reaction has reached equilibrium. In these circumstances, the effective concentration of drug decreases and its effect becomes (see Eq. (3.189))

$$E = E_0 + (E_{max} - E_0)\frac{(1 - K_{NS})C(t)}{K_D + (1 - K_{NS})C(t)} \tag{3.257}$$

3.5.2 Generic models

Previous section dealt with effects as a direct consequence of the drug binding to specific receptors. Some physiological evidence supports this approach, and system identification with these models aims at physiologically explaining the observed drug effects. However, a more pragmatic approach could be taken and suitable mathematical functions may be employed simply for the reason that they "fit" the data. There is no other justification for them apart their explanatory power in a particular case. These models can be used after acute dosing or when studying the steady state. Being, memoryless systems, they are assuming that there are no metabolites involved. A list of common mathematical functions used at this point is given below:

- Linear response:
$$E = E_0 + SC(t) \tag{3.258}$$
This model has the advantage that, thanks to Taylor expansion, it can be proved that any nonlinear effect response can be locally approximated by a linear function.

- Log-linear response:
$$E = m \log(C(t) + C_0) \tag{3.259}$$
C_0 is simply a parameter that helps to fit the model and does not have a real physiological meaning. However, it has been referred to as the "endogenous agonist" meaning by this that when there is no drug $C(t) = 0$, C_0 defines the basal response, and it would, in this way, represent a kind of "internal" concentration. Note that this model is unbounded (there is no limit for E), which violates physiological principles.

- Saturated effect:
$$E = E_0 + \frac{E_{max} C(t)}{EC_{50} + C(t)} \tag{3.260}$$
This model is the same as the one in Eq. (3.189). That one was obtained based on receptor binding considerations, while this one is simply used because it fits the data. EC_{50} is the drug concentration that causes an effect size that is 50% of the maximum response. EC_{50} is related to the **potency** of the drug: the lower EC_{50} the higher its potency (from the receptor binding model, the lower EC_{50}, the lower its dissociation constant and the higher its receptor affinity). This model represents an agonistic effect (the effect increases with the drug concentration). We may also model antagonistic effects (the effect decreases with the drug concentration) by simply changing the additive sign in the formula
$$E = E_0 - \frac{I_{max} C(t)}{IC_{50} + C(t)} \tag{3.261}$$

- Sigmoid effect:
$$E = E_0 + \frac{E_{max} C^h(t)}{EC_{50}^h + C^h(t)} \tag{3.262}$$
This model is similar to the one in Eq. (3.201) for multiple site binding and obviously we can construct its equivalent for inhibition as we did in the previous paragraph.

- Other sigmoid effects: A family of mathematical functions with sigmoidal shapes can be used:
  - Gompertz function:
$$E = \alpha \exp(-\exp(\beta - \gamma C(t))) \tag{3.263}$$
  - Logistic function: in its different "flavours"
$$\begin{aligned} E &= \frac{\alpha}{1+\exp(\beta-\gamma C(t))} \\ E &= \frac{1}{\alpha+\exp(\beta-\gamma C(t))} \\ E &= \frac{\alpha}{1+\beta\exp(-\gamma C(t))} \\ E &= \frac{1}{\alpha+\beta\exp(-\gamma C(t))} \end{aligned} \tag{3.264}$$

- Richards function:

$$E = \frac{\alpha}{(1 + \exp(\beta - \gamma C(t)))^{\frac{1}{\delta}}} \tag{3.265}$$

- Morgan-Mercer-Flodin function:

$$E = \frac{\beta\gamma + \alpha C^{\delta}(t)}{\gamma + C^{\delta}(t)} \tag{3.266}$$

- Weibull function:

$$E = \alpha - \beta \exp(-\gamma C^{\delta}(t)) \tag{3.267}$$

- Hyperbolic effect:

$$\begin{aligned} S &= S_0 + \frac{S_{max} C^{h_1}(t)}{S C_{50}^{h_1} + C^{h_1}(t)} \\ E &= E_0 + \frac{E_{max} S^{h_2}(t)}{E C_{50}^{h_2} + S^{h_2}(t)} \end{aligned} \tag{3.268}$$

  This is an easy way of producing steep responses with relatively low variations in concentration.

- Composite effect:

$$E = E_0 + \frac{E_{max,1} C^{h_1}(t)}{E C_{50,1}^{h_1} + C^{h_1}(t)} - \frac{I_{max,2} C^{h_2}(t)}{E C_{50,2}^{h_2} + C^{h_2}(t)} \tag{3.269}$$

  This equation models the effect of the drug on two different reactions. In one of the them the drug act as an agonist of the physiological response being studied, while in the other one, the drug acts as an antagonist. The overall result is a U-shaped effect response as a function of the drug concentration.

- Drug interactions:

$$E = E_0 + E_{max} \frac{\frac{C(t)}{EC_{50}} + \alpha \frac{C_A(t)}{EA_{50}} + \beta \frac{C(t) C_A(t)}{EAR_{50} EA_{50}}}{1 + \frac{C(t)}{EC_{50}} + \delta \frac{C_A(t)}{EA_{50}} + \gamma \frac{C(t) C_A(t)}{EAR_{50} E_{50}}} \tag{3.270}$$

  This model allows accounting for a single antagonist or agonist that interacts with the same receptor our drug binds to. See previous section for a complete discussion of competitive and non-competitive binding. Note that, in addition to the model developed in that section, two new constants (δ and γ have been added just for the sake of a better fitting; however, one would expect these constants to be close to 1).

Depending on the system, the model may be used with the total drug concentration $C(t)$ or with the unbound concentration $C_u(t)$ (see Sec. 3.4.4). In fact, in some examples it has been shown that the effect model based on unbound concentration is identical across different species.

3.5.3 Indirect models

Turnover driven models

Let us assume that the drug does not directly influence on the physiological variable of interest but on an intermediate. The drug concentration would affect the intermediate E and the intermediate would affect the final variable R. In this way, we may model time delays between the administration of the drug and its raise in concentration, and the changes observed in the final physiological variable. One of the most accepted models for the variable R is a linear differential equation

$$\frac{dR(t)}{dt} = k_0 - k_1 R(t) \tag{3.271}$$

Note that this model is identical to the one in Sec. 3.3.6 and that is why this effect model is sometimes referred to as **Turnover driven**. k_0 plays the role of internal generation and k_1 plays the role of elimination. However, their interpretation in this indirect pharmacodynamic context may not correspond to the turnover case.

The intermediate effect variable E affects either the order zero coefficient

$$\frac{dR(t)}{dt} = k_0 E(t) - k_1 R(t) \tag{3.272}$$

or the order one coefficient

$$\frac{dR(t)}{dt} = k_0 - k_1 E(t) R(t) \tag{3.273}$$

Depending on whether the effect $E(t)$ increases or decreases, the drug may act as an agonist or antagonist on R.

We may extend the model to incorporate enzymatic saturation effects, for instance, at its elimination, by substituting k_1 by $k_1 = \frac{V_{max}}{K_m + R(t)}$, or at its generation, by substituting k_0 by $k_0 = \frac{V_{max}}{K_m + R(t)}$.

An important extension is the so-called **irreversible turnover effect**. In this model, one of the constants (k_0 or k_1) is affected directly by the drug concentration so that they disappear when there is no drug. They become $k_0 = k_0 C(t)$ or $k_1 = k_1 C(t)$. A good example of this extension is the effect of an antibiotics: in the absence of drug there is no elimination of bacteria and their number stays or grows:

$$\frac{dR(t)}{dt} = -k_1 C(t) R(t) \tag{3.274}$$

where $R(t)$ represents the number of bacteria at a given time, and k_1 their elimination. This is called the Drecker function. We may add cell growth to the equation by simply adding a factor that depends on the current amount of bacteria

$$\frac{dR(t)}{dt} = k_0 R(t) - k_1 C(t) R(t) \tag{3.275}$$

An important concept at this point is the **Minimum inhibitory concentration** that is the concentration that stops the growth of bacteria. This happens when $\frac{dR(t)}{dt} = 0$ and it is required a drug concentration of

$$C(t) = \frac{k_0}{k_1} \tag{3.276}$$

We may also incorporate the dependence of k_0 on the substrate concentration:

$$\frac{dR(t)}{dt} = \frac{k_{0,max}C_S(t)}{K + C_S(t)}R(t) - k_1C(t)R(t) \tag{3.277}$$

as well as feedback on the maximum number of bacteria that can live in a certain environment, R_{max}. As the number of bacteria approaches this value, growth is progressively slowed down:

$$\frac{dR(t)}{dt} = \frac{k_{0,max}C_S(t)}{K + C_S(t)}\left(1 - \frac{R(t)}{R_{max}}\right)R(t) - k_1C(t)R(t) \tag{3.278}$$

Oscillatory turnover models

Some biological processes markedly follow periodic patterns. For instance, there are a clear periodicity of metabolic activity every 24h, women experience strong hormone cycles every 28 days, etc. These changes may strongly affect some of the effect constants in some of the equations (e.g., Eq. (3.273)). Values like k_0 or k_1 (or, in fact, any of the constants involved) are not constants any longer but have a cyclic behaviour. This is called oscillating turnover rates. These models are also called baseline models because the basal value of the variable R is not constant.

Note that a time varying coefficient is compatible with the methodology developed along the thesis. We simply need to use the corresponding value at each time during the simulation of the discrete time system. The identification of so many values from experimental data may be impossible if the data available is insufficient. However, since we expect a periodic behaviour, we may use a Fourier approximation. Fourier decomposition theorem states that any periodic signal (with some technical constraints like being square integrable within its period) can be exactly represented by an infinite sum of sinusoidal waves. Let us assume that we want to represent $k_0(t)$ whose period is T. Then, $k_0(t)$ can be exactly represented by the series

$$k_0(t) = a_0\sum_{i=1}^{\infty} a_k \cos\left(\frac{2\pi}{T}kt + \phi_k\right) \tag{3.279}$$

for some suitable constants a_k $(k = 0, 1, ...)$ and ϕ_k given by the theorem and out of the scope of the discussion required at this point. In practice, it has been found that we do not need infinite constants to approximate reasonably well periodic signals. This means that we do not need to identify those many values from the experimental data at hand. Instead, we may use a shorter representation

$$k_0(t) \approx a_0\sum_{i=1}^{N} a_k \cos\left(\frac{2\pi}{T}kt + \phi_k\right) \tag{3.280}$$

with only $2N + 1$ constants. In this way, we strongly simplify the identification problem. We may similarly decompose any other constant that is suspicious of periodic behaviour. By calculating the confidence interval (see Sec. 3.3.2) for the a_k coefficients we may correctly find the periodic dependence over time of each coefficient.

3.5.4 Link models

Link models are applicable to those drugs whose effect does not occur from the central compartment but from a peripheric one. In the language of link models, the compartment from which the effect is exerted is called the biophase. In this way, the concentration that must be used to calculate the effect is the concentration of the compartment and not the concentration in plasma. Note that being outside the central compartment involves a further delay due to the time needed to take the drug into that compartment. The model developed in this thesis is fully compatible with these models and we have already presented all the blocks needed to construct such a model. However, for completeness, we will illustrate this model with a three-compartment model (central, peripheral and tissue or biophase) with extravascular administration. We will assume that the effect is exerted from the biophase. We will start from Eq. (3.107) and add the necessary equations to include the pharmacodynamics model. We will assume a simple interaction of the drug with a single type of receptor as in Eq. (3.192). The full dynamics of the system is described by the following system of equations

$$\begin{aligned}
\frac{dA_e(t)}{dt} &= -(R_{in} + (K_a + K_d)A_{ev}(t)) + D_{po}(t) \\
V_c\frac{dC(t)}{dt} &= -(Cl + Cl_p + Cl_b)C(t) + Cl_pC_p(t) + Cl_bC_b(t) + R_{in} + K_aA_{ev}(t) \\
V_p\frac{dC_p(t)}{dt} &= -Cl_pC_p(t) + Cl_pC(t) \\
V_b\frac{dC_b(t)}{dt} &= -Cl_bC_b(t) + Cl_bC(t) + V_b(-k_fC_{R,free}(t)C_b(t) + k_bC_{DR}(t)) \\
\frac{dC_{DR}(t)}{dt} &= k_fC_{R,free}(t)C_b(t) - k_bC_{DR}(t) \\
\frac{dC_{R,free}(t)}{dt} &= -k_fC_{R,free}(t)C_b(t) + k_bC_{DR}(t) \\
p(t) &= \frac{C_{DR}(t)}{C_{DR}(t)+C_{R,free}(t)} \\
E &= E_{max}p(t)
\end{aligned} \tag{3.281}$$

Obviously identifying all parameters of this model at once can be a daunting task. Normally, the identification of these parameters can be performed by highly directed experiments particularly aimed at isolating individual effects (absorption, distribution among compartments, clearance and effect on the physiological variable of interest).

3.5.5 Transduction and transit compartment models

Transduction is the mechanism by which a certain signal (*e.g.* drug concentration) is effectively transformed into some measurable effect. During transduction, the stimulus may enter in a cascade of events that may include G-protein activation, second messengers, ion channel activation and gene translation that finally result in the measurable effect we are interested to. This cascade of events is normally modelled as a set of sequential systems that transform the initial effect into the n-th effect according to the following set of differential equations:

$$\begin{aligned}
\frac{dE_1(t)}{dt} &= \frac{1}{\tau}(E(t) - E_1(t)) \\
\frac{dE_2(t)}{dt} &= \frac{1}{\tau}(E_1(t) - E_2(t)) \\
&\dots \\
\frac{dE_n(t)}{dt} &= \frac{1}{\tau}(E_{n-1}(t) - E_n(t))
\end{aligned} \tag{3.282}$$

where τ is the mean transit time in each one of the event compartments. Fortunately, this model has an analytical solution in signal theory and we may calculate the final effect $E_n(t)$ without any differential equation. Let us make the Laplace transform of each one of the equations and calculate their system transfer function

$$\begin{array}{rcl} H_1(s) & = & \frac{E_1(s)}{E(s)} = \frac{\frac{1}{\tau}}{s+\frac{1}{\tau}} \\ H_2(s) & = & \frac{E_2(s)}{E_1(s)} = \frac{\frac{1}{\tau}}{s+\frac{1}{\tau}} \\ & ... & \\ H_n(s) & = & \frac{E_n(s)}{E_{n-1}(s)} = \frac{\frac{1}{\tau}}{s+\frac{1}{\tau}} \end{array} \tag{3.283}$$

So the overall transfer function is

$$H(s) = \frac{E_n(s)}{E(s)} = H_1(s)H_2(s)...H_n(s) = \left(\frac{\frac{1}{\tau}}{s+\frac{1}{\tau}}\right)^n \tag{3.284}$$

whose inverse Laplace transform is

$$h(t) = \frac{\frac{1}{\tau}}{(n-1)!}\left(\frac{t}{\tau}\right)^{n-1} e^{-\frac{t}{\tau}} u(t) \tag{3.285}$$

So, the final response is the convolution of the initial response and this function

$$E_n(t) = E(t) \star h(t) \tag{3.286}$$

3.5.6 Tolerance and rebound models

It is well known that certain drugs develop tolerance, that is, after regular administration, the drug ceases to cause the desired physiological effect or at least decreases its effect. This can be explained by several reasons: the number of receptors may be downregulated by cells, their affinity may change by some conformational change, some cofactors or precursors may be depleted, or the body may have developed antibodies against the drug (this effect is only valid for large drugs). Some of these actions correspond to the natural physiological response to maintain homeostasis. The time needed to develop tolerance may go from a few hours to several months. Rebound is an effect related to tolerance. Once the tolerance mechanism has been set, after ceasing the drug administration, the effect temporarily goes below the basal level and progressively recovers its basal line (see Fig. 3.18). Rebound is sometimes manifested as withdrawal symptoms or abstinence.

There are several ways to model tolerance. We may classify them as non-physiology based or physiology based

- Non-physiological
  - Negative feedback: If we consider any of the equations that relate the physiological effect E to the drug concentration $C(t)$ (e.g., Eq. (3.189)), we may add a negative feedback loop by subtracting an amount that depends on E.

$$\begin{array}{rcl} E(t) & = & E_0 + (E_{max} - E_0)\frac{C(t)}{K_D + C(t)} - E_f(t) \\ \frac{dE_f(t)}{dt} & = & k_1(E(t) - E_0) \end{array} \tag{3.287}$$

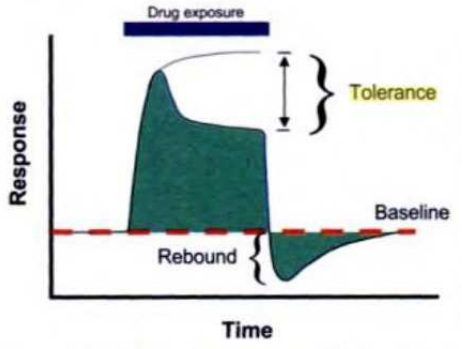

Figure 3.18: Illustration of the tolerance and rebound effects.

This model tends to stabilize the physiological variable around its basal value E_0. When $E(t) > E_0$, $E_f(t)$ becomes positive so that $E(t)$ decreases. On the contrary, when $E(t) < E0$, $E_f(t)$ is negative and $E(t)$ increases. We may add second or third order effects as in

$$\frac{dE_f(t)}{dt} = k_1(E(t) - E_0) + k_2(E(t) - E_0)^2 + k_3(E(t) - E_0)^3 \tag{3.288}$$

This model is very versatile but it has no physiological meaning. Moreover, it is based on an equation (Eq. (3.189)) that was obtained after the assumption that receptor binding is much faster than changes in the drug concentration, which may not hold in certain cases. We may also incorporate a negative feedback in an indirect model as (see Eq. (3.273))

$$\frac{dR(t)}{dt} = k_0 - k_1(R(t) - R_0)E(t)R(t) \tag{3.289}$$

or

$$\frac{dR(t)}{dt} = k_0(R_0 - R(t)) - k_1E(t)R(t) \tag{3.290}$$

The difference between $R(t)$ and R_0 controls the internal generation or elimination of the response variable.

– Time dependent parameters: We may model the decrease of response by making E_{max} or K_D to decay over time as in

$$E_{max}(t) = E_{max}(1 - Q(1 - e^{-Kt})) \tag{3.291}$$

or

$$K_D(t) = K_D(1 + Q(1 - e^{-Kt})) \tag{3.292}$$

and then using these varying constants in any of the models relating E to $C(t)$ (see, for instance, Eq. (3.189)). For indirect models, we may include the time decay in any of the parameters governing the differential equation for R (see Eq. (3.273))

$$\frac{dR(t)}{dt} = k_0 - k_1(1 - Q(1 - e^{-Kt}))E(t)R(t) \tag{3.293}$$

This model has the additional drawback that it is valid only for a single dose administration due to its exponential decay normally associated to a single "shot" effect.

- Physiological
  - Negative feedback through mediator: Let us assume that homeostasis is achieved through a mediator molecule that is controlled by the level of response R.

$$\frac{dC_M(t)}{dt} = k_{iM}R(t) - k_{oM}C_M(t) \tag{3.294}$$

Let us now incorporate this equation into the general dynamics of the system given by the equation system at Eqs. (3.192) and (3.273)

$$\begin{array}{rcl} \frac{dC_{DR}(t)}{dt} & = & k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\ \frac{dC_{R,free}(t)}{dt} & = & -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\ \frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) + Dose_{iv}(t) \\ p(t) & = & \frac{C_{DR}(t)}{C_{DR}(t)+C_{R,free}(t)} \\ E & = & E_{max}p(t) \\ \frac{dC_M(t)}{dt} & = & k_{iM}R(t) - k_{oM}C_M(t) \\ \frac{dR(t)}{dt} & = & k_0 - k_1 E(t)C_M(t) \end{array} \tag{3.295}$$

In this model, an increase of the response results in an increase of the mediator, which in its turn decreases the response. There might be variations to this model. For instance, we may substitute the last equation by

$$\frac{dR(t)}{dt} = k_0 - k_1 E(t)C_M(t)R(t) \tag{3.296}$$

which would reinforce the self-limitation of R. Note that this is not the only possible negative feedback mechanism. For instance, an increase of the response R may result in a decrease of the elimination of the mediator, which would result in an increase of the generation of R. The last two equations in the equation system above should be, then, substituted by

$$\begin{array}{rcl} \frac{dC_M(t)}{dt} & = & k_{iM} - k_{oM}R(t)C_M(t) \\ \frac{dR(t)}{dt} & = & k_0 C_M(t) - k_1 E(t)R(t) \end{array} \tag{3.297}$$

  - Antagonistic metabolite: Let us assume that the drug promotes the generation of a metabolite that has an antagonistic effect while the drug is agonistic. Let us assume that the metabolite concentration responds to the differential equation

$$\frac{dC_M(t)}{dt} = k_{iM}C(t) - k_{oM}C_M(t) \tag{3.298}$$

Let us now incorporate this equation into the general dynamics of

the system given by the equation system at Eq. (3.192)

$$\begin{array}{rcl}
\frac{dC_M(t)}{dt} & = & k_{iM}C(t) - k_{oM}C_M(t) - k_{f_M}C_{R,free}(t)C_M(t) + k_{b_M}C_{MR}(t) \\
\frac{dC_{DR}(t)}{dt} & = & k_f C_{R,free}(t)C(t) - k_b C_{DR}(t) \\
\frac{dC_{MR}(t)}{dt} & = & k_{f_M}C_{R,free}(t)C_M(t) - k_{b_M}C_{MR}(t) \\
\frac{dC_{R,free}(t)}{dt} & = & -k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) \\
 & & -k_{f_M}C_{R,free}(t)C_M(t) + k_{b_M}C_{MR}(t) \\
\frac{1}{V}\frac{dC(t)}{dt} & = & -ClC(t) - k_f C_{R,free}(t)C(t) + k_b C_{DR}(t) + Dose_{iv}(t) \\
p_D(t) & = & \frac{C_{DR}(t)}{C_{DR}(t)+C_{R,free}(t)+C_{MR}(t)} \\
p_M(t) & = & \frac{C_{MR}(t)}{C_{DR}(t)+C_{R,free}(t)+C_{MR}(t)} \\
E & = & E_{max}p_D(t) - E_{M,max}p_M(t)
\end{array} \tag{3.299}$$

– Precursor pool: In some situations, the physiological response R depends on some endogenous precursor, P, which may be depleted due to the action of the drug. Without any drug, the precursor and the response variable respond to the equation system:

$$\begin{array}{rcl}
\frac{dC_P(t)}{dt} & = & k_{0P} - k_{1P}C_P(t) \\
\frac{dR(t)}{dt} & = & k_{0R}C_P(t) - k_{1R}R(t)
\end{array} \tag{3.300}$$

It might also be that the level of precursor is controlled by the response variable as in

$$\frac{dC_P(t)}{dt} = (k_{0P} - k_{RP}R(t)) - k_{1P}C_P(t) \tag{3.301}$$

Whichever the case, the drug may affect positively or negatively on any of the constants (k_{0P}, k_{1P}, k_{0R}, k_{1R}, k_{RP}). In all cases, the overall effect of the drug on the response variable C exhibits some tolerance degree.

3.5.7 Discrete physiological response

All the pharmacodynamical analysis performed so far has assumed a continuous effect variable E. This is valid for variables like blood pressure or temperature, but it is not for a variable like death or not. Death is discrete variable that either takes a 0 value (no death) or a 1 value (death occurred). Our pharmacodynamical model must then account for the probability of the discrete variable taking value 1 at a given drug concentration. This is normally performed by a logistic regression. The probability of variable E taking a value 1 is $\pi(C(t))$ that is calculated as

$$\pi(C(t)) = \frac{e^{L(t)}}{e^{L(t)}+1} \tag{3.302}$$

where

$$L(t) = \alpha_0 + \alpha_1 C(t) + \beta_1 S_1 + \beta_2 S_2 + ... \tag{3.303}$$

S_1, S_2, ... are variables (discrete or not) that describe the patient situation (they may be discrete variables like sepsis or not, renal severe damage or not; or continuous variables like heart rate or blood pressure). The system identification task is now to identify the variables α_i and β_i which is done through a

logistic regression. Given pairs of values (C_i, π_i), logistic regression proceeds by transforming the probabilities through the logit function

$$l_i = \log\left(\frac{\pi_i}{1-\pi_i}\right) \tag{3.304}$$

and performing the standard Least-Squares regression of the data set (C_i, l_i).

The methodology developed in this thesis is compatible with this other kind of modelling. We would make use of the pharmacokinetical model to predict the behaviour of $C(t)$ and then change to the logistic regression to identify the drug effect on the physiological variable E.

3.6 Biopharmaceutics

Biopharmaceutics deals with the accurate modelling of the early stages of drug administration: release, dissolution and absorption. We will treat these effects separatedly and show how to include them in the modelling framework defended in the thesis. However, we have to note that the direct identification of drug absorption (biopharmaceutics), drug distribution (pharmacokinetics) and its effects (pharmacodynamics) is not straightforward from a single plasma concentration profile and it is recommended to perform multiple experiments aimed at isolating the different effects.

3.6.1 Diffusion

Diffusion is the process by which a certain molecule spreads in space, normally by random motion, from regions of high concentration to regions of low concentration. Diffusion laws are the biophysical way of modelling how the concentration of that molecule changes over time and space. Many biopharmaceutical equations are directly based on the diffusion laws and, for that reason, we will reproduce here a suitable derivation of those that will help us to further understand the accurate modelling in biopharmaceutics.

Fick's laws are the standard diffusion equations normally accepted. **Fick's first law** states that the flux of molecules $\mathbf{J}(\mathbf{r})$ at a given spatial location $\mathbf{r}$ (defined as amount of molecules per unit area and per unit time; its international units are $mol \cdot m^{-2} \cdot s^{-1}$) is proportional to the gradient of concentration $C(x)$ (whose units are $mol \cdot m^{-3}$):

$$\mathbf{J}(\mathbf{r}) = -D(\mathbf{r})\nabla C(\mathbf{r}) \tag{3.305}$$

Note that $C(\mathbf{r})$ is a scalar function while $\mathbf{J}$ is a vector function pointing in the direction of maximal (negative) gradient (in fact, the negative sign in Fick's first law indicates that the flux goes down the gradient (from regions of high concentration to regions of low concentration). The operator ∇ is defined as $\left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z}\right)$. $D(\mathbf{r})$ is the diffusivity and it is measured in $m^{-2} \cdot s^{-1}$. Einstein, in this theory of Brownian motion, proposed that dffusivity can be calculated as

$$D = \frac{kT}{6\pi\mu(\mathbf{r})a} \tag{3.306}$$

where k is Boltzmann's constant, T is the absolute temperature, $\mu(\mathbf{r})$ is the coefficient of viscosity for the solute and a is the radius of the solute molecule. For non spherical molecules, the formula can be generalized to

$$D = \frac{kT}{f} \tag{3.307}$$

where f is the Stokes frictional coefficient. The molecular weight of a spherical molecule of density ρ is $M = \frac{4}{3}\pi a^3 \rho$ from where we deduce that

$$D = \frac{kT}{3\mu}\left(\frac{\rho}{6\pi^2 M}\right)^{\frac{1}{3}} \tag{3.308}$$

The following table gives the diffusivity for some biochemical species

Substance	Molecular weight (Da)	D$(cm^{-2} \cdot s^{-1})$
Hydrogen ion	1	$4.5{\cdot}10^{-5}$
Oxygen	32	$2.1{\cdot}10^{-5}$
Glucose	192	$6.6{\cdot}10^{-6}$
Insulin	5,734	$2.1{\cdot}10^{-6}$
Ureasae	482,700	$3.5{\cdot}10^{-7}$

Fick's first law is a steady-state equation that gives the flux assuming that concentrations do not change over time. However, they normally do precisely due to the diffusion process. We may incorporate the time dependence as follows. Let us first do it in 1D, and we will extend it to 3D later. Consider a small slab in the x direction. Let $J(x)$ be the flux of molecules incoming into the slab and $J(x+dx)$ the flux of molecules going out. If they are not equal, then there is a raise or decrease of the concentration in the slab that we can write as

$$\frac{\partial C(x,t)}{\partial t} = \frac{J(x,t) - J(x+dx,t)}{dx} = -\frac{\partial J(x,t)}{\partial x} = \frac{\partial}{\partial x}\left(D(x,t)\frac{\partial C(x,t)}{\partial x}\right) \tag{3.309}$$

The general three-dimensional formula is

$$\frac{\partial C(\mathbf{r},t)}{\partial t} = \nabla \cdot (D(\mathbf{r},t)\nabla C(\mathbf{r},t)) \tag{3.310}$$

We may add an external input of molecules (our doses) which is diluted in a volume V

$$\frac{\partial C(\mathbf{r},t)}{\partial t} = \frac{Dose(\mathbf{r},t)}{V} + \nabla \cdot (D(\mathbf{r},t)\nabla C(\mathbf{r},t)) \tag{3.311}$$

In case that the diffusivity is a constant we get

$$\frac{\partial C(\mathbf{r},t)}{\partial t} = \frac{Dose(\mathbf{r},t)}{V} + D\nabla^2 C(\mathbf{r},t) \tag{3.312}$$

which is the most standard form of **Fick's second law**. ∇^2 is often referred to as the Laplacian operator and represented by $\triangle$. On the other extreme, we may consider the diffusivity to be anisotropic, time and space dependent. Then $D(\mathbf{r},t)$ becomes a tensor with different values at each location and time.

This law has been obtained under certain assumptions: 1) Pressure is constant; 2) There is no thermal diffusion; 3) There is no chemical reaction. The

first two conditions are reasonable for a drug. However, the last one is not because the drug can be readily modified by enzymes at the absorption site. This problem is then called of diffusion-reaction and it is studied below.

Let us consider the case in which the drug is irreversably degraded according to the equation

$$D \rightarrow D' \tag{3.313}$$

where D' has no therapeutic effect. Let k_1 be the reaction rate of the degradation, which is assumed to be constant along time and space. Then, Fick's second law has to include the lost of concentration due to the degradation

$$\frac{\partial C(\mathbf{r},t)}{\partial t} = \frac{Dose(\mathbf{r},t)}{V} + D\nabla^2 C(\mathbf{r},t) - k_1 C(\mathbf{r},t) \tag{3.314}$$

In the same way we could model reversible binding to some biochemical substance that is also diffusing

$$D + S \leftrightarrow DS \tag{3.315}$$

only that now we have to keep track of the concentrations of D, S and DS:

$$\begin{array}{rcl} \frac{\partial C_D(\mathbf{r},t)}{\partial t} & = & \frac{Dose(\mathbf{r},t)}{V} + D_D\nabla^2 C_D(\mathbf{r},t) - k_1 C_D(\mathbf{r},t)C_S(\mathbf{r},t) + k_{-1}C_{DS}(\mathbf{r},t) \\ \frac{\partial C_S(\mathbf{r},t)}{\partial t} & = & D_S\nabla^2 C_S(\mathbf{r},t) - k_1 C_D(\mathbf{r},t)C_S(\mathbf{r},t) + k_{-1}C_{DS}(\mathbf{r},t) \\ \frac{\partial C_{DS}(\mathbf{r},t)}{\partial t} & = & D_S\nabla^2 C_{DS}(\mathbf{r},t) + k_1 C_D(\mathbf{r},t)C_S(\mathbf{r},t) - k_{-1}C_{DS}(\mathbf{r},t) \end{array} \tag{3.316}$$

In the equation above we have assumed that the molecule S is much larger than D (which is often the case for small drug molecules bound to a protein) and, consequently, the diffusivity of S is approximately the same as the diffusivity of DS. If we sum the second and third equations we get

$$\frac{\partial C_S(\mathbf{r},t)+C_{DS}(\mathbf{r},t)}{\partial t} = D_S\nabla^2(C_S(\mathbf{r},t) + C_{DS}(\mathbf{r},t)) \tag{3.317}$$

But the concentration of $C_S(\mathbf{r},t) + C_{DS}(\mathbf{r},t)$ does not change in a differential time t (because the S molecules are either free or bound, but their total amount in a given space location does not change over time). This means that

$$0 = \nabla^2(C_S(\mathbf{r},t) + C_{DS}(\mathbf{r},t)) \tag{3.318}$$

In fact, at $t = 0$ (when the drug is supposed to be given), this equation must also hold for C_S because $C_{DS}(\mathbf{r},0) = 0$:

$$0 = \nabla^2(C_S(\mathbf{r},0)) \tag{3.319}$$

If the binding reaction between S and D is much faster than the changes in concentration of D, then the reaction is at equilibrium, that is at any point

$$k_1 C_D(\mathbf{r},t)C_S(\mathbf{r},t) = k_{-1}C_{DS}(\mathbf{r},t) \tag{3.320}$$

Additionally, locally the concentration of S and DS molecules must remain constant (since their sum do not change over time)

$$C_S(\mathbf{r},t) + C_{DS}(\mathbf{r},t) = C_S(\mathbf{r},0) \Rightarrow C_{DS}(\mathbf{r},t) = C_S(\mathbf{r},0) - C_S(\mathbf{r},t) \tag{3.321}$$

So we may calculate at any time the concentration of unbound S as

$$k_1 C_D(\mathbf{r},t) C_S(\mathbf{r},t) = k_{-1}(C_S(\mathbf{r},0) - C_S(\mathbf{r},t)) \Rightarrow C_S(\mathbf{r},t) = \frac{K_{eq} C_S(\mathbf{r},0)}{K_{eq} + C_D(\mathbf{r},t)} \tag{3.322}$$

where $K_{eq} = \frac{k_{-1}}{k_1}$. If we subtract the first and second equations of Eq. (3.316) we get

$$\frac{\partial (C_D(\mathbf{r},t) - C_S(\mathbf{r},t))}{\partial t} = \frac{Dose(\mathbf{r},t)}{V} + D_D \nabla^2 C_D(\mathbf{r},t) - D_S \nabla^2 C_S(\mathbf{r},t) \tag{3.323}$$

By substituting $C_S(\mathbf{r},t)$ by its value, we get the nonlinear equation

$$\frac{\partial}{\partial t}\left(C_D(\mathbf{r},t) - \frac{K_{eq} C_S(\mathbf{r},0)}{K_{eq} + C_D(\mathbf{r},t)}\right) = \frac{Dose(\mathbf{r},t)}{V} + D_D \nabla^2 C_D(\mathbf{r},t) - D_S \nabla^2 \left(\frac{K_{eq} C_S(\mathbf{r},0)}{K_{eq} + C_D(\mathbf{r},t)}\right) \tag{3.324}$$

As we have done along the thesis, this equation may be discretized, both in time and space, and be numerically simulated for any arbitrary dose regime.

3.6.2 Dissolution

Many times drugs are orally given in tablets that dissolve in the stomach and intestine. Epidermal patches also dissolve their content through the skin till it reaches the bloodstream. In both cases, diffusion is the key process that mainly describes the drug spread into the intestine fluids or the dermis. Let us generally refer to the tablet or the patch as the pharmaceutical dosage form and for consistency with the notation in Sec. 3.3.3 let us refer to the amount of drug available for absorption as $A_g(t)$. For the moment, we will not consider the absorption process and we will concentrate on how the dosage dissolves and becomes available for absorption. Several models have been proposed for this dissolution process (Costa and Sousa Lobo, 2001; Zarzycki et al., 2010):

- Zero-order kinetics: This model is valid for pharmaceutical dosages that do not disaggregate and release the drug slowly (assuming the release area does not change over time). The amount of drug available for absorption at a given differential time dt is constant

$$\frac{dA_g(t)}{dt} = K \tag{3.325}$$

  This equation is valid till the total dose in the dosage is available. The integration of the equation above gives:

$$A_g(t) = Ktu(t) \tag{3.326}$$

- First-order kinetics: This model explicitly considers the solubility, C_{max} of the drug in the fluid in which it is being dissolved and is applicable to water-soluble drugs in porous matrices. The solubility determines the maximum concentration achievable in the fluid. The differential equation governing this solution process is

$$\frac{dA_g(t)}{dt} = \frac{DS}{Vh}(VC_{max} - A_g(t)) \tag{3.327}$$

where D the drug diffusivity, S is the surface through which the drug is diffused, V is the volume in which the drug is dissolved, and h is the width of the diffusion layer. Its solution is

$$A_g(t) = VC_{max}\left(1 - \exp\left(-\frac{DS}{Vh}t\right)\right)u(t) \tag{3.328}$$

- Fractional-order kinetics: We may generalize the model above to any fractional order. These models propose that $A_g(t)$ fulfills

$$A_{max}^{\alpha} - (A_{max} - A_g(t))^{\alpha} = \alpha\frac{DS}{Vh}t \tag{3.329}$$

where A_{max} is the total amount of drug in the dosage. One of the most accepted models is for $\alpha = \frac{1}{3}$ that is called the cube-root law (Macheras and Iliadis, 2006)[Sec. 5.1.1]. Let us derive this equation with respect to t

$$-\alpha(A_{max} - A_g(t))^{\alpha-1}\left(-\frac{dA_g(t)}{dt}\right) = \alpha\frac{DS}{Vh} \tag{3.330}$$

from where

$$\frac{dA_g(t)}{dt} = \frac{DS}{Vh}(A_{max} - A_g(t))^{1-\alpha} \tag{3.331}$$

We see that this equation is the generalization of Eq. (3.327) for any arbitrary order. This fractional order appears after making fractal considerations on the structure of the matrix.

- Weibull model: This model simply fits a curve to the amount of drug available. This model states that the amount of drug available is

$$A_g(t) = A_{max}\left(1 - \exp\left(-\lambda t^b\right)\right)u(t) \tag{3.332}$$

where A_{max} is the total amount in the dosage. If $b = 1$, this model is the same as the first order kinetics. If $b > 1$, the amount available has a sigmoid shape. If $b < 1$, the model is said to be parabolic. We may set this model in the differential equation modelling framework of this thesis by differentiating the curve

$$\frac{dA_g(t)}{dt} = A_{max}\lambda b t^{b-1}\exp\left(-\lambda t^b\right) \tag{3.333}$$

This seemingly empirical model is actually based on chemical kinetics theory (Macheras and Iliadis, 2006)[Sec. 5.1.3]. Assume that the layer surrounding the pharmaceutical dosage is homogeneous, and that its intrinsic dissolution rate constant can be modelled as

$$k(t) = k_0\left(\frac{t}{t_0}\right)^{-\gamma} \tag{3.334}$$

for some suitable constants k_0, t_0 and γ. Then, it can be proved (Macheras and Iliadis, 2006)[Sec. 5.1.3] that the amount of drug released follows a Weibull model with

$$\begin{aligned} \lambda &= \tfrac{1}{t_0}\left(\tfrac{k_0 t_0}{1-\gamma}\right)^{\frac{1}{1-\gamma}} \\ b &= 1 - \gamma \end{aligned} \tag{3.335}$$

- Higuchi model: Higuchi developed several methods to describe drug release from porous matrices. One of his models dealed with drug release through a planar surface that does not change over time (e.g., an epidermal patch). The amount of drug released is

$$A_g(t) = \sqrt{tDC_{max}(2C_0 - C_{max})}u(t) \tag{3.336}$$

where D is the diffusivity of the drug, C_{max} its solubility, and C_0 is the initial drug concentration in the patch. We may also include parameters describing the matrix pores. Particularly, its tortuosity factor τ and the matrix porosity ϵ:

$$A_g(t) = \sqrt{tDC_{max}\frac{\epsilon}{\tau}(2C_0 - \epsilon C_{max})}u(t) \tag{3.337}$$

In any of the two cases the model is of the form:

$$A_g(t) = A_{max}K_H t^{\frac{1}{2}}u(t) \tag{3.338}$$

where K_H is called the Higuchi constant. We can put this model in a differential equation framework as

$$\frac{dA_g(t)}{dt} = A_{max}\frac{K_H}{2}t^{-\frac{1}{2}} \tag{3.339}$$

It has been argued that the conditions under which the Higuchi model is valid do not hold when 60% of A_{max} has been released (Macheras and Iliadis, 2006). For this reason it is recommended that the model is fitted with data before this critical point.

- Korsmeyer-Peppas model: Korsmeyer and Peppas proposed that a generic model would be

$$A_g(t) = A_{max}at^m \tag{3.340}$$

where a and m are empirical parameters to be determined. The release exponent m is particularly interesting because it is directly linked to the release mechanism. For a thin film, if $m = 0.5$ drug dissolution perfectly follows Fick's diffusion laws; drug transport within the pharmaceutical dosage is referred to as Case I. If $m = 1$, the polymer in which the drug is embedded swells and the drug release follow a zero-order kinetics, much faster than what would be predicted only by diffusion; the transport of drug within the dosage is referred to as Case II. If $0.5 < m < 1$, then the transportation is said to be anomalous and it is between Case I and Case II and this response is normally related to the penetration of solvent fluid into the pharmaceutical form. If $m > 1$, it is Supercase II. The following table shows the limits normally accepted for Case I and Case II transports and different geometries (Macheras and Iliadis, 2006)

Thin film	Cylinder	Sphere	Release mechanism
$m = 0.5$	$m = 0.45$	$m = 0.43$	Case I
$0.5 < m < 1.0$	$0.45 < m < 0.89$	$0.43 < m < 0.85$	Anomalous transport
$m = 1.0$	$m = 0.89$	$m = 0.85$	Case II

The release dynamics is given by the following differential equation:

$$\frac{dA_g(t)}{dt} = A_{max} a m t^{m-1} \tag{3.341}$$

As with Higuchi's model, it has been argued that parameter fitting should be performed before 60% of the dosage is released (Costa and Sousa Lobo, 2001) although some other authors defend that the model is valid for the full data range (Macheras and Iliadis, 2006).

- Hixson-Crowell model: Hixson and Crowell recognized that the tablet area is proportional to the cubic root of its volume. As the drug dissolves, its volume decreases and so does its interface area. With this effect in mind, they proposed the following model for the amount of drug available

$$A_g(t) = A_{max}(1 - (1 - K_\beta t)^3) \tag{3.342}$$

where K_β is a constant that depends on the surface, shape and density of the tablet, the drug diffusivity, its solubility and the width of the diffusion layer. It has been proposed (Macheras and Iliadis, 2006) that for spherical dosages $K_\beta = \frac{k_0}{C_0 r_0}$ where k_0 is the Case II relaxation constant, C_0 is the initial concentration of the drug in the matrix and r_0 is the radius of the sphere. This function obeys the differential equation

$$\frac{dA_g(t)}{dt} = A_{max} 3 K_\beta (1 - K_\beta t)^2 \tag{3.343}$$

- Baker-Lonsdale model: this model describes the drug controlled release from a spherical matrix. It responds to the equation

$$\frac{3}{2}\left(1 - \left(1 - \frac{A_g(t)}{A_{max}}\right)^{\frac{2}{3}}\right) - \frac{A_g(t)}{A_{max}} = \frac{3 D_m C_{max}}{r_0^2 C_0} t \tag{3.344}$$

where D_m is the diffusivity of the drug in the matrix, C_{max} is the solubility of the drug in the matrix, r_0 is the radius of the spherical matrix, and C_0 is the initial concentration of the drug in the matrix. Note that this model does not provide a closed-form for $A_g(t)$ and, consequently, it is not easy to fit the parameters and simulate this function. However, in the framework defended in this thesis with differential equations, it is rather easy. Let us derive with respect to t both sides of the equation above

$$\begin{gathered} -\left(1 - \frac{A_g(t)}{A_{max}}\right)^{-\frac{1}{3}} \left(-\frac{1}{A_{max}} \frac{dA_g(t)}{dt}\right) - \frac{1}{A_{max}} \frac{dA_g(t)}{dt} = \frac{3 D_m C_{max}}{r_0^2 C_0} \\ \frac{dA_g(t)}{dt} = A_{max} \frac{3 D_m C_{max}}{r_0^2 C_0} \left(\left(1 - \frac{A_g(t)}{A_{max}}\right)^{-\frac{1}{3}} - 1\right)^{-1} \end{gathered} \tag{3.345}$$

Note that the slope of $A_g(t)$ for $t = 0$ is infinite because $A_g(0) = 0$. This is a problem if we want to approximate the continuous system by a causal discrete system but it is not if we approximate it by an anticausal discrete system. Since this is not normally the case, we will omit the mathematics needed to simulate this process. Note that the approximation normally adopted that $\frac{A_g(t)}{A_{max}} \approx kt$ for low t aducing a linearization of $A_g(t)$ is incorrect, because the Taylor approximation of the left-hand side of Eq. (3.344) lacks a linear term.

- Hopfenberg model: This model addresses drug release from an erodible matrix. The amount of drug released is supposed to follow

$$A_g(t) = A_{max}\left(1 - \left(1 - \frac{k_0 t}{C_0 a_0}\right)^n\right) \tag{3.346}$$

where k_0 is the erosion rate constant, C_0 the initial drug concentration in the matrix, a_0 is the radius of the sphere or cylinder (assuming the matrix has this shape) or half the size of a slab (e.g. a patch), and n is 1 for a slab, 2 for a cylinder, and 3 for a sphere. The corresponding differential equation is

$$\frac{dA_g(t)}{dt} = A_{max}\left(1 - \frac{k_0 t}{C_0 a_0}\right)^{n-1}\frac{k_0}{C_0 a_0} \tag{3.347}$$

There are two important effects that may be important depending on the specific situation:

- Lag time: Sometimes, the drug has to traverse a membrane before becoming available for absorption. We may include the time needed by the drug to go through the membrane as a time delay and subsitute t by $t - t_{lag}$ in all the expressions above, where

$$t_{lag} = \frac{h^2}{6D} \tag{3.348}$$

being h the membrane thickness and D the drug diffusivity in the membrane.

- Initial burst: Initial burst occurs when the drug desorpbs from the matrix at the beginning. This is normally solved by better manufacturing processes and more controlled release rates are normally sought. If initial burst is important, then we may add an exponential term to the differential equation. For instance, for the Korsmeyer-Peppas model above we would have

$$\frac{dA_g(t)}{dt} = A_{max} a n t^{n-1} + B\exp(-kt) \tag{3.349}$$

Along this section we have disregarded the possibility of having multiple doses. For including this possibility, let us introduce a new variable that we will refer to as the available amount $A_a(t)$ and let us consider any of the models above, for instance, the first order kinetics. One possible way of considering multiple doses and drug release at the same time is by the following system of differential equations

$$\begin{array}{rcl} \frac{dA_a(t)}{dt} & = & Dose(t) - \frac{DS}{Vh}(VC_{max} - A_g(t)) \\ \frac{dA_g(t)}{dt} & = & \frac{DS}{Vh}(VC_{max} - A_g(t)) \end{array} \tag{3.350}$$

This equation system is valid as long as $A_a(t) > 0$. When the amount of drug available is depleted ($A_a(t) = 0$), then

$$\frac{dA_a(t)}{dt} = \frac{dA_g(t)}{dt} = 0 \tag{3.351}$$

3.6.3 Absorption

Absorption is the process by which the drug molecules enter into the bloodstream from the location where they are administered so that they are distributed to all the body. We already introduced one of the most widely used models in Sec. 3.3.3, namely 1st order absorption, which we reproduce here for convenience:

$$\frac{dA_g(t)}{dt} = -K_a A_g(t) \tag{3.352}$$

The previous equation explains how the amount of drug available for absorption disappears over time. It has two contributions, one from the absorption process itself, K_a, and another by some first order degradation, K_d, that we have assumed to be 0 for illustration purposes. If we compare this equation with Fick's first law (see Eq. 3.305) for the diffusion across a membrane of thickness h and diffusivity D, we would have

$$J = -D\frac{C_g(t) - C(t)}{h} \tag{3.353}$$

being J the flux (in $mol \cdot m^{-2} \cdot s^{-1}$) from the gut (where we assume that we have administered the dosage, although the reasoning is valid for any other location) to the central compartment. If we now multiply by the area, A, at which this absorption takes place we have

$$JA = -DA\frac{C_g(t) - C(t)}{h} \tag{3.354}$$

JA is exactly the amount of drug that is lost in the instestine. Rearranging the different terms we have

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & JA \\ & = & -DA\frac{C_g(t)-C(t)}{h} \\ & = & -\frac{DA}{h}C_g(t) + \frac{DA}{h}C(t) \\ & = & -\frac{DA}{h}\frac{A_g(t)}{V_g} + \frac{DA}{h}C(t) \\ & = & -\frac{DA}{hV_g}A_g(t) + \frac{DA}{h}C(t) \end{array} \tag{3.355}$$

Comparing Eq. (3.352) and Eq. (3.355) we see that they are identical when $K_a = \frac{DA}{hV_g}$ and $C(t) = 0$, that is, first order absorption in Sec. 3.3.3 is a simplified absorption process in which it is assumed that drug concentration in blood is much smaller than drug concentration at the absorption site. This is most likely correct for most drug dosages, although we now know which are the limitations of this assumption. Actually, since there is a variation of the drug concentrations over time, Fick's second law should have been used instead of Fick's first law.

An important issue in drug absorption is that electrically neutral drugs are better absorbed than ionized drugs because they are more lipophilic. The small intestine, where most drugs are absorbed, is slightly acidic (pH=6.5). Consider the following reactions for a acidic drug HD_a and a basic drug D_b

$$\begin{array}{rcl} HD_a & \leftrightarrow & H^+ + D_a^- \\ H^+ + D_b & \leftrightarrow & D_b H^+ \end{array} \tag{3.356}$$

The Henderson-Hasselbach equation states the equilibrium condition for both reactions

$$\begin{array}{rcl} pH & = & pKa + \log_{10} \frac{C_{D_a^-}}{C_{HD_a}} \Rightarrow C_{HD_a} = C_{D_a^-} 10^{pH-pKa} \\ pH & = & pKa + \log_{10} \frac{C_{D_b}}{C_{D_b H^+}} \Rightarrow C_{D_b} = C_{D_b H^+} 10^{pKa-pH} \end{array} \tag{3.357}$$

The corresponding fraction of unionized drug is

$$\begin{array}{rcl} f_{un} & = & \frac{C_{HD_a}}{C_{HD_a}+C_{D_a^-}} = \frac{10^{pH-pKa}}{10^{pH-pKa}+1} \\ f_{un} & = & \frac{C_{D_b}}{C_{D_b}+C_{D_b H^+}} = \frac{10^{pKa-pH}}{10^{pKa-pH}+1} \end{array} \tag{3.358}$$

The actual diffusion coefficient across the membrane is given a weighted average between the diffusion coefficient for the unionized form and the diffusion coefficient for the ionized form:

$$D = f_{un} D_{un} + (1 - f_{un}) D_i \tag{3.359}$$

Since the gastrointestinal tract moves forward its fluids by a peristaltic movement, we may also model the longitudinal evolution of drug concentration that is available for absorption. Let us name L the total length of the small intestine, and v the speed of the fluids in the small intestine (typically, $L = 4m$ and $v = 1.76cm \cdot min^{-1}$). Let us assume that the amount of drug dissolved has a z dependence, where z is a spatial variable that goes from 0 to L and denotes the location within the small intestine. We may modify any of the dissolution models above to include the spatial dependence as follows. $\frac{dA_g(t)}{dt}$ gives the amount of drug that becomes available at time t. However, this drug is distributed in a spatial region

$$\frac{\partial A_{g,l}(z,t)}{\partial t} = \frac{dA_g(t)}{dt} \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{1}{2} \left(\frac{z - vt}{\sigma} \right)^2 \right) \tag{3.360}$$

that is, the drug dissolution is delocalized in a Gaussian of variance σ^2 whose center is at $z = vt$. $A_{g,l}$ is the linear density of mass. If we now divide by the lumen area, S, we have the contribution to the local concentration as $\frac{1}{S} \frac{\partial A_{g,l}(z,t)}{\partial t}$. Overall, the concentration at any point z changes as

$$\frac{\partial C_g(z,t)}{\partial t} = D \frac{\partial^2 C_g(z,t)}{\partial z^2} - v \frac{\partial C_g(z,t)}{\partial z} - k_a (C_g(t) - C(t)) - k_d C_g(t) + \frac{1}{S} \frac{\partial A_{g,l}(z,t)}{\partial t} \tag{3.361}$$

The first term accounts for diffusion along the intestine, the second term explains the movement of fluids towards the large intestine, the third one explains absorption, the fourth one drug degradation, and the fifth one the increase of concentration thanks to drug dissolution.

Wagner-Nelson method (Wagner and Nelson, 1963) is a widespread method to evaluate the amount of absorbed drug and the kinetics order of its absorption. It presumes that all absorbed drug at a given moment, $\frac{dA_b(t)}{dt}$, either remains in the body or it is excreted. In this way,

$$\frac{dA_b(t)}{dt} = V \frac{dC(t)}{dt} + ClC(t) \tag{3.362}$$

Equivalently

$$dA_b(t) = VdC(t) + ClC(t)dt \tag{3.363}$$

Integrating the equation

$$A_b(t) = VC(t) + \int_{-\infty}^{t} ClC(\tau)d\tau \tag{3.364}$$

If the dose consists of a single dose at time $t = 0$, it must be $\lim_{t\to\infty} C(t) = 0$, then the total amount of drug absorbed is

$$A_b^{max} = \lim_{t\to\infty} A_b(t) = Cl \int_{-\infty}^{\infty} C(\tau)d\tau \tag{3.365}$$

If we calculate now the difference to the known dose, A, we can calculate the bioavailability f

$$A_b^{max} = fA \tag{3.366}$$

and the remaining amount in the absorption site (e.g., the gut)

$$A_g(t) = A - \frac{1}{f}A_b(t) \tag{3.367}$$

tell us the kinetics order (order 0, 1, ...) of the absorption. For instance, if the absorption is of order 1, then

$$A_g(t) = A - \frac{1}{f}A_b(t) = Ae^{-K_a t}u(t) \tag{3.368}$$

3.7 Technical details

There are some important technical issues that deserve our attention: how to choose the sampling rate (T_s) so that our approximation is accurate and stable and how accurate our estimates of the system parameters will be. The following two sections cover those two aspects.

3.7.1 Selection of the sampling rate

As already introduced in Section 1.2, the approach defended in this thesis is the general use of numerical methods to solve the set of non-homogeneous differential equations. We have set the problem as one of discrete systems. However, we could have set it equivalently using the standard notation of the numerical solution of differential equations. In fact, the two procedures that we have mostly used along the thesis are the explicit and implicit Euler methods. The problem of how to choose the sampling rate (the step size in the terminology of numerical differential equations) have been extensively studied. The main goal of the discretization is to be able to calculate the solution with a computer. At the same time, we want this calculation to be accurate (it faithfully represents the correct answer) and stable (it does not blow up or oscillates around the correct answer). In fact the two concepts are very much related and we will address the two aspects at the same time.

The selection of the sampling rate (T_s, the time difference between two consecutive samples in the discretization) depends on:

1. the numerical method that is being used, which determines the local truncation error.

2. how smooth the solution is, which determines how large the high-order derivatives are.

3. the required accuracy, which in its turn is determined the measurement noise.

4. the stability of the numerical method.

We already saw in Section 3.3.2 that we could have a precision in the solution ranging from 0.1 to $10^{-10}\%$. In this section we will show how the sampling rate determines this accuracy and numerical stability.

In order to perform a rigorous approch to the subject, let us define a number of concepts (we will here assume the numerical literature notation and call h to T_s; $\mathbf{y}$ to the functions that are bound by the differential equation, in our case, they normally are concentrations; t to the continuous time variable and n to the discrete time variable):

- Vector sequence: A vector sequence, $\mathbf{a} = \{\mathbf{a}_n\}$ (with $n = 1, 2, 3, ...$), is a finite or infinite collection of vectors.

- Norm of a vector sequence: the l_∞ norm of a sequence is its maximum module
$$\|\mathbf{a}\|_\infty = \max_n \|\mathbf{a}_n\| \tag{3.369}$$
while the module of a vector is defined in the standard way in a Hilbert space $\|\mathbf{a}_n\| = \sqrt{\langle \mathbf{a}_n, \mathbf{a}_n \rangle}$.

- Initial Value Problem (IVP) of first order: most problems treated in this thesis are of this kind. All of them can be stated as
$$\frac{\mathbf{y}(t)}{dt} = \mathbf{f}(t, \mathbf{y}(t)) \tag{3.370}$$
with the initial value $\mathbf{y}(t_0) = \mathbf{y}_0$.

- Existence and uniqueness: before attempting to numerically calculate a solution of the IVP, we must be sure that such a solution exists and, preferrably, that it is unique. If $\mathbf{f}(t, \mathbf{y}(t))$ is continuous in the interval $t \in [t_0, t_F]$ and satisfies the Lipschitz condition
$$\|\mathbf{f}(t, \mathbf{y}_1(t)) - \mathbf{f}(t, \mathbf{y}_2(t))\| \leq L(\mathbf{f})\|\mathbf{y}_1(t) - \mathbf{y}_2(t)\| \tag{3.371}$$
for all $t, \mathbf{y}_1(t), \mathbf{y}_2(t)$ and some constant $L(\mathbf{f})$ that depends on $\mathbf{f}$, then there exists a unique solution to the Initial Value Problem in the interval $[t_0, t_F]$ for every initial value $\mathbf{y}(t_0) = \mathbf{y}_0$. Fortunately, all problems encountered in pharmacokinetics, pharmacodynamics and biopharmaceutics meet this Lipschitz condition.

- Numerical IVP method: is any method that estimates $\mathbf{y}$ at discrete time points $(t_n, \mathbf{y}_n)$. Normally, t_n is within a time frame $t_n \in T = [t_0, t_F]$ such

that $t_n = t_0 + nh$. Let us consider as an example the explicit Euler method (its justification is explained below). It can be written as

$$\mathbf{y}_{n+1} = \mathbf{y}_n + h\mathbf{f}(t_n, \mathbf{y}_n) \tag{3.372}$$

or what is the same

$$\mathbf{y}_{n+1} = \mathbf{y}_n + hF(t_n, \mathbf{y}_n, h, \mathbf{f}) \tag{3.373}$$

where F is referred to as the increment function and allows as to move from one time point to the next. It can be shown (Epperson, 2014)[p. 375] that any numerical method for a single unknown ($y(t)$) can be written in the form

$$y_{n+1} = \sum_{k=0}^{N} a_k y_{n-k} + h \sum_{k=-1}^{M} b_k f(t_{n-k}, y_{n-k}) \tag{3.374}$$

- Convergence: a numerical IVP method is convergent if its global error within a time frame T satisfies

$$\|\boldsymbol{\epsilon}_n\|_\infty \leq Ch^p \tag{3.375}$$

where $\boldsymbol{\epsilon}_n$ is the global error at t_n

$$\boldsymbol{\epsilon}_n = \mathbf{y}(t_n) - \mathbf{y}_n \tag{3.376}$$

C is a constant and p is the order of convergence. Note that if a method is convergent, then

$$\lim_{h \to 0} \|\boldsymbol{\epsilon}_n\|_\infty = 0 \tag{3.377}$$

Intituively, a method is convergent if it gives the correct answer to the differential equation when the time step goes to 0.

- Consistency: a numerical IVP method is consistent if its local error (that is the error introduced at a single time step) is $o(h)$. The local error is defined as the difference between the correct value $\mathbf{y}(t_{n+1})$ and $\mathbf{y}_{n+1}$ assuming that we had perfect knowledge of $\mathbf{y}(t)$ up to $t = t_n$. In this is the case, our estimate would be

$$\mathbf{y}^*_{n+1} = \mathbf{y}(t_n) + hF(t_n, \mathbf{y}(t_n), h, \mathbf{f}) \tag{3.378}$$

The difference between $\mathbf{y}^*_{n+1}$ and $\mathbf{y}(t_{n+1})$ is the local error:

$$\boldsymbol{\tau}_{n+1} = \mathbf{y}(t_{n+1}) - \mathbf{y}^*_{n+1} \tag{3.379}$$

If the local error is $o(h)$ it means that

$$\forall \epsilon > 0 \Rightarrow \exists H > 0 \quad \text{such that} \quad \forall 0 < h < H \Rightarrow \|\boldsymbol{\tau}_{n+1}\| < \epsilon h \tag{3.380}$$

Intituively, a method is consistent if it gives the correct prediction for t_{n+1} if we know exactly the solution of the differential equation up to t_n. It can be shown that if F is differentiable, a numerical IVP is consistent if and only if $F(t, \mathbf{y}, 0, \mathbf{f}) = \mathbf{f}(t, \mathbf{y})$ (Suli and Mayers, 2003)[p.321].

- Stability: A method is said to be stable if

$$\|\boldsymbol{\tau}_{n+1}\| \leq (1+\rho(h))\|\boldsymbol{\epsilon}_n\| \tag{3.381}$$

for some constant $\rho(h)$ that depends on h and that it tends to 0 as h tends to 0 ($\lim_{h\to 0}\rho(h)=0$).

- Zero-stability: Let us assume that $\mathbf{y}_n$ is the solution found by a numerical IVP method to the initial value problem

$$\frac{\mathbf{y}(t)}{dt} = \mathbf{f}(t,\mathbf{y}(t)) \tag{3.382}$$

with the initial value $\mathbf{y}(t_0)=\mathbf{y}_0$. Let us assume that we perturb the initial condition so that $\mathbf{y}(t_0)=\mathbf{y}_0+\boldsymbol{\delta}_0$ and that we perturb every evaluation of $\mathbf{f}$ by adding some small noise ($\boldsymbol{\delta}_n$; this is a roundoff error). Then, the numerical IVP method is zero-stable if there exist $h_0>0$, $K>0$ and $\epsilon_0>0$ such that $\forall h\in(0,h_0]$, $\forall\epsilon\in(0,\epsilon_0]$ it is verified that if $\|\boldsymbol{\delta}_n\|\leq\epsilon$, then the new solution $\{\tilde{\mathbf{y}}_n\}$ fulfills

$$\|\tilde{\mathbf{y}}_n-\mathbf{y}_n\| < Kh \tag{3.383}$$

Intuitively, a numerical IVP method is zero-stable if the round-off error does not make the solution to blow up. The importance of zero-stability is that it can be shown that if a numerical IVP method is zero-stable and consistent, then it is convergent (Lax and Richtmyer, 1956).

$$\text{zero-stability} + \text{consistency} \Rightarrow \text{convergence}$$

- Absolute stability: Zero-stability is sometimes difficult to verify. Alternatively, an easy way to determine the stability of our method is through the absolute stability that is defined considering the following IVP problem

$$\frac{y(t)}{dt} = \lambda y(t) \tag{3.384}$$

with the initial value $y(t_0)=y_0$ and with $\lambda\in\mathbb{C}$ (its exact solution is $y(t)=y_0e^{\lambda}t$). This problem is referred to as the *model problem*.

For a numerical IVP method in the form of Eq. (3.374) we define the stability polynomial as

$$\sigma(z) = z^{N+1} - \sum_{k=0}^{N} a_k z^{N-k} \tag{3.385}$$

The numerical IVP method is stable if all its roots z_k ($k=1,...,N+1$) fulfill the root condition

$$|z_k| \leq 1 \tag{3.386}$$

and if $|z_k|=1$, then it is a simple root.

For instance, when we apply the explicit Euler method (Eq. (3.372)) to the model problem, we get the recursion

$$y_{n+1} = y_n + h\lambda y_n = (1+h\lambda)y_n \tag{3.387}$$

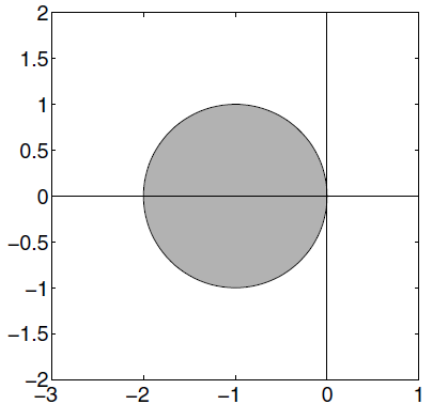

Figure 3.19: Stability region for the explicit Euler method. If the product $h\lambda$ is within the shaded region, then the method is stable.

Its stability polynomial is given by

$$\sigma(z) = z - (1 + h\lambda) \tag{3.388}$$

Its single root is $1 + h\lambda$ (remind that λ is a complex number). So the region of stability is defined for those h that fulfill (see Fig. 3.19)

$$|1 + h\lambda| \leq 1 \tag{3.389}$$

A numerical IVP method is absolutely stable (A-stable) if and only if the region of stability contains the left semiplane (h$\lambda < 0$). For an extensive review of A-stability see LeVeque (2007)[Chap. 7] and Ascher and Petzold (1998)[Chaps. 4 and 5]. A consequence of this definition is that the solution of any A-stable numerical IVP method to a model problem with $\mathbf{Re}\{\lambda\} < 0$ fulfills

$$\lim_{n\to\infty} y_n = 0 \tag{3.390}$$

It may seem that the absolute stability has been defined for a rather limited problem. However, this definition has a great importance in practical terms because all differential equations locally behave like the model problem. To show this let us linearize any Initial Value Problem around a given point $(t_n, y(t_n))$. Let us consider a nearby point that is also a solution of the differential equation defined as $(t_n + t', y(t_n) + y')$. Let us call $t = t_n + t'$ and $y(t) = y(t_n) + y'$. Since the new $y(t)$ is also a solution of the differential equation, it must fulfill

$$\begin{array}{rcl} \frac{dy(t)}{dt} & = & f(t, y(t)) \\ & = & f(t_n + t', y(t_n) + y') \end{array} \tag{3.391}$$

The left hand side can be calculated as

$$\begin{array}{rcl} \frac{dy(t)}{dt} & = & \frac{d(y(t_n)+y')}{dt'}\frac{dt'}{dt} \\ & = & \frac{dy(t_n)}{dt'}\frac{dt'}{dt} + \frac{dy'}{dt'}\frac{dt'}{dt} \end{array} \tag{3.392}$$

But $t' = t - t_n$, so $\frac{dt'}{dt} = 1$ and $y(t_n)$ is a constant, so $\frac{dy(t_n)}{dt'} = 0$. Finally we have

$$\frac{dy(t)}{dt} = \frac{dy'}{dt'} \tag{3.393}$$

For the right hand side, let us perform a Taylor approximation of first order of f around the point $(t_n, y(t_n))$

$$f(t_n + t', y(t_n) + y') \approx f(t_n, y(t_n)) + \frac{\partial f(t,y(t))}{\partial t'}\Big|_{t_n, y(t_n)} t' + \frac{\partial f(t,y(t))}{\partial y'}\Big|_{t_n, y(t_n)} y' \tag{3.394}$$

Normally $\frac{\partial f(t,y(t))}{\partial t'} = 0$ because otherwise we would have a time-varying system (its response depends on the time of excitation), so

$$f(t_n + t', y(t_n) + y') \approx f(t_n, y(t_n)) + \frac{\partial f(t,y(t))}{\partial y'}\Big|_{t_n, y(t_n)} y' \tag{3.395}$$

Combining Eqs. (3.393) and (3.395) we have

$$\frac{dy'}{dt'} \approx f(t_n, y(t_n)) + \frac{\partial f(t,y(t))}{\partial y'}\Big|_{t_n, y(t_n)} y' \tag{3.396}$$

This is a non-homogeneous differential equation whose stability properties are determined by its homogeneous equation. If we call $\lambda(t_n, y(t_n)) = \frac{\partial f(t,y(t))}{\partial y'}\Big|_{t_n, y(t_n)}$, then the homogeneous equation becomes

$$\frac{dy'}{dt'} = \lambda(t_n, y(t_n))y' \tag{3.397}$$

That is, locally, if we linearize the right hand side of the differential equation, any differential equation behaves as the model problem. This is also true for differential equation systems. In this case, instead of a scalar value (as λ above), what we obtain is a matrix $J(t_n, \mathbf{y}_n)$

$$\frac{d\mathbf{y}'}{dt'} = J(t_n, \mathbf{y}(t_n))\mathbf{y}' \tag{3.398}$$

where $J(t_n, \mathbf{y}_n)$ is the Jacobian of $\mathbf{f}$

$$J(t_n, \mathbf{y}_n) = \begin{pmatrix} \frac{\partial \mathbf{f}_1(t,\mathbf{y})}{\partial y_1} & \frac{\partial \mathbf{f}_1(t,\mathbf{y})}{\partial y_2} & \cdots & \frac{\partial \mathbf{f}_1(t,\mathbf{y})}{\partial y_p} \\ \frac{\partial \mathbf{f}_2(t,\mathbf{y})}{\partial y_1} & \frac{\partial \mathbf{f}_2(t,\mathbf{y})}{\partial y_2} & \cdots & \frac{\partial \mathbf{f}_2(t,\mathbf{y})}{\partial y_p} \\ \cdots & \cdots & \cdots & \cdots \\ \frac{\partial \mathbf{f}_p(t,\mathbf{y})}{\partial y_1} & \frac{\partial \mathbf{f}_p(t,\mathbf{y})}{\partial y_2} & \cdots & \frac{\partial \mathbf{f}_p(t,\mathbf{y})}{\partial y_p} \end{pmatrix} \tag{3.399}$$

Additionally, we can always diagonalize the Jacobian as

$$J(t_n, \mathbf{y}(t_n)) = P(t_n, \mathbf{y}(t_n))D(t_n, \mathbf{y}(t_n))P(t_n, \mathbf{y}(t_n))^{-1} \tag{3.400}$$

Make a change of variables

$$\mathbf{y}'' = P^{-1}(t_n, \mathbf{y}(t_n))\mathbf{y}' \tag{3.401}$$

so that the differential equation system becomes

$$\frac{d\mathbf{y}''}{dt'} = D(t_n, \mathbf{y}(t_n))\mathbf{y}'' \tag{3.402}$$

In this new equation system, each variable y_i'' is independent of the rest, and its differential equation is exactly the model problem

$$\frac{dy_i''}{dt'} = \lambda_i(t_n, \mathbf{y}(t_n))y_i'' \tag{3.403}$$

In fact, when analyzing differential equation systems, instead of a single differential equation, the concept of stiffness appears. A problem is stiff if the ratio between the largest and the smallest eigenvalues of the matrix $J(t_n, \mathbf{y}(t_n))$ is much larger than 1. In this case, the largest eigenvalue determines which is the maximum time step (h) we can use in the discretization. Additionally, the eigenvalues of the Jacobian may determine the behaviour of the system. For instance, if the real part of any of the eigenvalues is positive, there cannot be any stable method dealing with this problem. Also, there cannot be any stable solution if there are multiple roots (roots with multiplicity larger than 1) and the associated eigenvectors are not linearly independent.

Given all these definitions we are now ready to study the stability of the discretization procedures used in the thesis, mostly, implicit or explicit Euler methods. Let us at this point establish the difference between an explicit and an implicit method. Remind that the problems handled in the thesis are first-order differential equation systems with some initial value as shown in Eq. (3.370) and reproduced here for convenience

$$\frac{\mathbf{y}(t)}{dt} = \mathbf{f}(t, \mathbf{y}(t)) \tag{3.404}$$

We may discretize the equation in two different ways

$$\begin{array}{rcl} \frac{\mathbf{y}_{n+1}-\mathbf{y}_n}{h} & = & \mathbf{f}(t_n, \mathbf{y}_n) \\ \frac{\mathbf{y}_{n+1}-\mathbf{y}_n}{h} & = & \mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) \end{array} \tag{3.405}$$

That give the two recursions

$$\begin{array}{rcl} \mathbf{y}_{n+1} & = & \mathbf{y}_n + h\mathbf{f}(t_n, \mathbf{y}_n) \\ \mathbf{y}_{n+1} & = & \mathbf{y}_n + h\mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) \end{array} \tag{3.406}$$

The first equation is very straightforward to calculate since to calculate the sample at $n+1$ we only need to perform calculations on the current values at n. However, the second one is not that simple because to calculate $\mathbf{y}_{n+1}$ we need to evaluate $\mathbf{f}$ at $\mathbf{y}_{n+1}$ (which we do not know). In general, if $\mathbf{f}$ is a nonlinear function, this recursion implies solving a nonlinear equation system for which again we need some numerical method. However, paying this extra cost may have its benefits in terms of stability. We have already seen in Eq. (3.388) that the stability region of the explicit Euler method is restricted to the region $|1+h\lambda| \leq 1$. However, the recursion of the Euler implicit method for the model problem is

$$y_{n+1} = \frac{1}{1-\lambda h} y_n \tag{3.407}$$

and its the stability polynomial

$$\sigma(z) = z - \frac{1}{1-\lambda h} \tag{3.408}$$

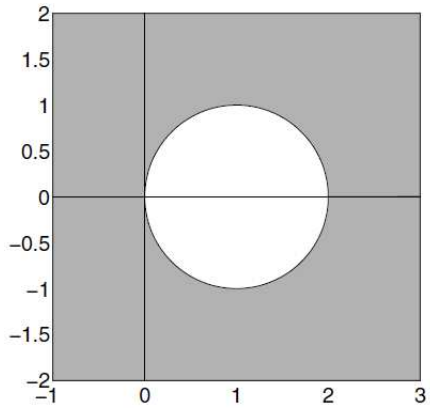

Figure 3.20: Stability region for the implicit Euler method. If the product $h\lambda$ is within the shaded region, then the method is stable.

The condition for stability is then $\left|\frac{1}{1-\lambda h}\right| \leq 1$ (see Fig. 3.20). That is, the method is absolutely stable.

Different discretization schema have different stability properties. Generally speaking, all implicit methods are A-stable (LeVeque, 2007)[Chap. 7] although their regions of stability normally differ. It can also be shown that both Euler methods are consistent and convergent, that is, if the sampling rate is small enough, the calculations are very accurate.

The model problem for differential equation systems is

$$\frac{d\mathbf{y}(t)}{dt} = A\mathbf{y}(t) \tag{3.409}$$

with the initial condition $\mathbf{y}(0) = \mathbf{y}_0$. The corresponding explicit Euler recursion would be

$$\mathbf{y}_{n+1} = \mathbf{y}_n + hA\mathbf{y}_n = (I + hA)\mathbf{y}_n = (I + hA)^n\mathbf{y}_0 \tag{3.410}$$

To be stable, we need that all eigenvalues of the matrix $I+hA$ have unit module or a module smaller than 1. Otherwise, the term $(I+hA)^n$ would explode with increasing n.

Let's for the moment put aside this discussion on stability and concentrate on the other constraint for the sampling rate, approximation accuracy, we would like the discrete system to represent faithfully the continuous system. In the terminology of differential equations this is related to the local error. Let us study how some of the methods are derived to understand how the approximation error is treated in each case. Let us start with the two Euler methods. The two recursions

$$\begin{aligned} \mathbf{y}_{n+1} &= \mathbf{y}_n + h\mathbf{f}(t_n, \mathbf{y}_n) \\ \mathbf{y}_{n+1} &= \mathbf{y}_n + h\mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) \end{aligned} \tag{3.411}$$

mean that for moving from t_n to t_{n+1} we should move a distance h in the direction of the gradient at the time t_n (explicit) or t_{n+1} (implicit). Let's analyze the local error of each one. If we knew exactly $\mathbf{y}(t_n)$, the explicit Euler method would make the prediction

$$\mathbf{y}^*_{n+1} = \mathbf{y}(t_n) + h\mathbf{f}(t_n, \mathbf{y}_n) \tag{3.412}$$

But we know from Taylor expansion at $t = t_n$ that

$$\begin{aligned} \mathbf{y}(t_{n+1}) &= \mathbf{y}(t_n) + hD\mathbf{y}(t_n) + O(h^2) \\ &= \mathbf{y}(t_n) + h\mathbf{f}(t_n, \mathbf{y}_n) + O(h^2) \end{aligned} \tag{3.413}$$

where we have defined the operator $D\mathbf{y}(t) = \frac{d\mathbf{y}(t)}{dt}$ and $D\mathbf{y}(t_n)$ is the value of the operator applied on $\mathbf{y}(t)$ and then evaluated at $t = t_n$. So, the local error is

$$\boldsymbol{\tau}(t_{n+1}) = \mathbf{y}(t_{n+1}) - \mathbf{y}^*_{n+1} = O(h^2) \tag{3.414}$$

When the local error is $O(h^p)$ the method is said to be of consistency order $p-1$ (it can be shown that when the local error is $O(h^p)$, then the global error is $O(h^{p-1})$ due to the error propagation effect). So, the explicit Euler method is of order 1.

The implicit Euler method is also of consistency order 1. From the Taylor expansion at $t = t_{n+1}$ we know

$$\begin{aligned} \mathbf{y}(t_n) &= \mathbf{y}(t_{n+1}) - hD\mathbf{y}(t_{n+1}) + O(h^2) \\ &= \mathbf{y}(t_{n+1}) - h\mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) + O(h^2) \end{aligned} \tag{3.415}$$

Rearranging the terms we get

$$\mathbf{y}(t_{n+1}) = \mathbf{y}(t_n) + h\mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) + O(h^2) \tag{3.416}$$

Proceeding analogously as to the explicit Euler method we could calculate

$$\boldsymbol{\tau}(t_{n+1}) = \mathbf{y}(t_{n+1}) - \mathbf{y}^*_{n+1} = O(h^2) \tag{3.417}$$

Consequently, we see that the fact that the method is implicit or explicit does not affect its consistency, it only affects its stability.

Let us try to get a more accurate recursion. Instead of moving in the direction of the gradient at t_n (as the explicit method), or the gradient at (t_{n+1}), let us try to move in the direction of the average of the two

$$\mathbf{y}_{n+1} = \mathbf{y}_n + h\frac{\mathbf{f}(t_n, \mathbf{y}_n) + \mathbf{f}(t_{n+1}, \mathbf{y}_{n+1})}{2} \tag{3.418}$$

Let us calculate its order of consistency. If we knew exactly the data up to t_{n+1} our prediction would be

$$\mathbf{y}^*_{n+1} = \mathbf{y}(t_n) + h\frac{\mathbf{f}(t_n, \mathbf{y}(t_n)) + \mathbf{f}(t_{n+1}, \mathbf{y}(t_{n+1}))}{2} \tag{3.419}$$

Let us now repeat the Taylor expansion at $t = t_{n+\frac{1}{2}}$

$$\begin{aligned} \mathbf{y}(t_{n+1}) &= \mathbf{y}(t_{n+\frac{1}{2}}) + \tfrac{h}{2}D\mathbf{y}(t_{n+\frac{1}{2}}) + \frac{\left(\frac{h}{2}\right)^2}{2}D^2\mathbf{y}(t_n)\mathbf{1} + O(h^3) \\ \mathbf{y}(t_n) &= \mathbf{y}(t_{n+\frac{1}{2}}) - \tfrac{h}{2}D\mathbf{y}(t_{n+\frac{1}{2}}) + \frac{\left(\frac{h}{2}\right)^2}{2}D^2\mathbf{y}(t_n)\mathbf{1} + O(h^3) \end{aligned} \tag{3.420}$$

If we now subtract the two equations we have

$$\mathbf{y}(t_{n+1}) - \mathbf{y}(t_n) = hD\mathbf{y}(t_{n+\frac{1}{2}}) + O(h^3) \tag{3.421}$$

Let us now compute the Taylor expansion of $D\mathbf{y}(t)$ at $t = t_{n+\frac{1}{2}}$

$$\begin{aligned} D\mathbf{y}(t_{n+1}) &= D\mathbf{y}(t_{n+\frac{1}{2}}) + \tfrac{h}{2}D^2\mathbf{y}(t_{n+\frac{1}{2}}) + \frac{\left(\frac{h}{2}\right)^2}{2}D^3\mathbf{y}(t_n) + O(h^4) \\ D\mathbf{y}(t_n) &= D\mathbf{y}(t_{n+\frac{1}{2}}) - \tfrac{h}{2}D^2\mathbf{y}(t_{n+\frac{1}{2}}) + \frac{\left(\frac{h}{2}\right)^2}{2}D^3\mathbf{y}(t_n)\mathbf{1} + O(h^4) \end{aligned} \tag{3.422}$$

Let us now sum the two equations and divide by 2

$$\frac{D\mathbf{y}(t_n)+D\mathbf{y}(t_{n+1})}{2} = D\mathbf{y}(t_{n+\frac{1}{2}}) + \frac{\left(\frac{h}{2}\right)^2}{2} D^3\mathbf{y}(t_n) + O(h^5) \tag{3.423}$$

Now we solve for $D\mathbf{y}(t_{n+\frac{1}{2}})$

$$D\mathbf{y}(t_{n+\frac{1}{2}}) = \frac{D\mathbf{y}(t_n)+D\mathbf{y}(t_{n+1})}{2} + O(h^3) \tag{3.424}$$

and substitute in Eq. (3.421)

$$\mathbf{y}(t_{n+1}) - \mathbf{y}(t_n) = h\frac{D\mathbf{y}(t_n)+D\mathbf{y}(t_{n+1})}{2} + O(h^3) \tag{3.425}$$

From where we get

$$\begin{aligned} \mathbf{y}(t_{n+1}) &= \mathbf{y}(t_n) + h\frac{D\mathbf{y}(t_n)+D\mathbf{y}(t_{n+1})}{2} + O(h^3) \\ &= \mathbf{y}(t_n) + h\frac{\mathbf{f}(t_n,\mathbf{y}(t_n))+\mathbf{f}(t_{n+1},\mathbf{y}(t_{n+1}))}{2} + O(h^3) \end{aligned} \tag{3.426}$$

If we now calculate the local error

$$\boldsymbol{\tau}(t_{n+1}) = \mathbf{y}(t_{n+1}) - \mathbf{y}^*_{n+1} = O(h^3) \tag{3.427}$$

So, the trapezoidal rule has a consistency rate of 2.

The trapezoidal method is implicit because in order to calculate $\mathbf{y}_{n+1}$ we need to evaluate $\mathbf{f}$ at $(t_{n+1}, \mathbf{y}_{n+1})$. Predictor-corrector methods use a similar strategy to the trapezoidal rule but they try to avoid the implicit scheme. They use a explicit Euler method to predict the $\mathbf{y}_{n+1}$

$$\text{Predictor:}\tilde{\mathbf{y}}_{n+1} = \mathbf{y}_n + h\mathbf{f}(t_n, \mathbf{y}_n) \tag{3.428}$$

Then, they use this predicted value to evaluate the trapezoidal rule

$$\text{Corrector:}\mathbf{y}_{n+1} = \mathbf{y}_n + h\frac{\mathbf{f}(t_n,\mathbf{y}_n)+\mathbf{f}(t_{n+1},\tilde{\mathbf{y}}_{n+1})}{2} \tag{3.429}$$

It can be proved that the scheme is still of consistency rate 2, but now the scheme is not implicit. Actually, we can be very creative and use many different prediction-correction formulas to obtain different levels of accuracy.

Alternatively, we could have linearized the evaluation of $\mathbf{f}$ at $(t_{n+1}, \mathbf{y}_{n+1})$ by performing a Taylor expansion

$$\mathbf{f}(t_{n+1}, \mathbf{y}_{n+1}) \approx \mathbf{f}(t_{n+1}, \mathbf{y}_n) + J(t_{n+1}, \mathbf{y}_n)(\mathbf{y}_{n+1} - \mathbf{y}_n) \tag{3.430}$$

Note that we still keep t_{n+1} in the evaluation of the Jacobian and $\mathbf{f}$ since we know t_{n+1}, what we do not know is $\mathbf{y}_{n+1}$. Then the trapezoidal rule becomes

$$\mathbf{y}_{n+1} \approx \mathbf{y}_n + h\frac{\mathbf{f}(t_n,\mathbf{y}_n)+\mathbf{f}(t_{n+1},\mathbf{y}_n)+J(t_{n+1},\mathbf{y}_n)(\mathbf{y}_{n+1}-\mathbf{y}_n)}{2} \tag{3.431}$$

Knowing that in pharmaceutical problems, the system is time invariant we have that $\mathbf{f}(t_n, \mathbf{y}_n) = \mathbf{f}(t_{n+1}, \mathbf{y}_n)$ and $J(t_{n+1}, \mathbf{y}_n) = J(t_n, \mathbf{y}_n)$. So we may explicitly solve for $\mathbf{y}_{n+1}$ by computing

$$\mathbf{y}_{n+1} = \mathbf{y}_n + h(I - \tfrac{h}{2}J(t_n, \mathbf{y}_n))^{-1}\mathbf{f}(t_n, \mathbf{y}_n) \tag{3.432}$$

Notwithstanding, Runge-Kutta's methods are normally the preferred explicit and implicit methods for high-order accuracy. They are developed on similar

grounds to the error analysis developed so far and their theory will not be developed here. It is enough to know that they respond to the general scheme

$$\mathbf{y}_{n+1} = \mathbf{y}_n + \sum_{i=1}^{p} b_i \mathbf{k}_i \tag{3.433}$$

where

$$\mathbf{k}_i = h\mathbf{f}\left(t_n + c_i h, \mathbf{y}_n + \sum_{j=1}^{p} a_{ij}\mathbf{k}_j\right) \tag{3.434}$$

If $a_{ij} = 0$ for $i < j$, then the scheme is explicit; otherwise, it is implicit. Coefficients c_i, b_i and a_{ij} are calculated for each Runge-Kutta method so that they reach an order of accuracy of p.

All the methods presented so far are one-step methods in which an increase of accuracy is achieved by a clever combination of points beyond t_n. They are called one-step because from t_n we try to predict the value at t_{n+1}. However, there is another family of methods in which the increase in accuracy is achieved by using previous information $(t_n, t_{n-1}, t_{n-2}, ...)$, they are called linear multistep methods (LMMs). Among these methods the most popular are Adams-Bashforth (explicit), Adams-Moulton (implicit), and BDF (backwards differentiation formula; implicit). We will not enter into the details of these methods. Instead it is enough to state that they all respond to the general scheme

$$\mathbf{y}_{n+1} = \sum_{k=0}^{N} a_k \mathbf{y}_{n-k} + h \sum_{k=-1}^{M} b_k \mathbf{f}(t_{n-k}, \mathbf{y}_{n-k}) \tag{3.435}$$

In the following we will show how to apply all these ideas to the selection of the sampling rate in a practical pharmaceutical problem. For doing so, we will make use of the non-linear example of a drug with autoinduction (see Section 3.4.2). The system can be modelled with a pair of differential equations (Eq. (3.158)) and reproduced here for convenience:

$$\begin{array}{rcl} \frac{dC_E(t)}{dt} & = & R_E - Cl_E\left(1 - \frac{S_{max}C(t)}{SC_{50}+C(t)}\right)C_E(t) \\ \frac{dC(t)}{dt} & = & -\frac{1}{V}\frac{Q_H a C_E(t) f_u}{aC_E(t)f_u + C(t)Q_H + K_m Q_H}C(t) + \frac{dose_{iv}(t)}{V} \end{array} \tag{3.436}$$

Let us assume the system parameters: $S_{max} = 0.3$, $R_E = 0.01$ (mg/min), $Cl_E = 0.005$ (L/min), $SC_{50} = 0.02$ (mg/L), $V = 10$ (L), $Q_H = 1.45$ (L/min), $f_u = 1$, $a = 0.01$ (1/min), $T_s = 1$ (min), and $K_m = 0.1$ (mg/L) (see Fig. 3.16). We may write this equation in the form

$$\frac{d\mathbf{y}(t)}{dt} = \mathbf{f}(t, \mathbf{y}(t)) + \mathbf{f_0}(t, \mathbf{x}(t)) \tag{3.437}$$

where $\mathbf{y}(t)$ is the response variable in which we are interested in, and $\mathbf{x}(t)$ is the excitation to the system. We simply need to make the following assignments

$$\begin{array}{rcl} \mathbf{y}(t) & = & \begin{pmatrix} C_E(t) \\ C(t) \end{pmatrix} \\ \mathbf{x}(t) & = & \begin{pmatrix} 1 \\ dose_{iv}(t) \end{pmatrix} \\ \mathbf{f}(t, \mathbf{y}(t)) & = & \begin{pmatrix} -Cl_E\left(1 - \frac{S_{max}C(t)}{SC_{50}+C(t)}\right)C_E(t) \\ -\frac{1}{V}\frac{Q_H a C_E(t) f_u}{aC_E(t)f_u + C(t)Q_H + K_m Q_H}C(t) \end{pmatrix} \\ \mathbf{f}_0(t, \mathbf{x}(t)) & = & \begin{pmatrix} R_E \\ \frac{dose_{iv}(t)}{V} \end{pmatrix} \end{array} \tag{3.438}$$

The stability analysis is determined by the homogeneous part of the equation, that, is that defined by $\mathbf{f}(t, \mathbf{y}(t))$. As stated in Eq. (3.398), the stability properties can be studied through the Jacobian of the function $\mathbf{f}$.

$$\begin{aligned} J(t_n, \mathbf{y}_n) &= \left(\begin{array}{cc} \frac{\partial \mathbf{f}_1(t,\mathbf{y})}{\partial C_E(t)} & \frac{\partial \mathbf{f}_1(t,\mathbf{y})}{\partial C(t)} \\ \frac{\partial \mathbf{f}_2(t,\mathbf{y})}{\partial C_E(t)} & \frac{\partial \mathbf{f}_2(t,\mathbf{y})}{\partial C(t)} \end{array} \right) \Bigg|_{t=t_n, \mathbf{y}(t)=\mathbf{y}_n} \\ &= \left(\begin{array}{cc} -Cl_E \left(1 - \frac{S_{max} C[n]}{SC_{50}+C[n]}\right) & S_{max} Cl_E \frac{C_E[n] SC_{50}}{(SC_{50}+C[n])^2} \\ -\frac{1}{V} \frac{C[n] Q_H^2 a f_u (C[n]+K_m)}{(aC_E[n] f_u + C[n] Q_H + K_m Q_H)^2} & -\frac{1}{V} \frac{C_E[n] Q_H a f_u (K_m Q_H + C_E[n] a f_u)}{(aC_E[n] f_u + C[n] Q_H + K_m Q_H)^2} \end{array} \right) \end{aligned} \tag{3.439}$$

Remind that the condition for the explicit Euler method to be stable was that $|1 + h\lambda| \leq 1$ where λ is any of the eigenvalues of $J(t_n, \mathbf{y}_n)$. It is easy to show that this condition is equivalent to

$$T_s = h \leq -\frac{\mathrm{Re}\{\lambda\}}{\|\lambda\|^2} \tag{3.440}$$

Figs. 3.21 and 3.22 show the real part of the two eigenvalues of $J(t_n, \mathbf{y}_n)$ and the maximum sampling rate allowed at each moment. As it should, the two eigenvalues have negative real parts (otherwise, the system would not be stable; the drug concentration would grow indefinitely which does not make sense in a pharmaceutical setting). As stated in Fig. 3.22, the sampling rate cannot be larger than 1.92 if we want to use the explicit Euler scheme in a stable way. Interestingly, before one hour (approximately) the two eigenvalues are complex conjugates of each other, while beyond one hour they are not anymore. This has a number of implications in the properties of the dynamic system and it is related to bifurcation theory. This theory is beyond the scope of this thesis and the interested reader is referred to Hirsch et al. (2013).

The other aspect we have been discussing in this section is accuracy. Fig. 3.23 shows the order of magnitude of each one of the three first terms in the Taylor expansion of the concentration $C(t)$ (it shows the logarithm in base 10 of $|C(t)|$, $\left|h\frac{dC(t)}{dt}\right|$ and $\left|\frac{h^2}{2}\frac{d^2C(t)}{dt^2}\right|$). At the sight of this figure we can safely state that the second order terms are much smaller (2 orders of magnitude smaller) than the first order terms, and consequently, it is safe to use the explicit Euler method to discretize the continuous system. However, if we still wanted a larger accuracy we should have used an order 2 or higher numerical scheme.

3.7.2 Sensitivity and identifiability

Along the thesis we have concentrated on establishing a model that represents our data and estimating its parameters. Let's say we already got an estimate of those parameters. In the Statistical literature, this is called a point estimate. In this section we address the question "how sure can I be that these are the true parameters and not others?". This question can be answered in two ways:

1. If I change a little bit the parameters, does my response change a lot? This question is answered by a sensitivity analysis (Saltelli et al., 2004). We study which is the variation of the measurements (normally concentrations) if we change the parameters. We will discover that there are parameters that are very sensitive and parameters that are not sensitive

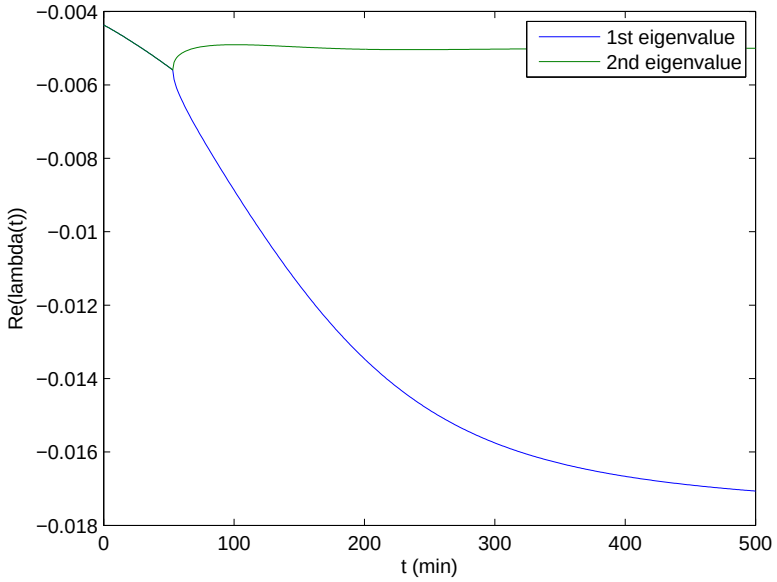

Figure 3.21: Real part of the two eigenvalues of the Jacobian of $\mathbf{f}$

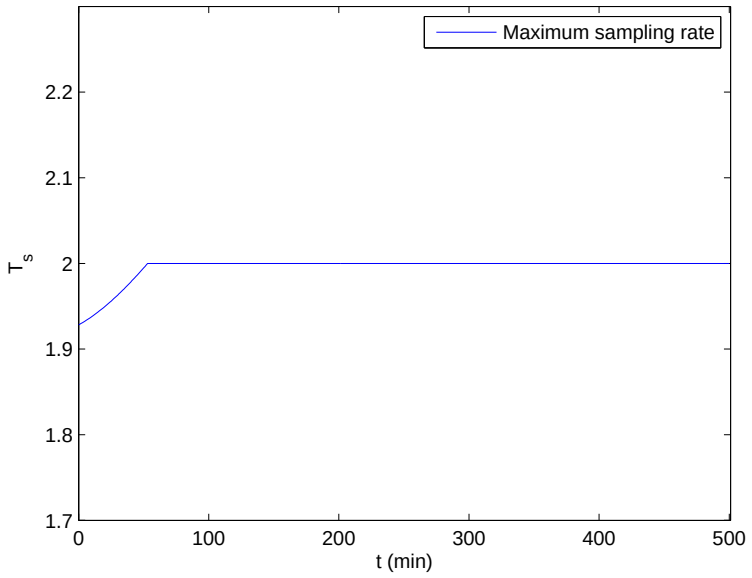

Figure 3.22: Maximum sampling rate for the stability of the explicit Euler method.

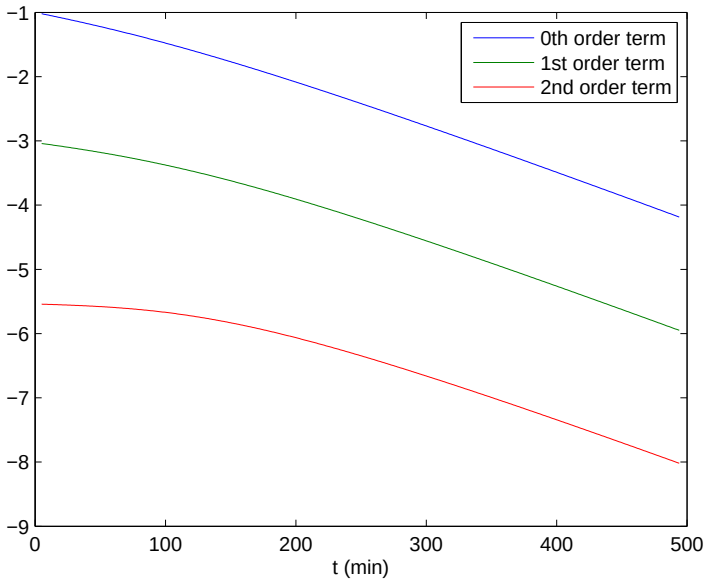

Figure 3.23: Order of magnitude of each one of terms in the Taylor expansion of $C(t)$.

at all. These latter are specially bad news, because that means that we can change the parameter in a wide range and still do not see any significant variation in the drug concentrations. Sensitivity analysis is something that has already been addressed by the pharmaceutical literature (Bonate, 2011)[p. 564], (Kimko and Duffull, 2003)[p. 153], (Parrott and Lavé, 2002), although except in a few exceptions it is restricted to a static, linear analysis. We will show how sensitivity changes over time and how the linear approach cannot predict the correct behaviour of non-linear systems. We will also perform a second-order approach, in which we will pose the question if I change one parameter, can I move another parameter in a different direction to compensate?

2. If I change a little bit the measurements, do my parameters change a lot? This is the reverse question. We can imagine that the uncertainty (measurement noise) that we have in our observed variables translate into an uncertainty in the model parameters. When we perform a model fitting with a standard pharmacokinetics program, it reports the variance of each parameter. We will show that there is a theoretical minimum for this variance, that is for a certain amount of measurement noise, there is a minimum amount of variance for a given parameter, no matter how sophisticated is our estimation method. This is called the Crámer-Rao bound and it has been largely overlooked in the pharmaceutical literature (Ette and Williams, 2007)[Sec. 5.2.4.3].

From parameters to measurements

Traditional sensitivity analysis in pharmacokinetics has concentrated on the effect of a given parameter on drug concentration using closed-form formulas (Bonate, 2011)[p. 564],(Kimko and Duffull, 2003)[p. 153]. For instance, let us consider the closed-form formula of the response with an extravascular dose (Eq. (3.89)) and reproduced here for convenience

$$C(t) = \frac{K_a D_{po}}{V(K_a + K_d) - Cl} \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) u(t) \tag{3.441}$$

Linear sensitivity analysis follows from a first order Taylor expansion about a given time point and the realization that $C(t)$ is actually a convenient short-name for a function that actually depends on t and all the model parameters

$$C(t, \boldsymbol{\Theta}_0 + \Delta\boldsymbol{\Theta}) = C(t, \boldsymbol{\Theta}_0) + \sum_{i=1}^{p} \left. \frac{\partial C(t,\boldsymbol{\Theta})}{\partial \Theta_i} \right|_{t,\boldsymbol{\Theta}_0} \Delta\Theta_i + O(\|\Delta\boldsymbol{\Theta}\|^2) \tag{3.442}$$

The term $\left. \frac{\partial C(t,\boldsymbol{\Theta})}{\partial \Theta_i} \right|_{t,\boldsymbol{\Theta}_0}$ is the so-called sensitivity index, that is normally taken in absolute value since at this point we do not care if the function increases or decreases when the parameter increases. In this particular case, we have

$$\begin{aligned}
S_{D_{po}}(t) &= \left| \frac{\partial C(t)}{\partial D_{po}} \right| = \left| \frac{K_a}{V(K_a+K_d)-Cl} \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) \right| \\
S_{K_a}(t) &= \left| \frac{\partial C(t)}{\partial K_a} \right| = \left| \frac{D_{po}}{V(K_a+K_d)-Cl} \right| \left| \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) \left(1 - \frac{1}{V(K_a+K_d)-Cl} \right) \right. \\
&\quad \left. + K_a t e^{-(K_a+K_d)t} \right| \\
S_{K_d}(t) &= \left| \frac{\partial C(t)}{\partial K_d} \right| = \left| \frac{D_{po}K_a}{V(K_a+K_d)-Cl} \right| \left| te^{-(K_a+K_d)t} - \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) \frac{V}{V(K_a+K_d)-Cl} \right| \\
S_{Cl}(t) &= \left| \frac{\partial C(t)}{\partial K_d} \right| = \left| \frac{D_{po}K_a}{V(K_a+K_d)-Cl} \right| \left| \frac{t}{V} e^{-\frac{Cl}{V}t} + \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) \frac{V}{V(K_a+K_d)-Cl} \right| \\
S_V(t) &= \left| \frac{\partial C(t)}{\partial K_d} \right| = \left| \frac{D_{po}K_a}{V(K_a+K_d)-Cl} \right| \left| \frac{Clt}{V^2} e^{-\frac{Cl}{V}t} + \left(e^{-\frac{Cl}{V}t} - e^{-(K_a+K_d)t} \right) \frac{1}{V(K_a+K_d)-Cl} \right|
\end{aligned} \tag{3.443}$$

An important aspect of this sensitivity analysis is that sensitivity has a strong dependence on time. This is easly seen in Fig. 3.24 in which we see the time dependence of all these sensitivities for $D_{po} = 10$ mg, $Cl = 3$ L/h, $V = 10$ L, $K_a = 0.6$ h^{-1}, $K_d = 1.8$ h^{-1}. The first 5-6 hours is the region with larger sensitivity and where these parameters are more accurately determined.

In the previous experiment D_{po} and V are apparently the most difficult parameters to determine. This is simply because their nominal values are much larger than the rest. To account for this fact, the normalized sensitivity index has been defined (Kimko and Duffull, 2003)[p. 153] as

$$\tilde{S}_{\Theta_i}(t) = \left| \frac{\partial C(t)/C(t)}{\partial \Theta_i/\Theta_i} \right| = \left| \frac{\Theta_i}{C(t)} \frac{\partial C(t)}{\partial \Theta_i} \right| = \left| \frac{\Theta_i}{C(t)} \right| S_{\Theta_i}(t) \tag{3.444}$$

Although mathematically appealing because there is a double normalization, it gives the false impression that parameters affect equally at all times (see Fig. 3.25). For instance, note how $\tilde{S}_{D_{po}}$ is a constant; however, it does not equally affect the drug concentration just after administration than after a long period.

Instead we propose to normalize only by the parameter value (as is done in other fields like filter design with the Q factor (Siebert, 1985)[p.497]); see Fig. 3.26.

$$\tilde{S}_{\Theta_i}(t) = |\Theta_i| \, S_{\Theta_i}(t) \tag{3.445}$$

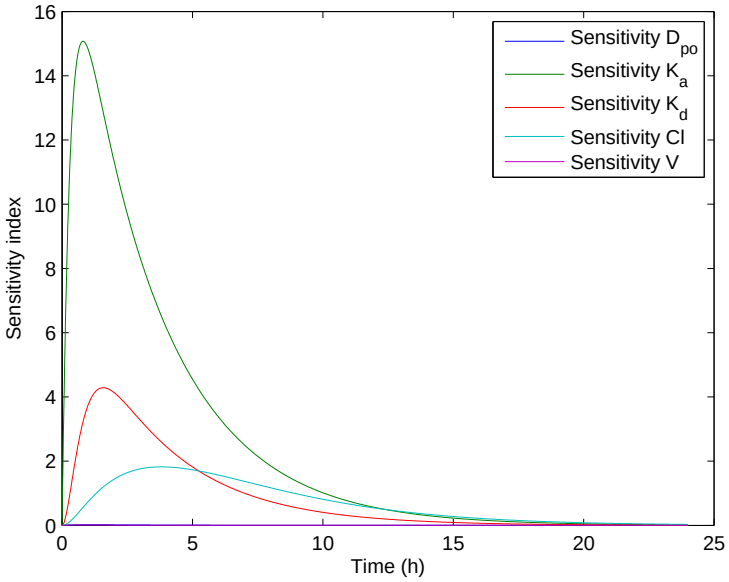

Figure 3.24: Sensitivity index for each one of the parameters of the oral dose.

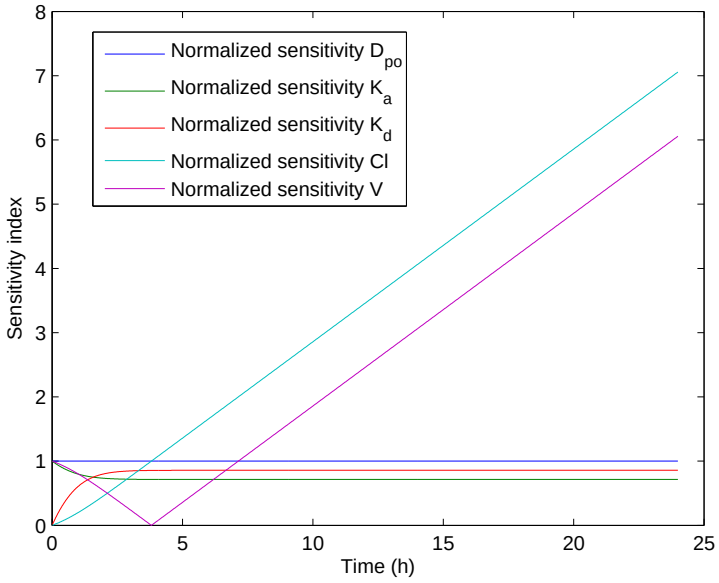

Figure 3.25: Doubly normalized sensitivity index for each one of the parameters of the oral dose.

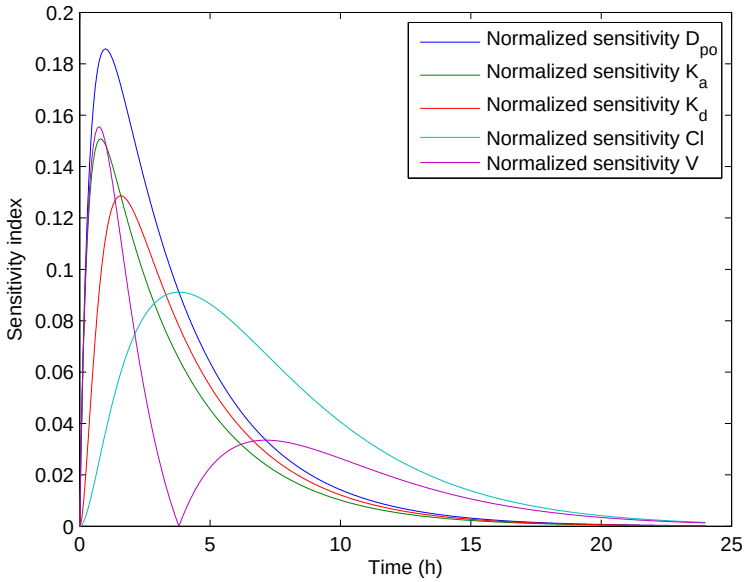

Figure 3.26: Singly normalized sensitivity index for each one of the parameters of the oral dose.

This normalization is more in accordance with the Taylor expansion since all we are doing is normalizing the variations of the parameters to set them in unitary changes.

$$C(t,\boldsymbol{\Theta}_0+\Delta\boldsymbol{\Theta}) \quad = \quad C(t,\boldsymbol{\Theta}_0)+\sum_{i=1}^{p}\left.\frac{\partial C(t,\boldsymbol{\Theta})}{\partial\Theta_i}\right|_{t,\boldsymbol{\Theta}_0}\Theta_i\frac{\Delta\Theta_i}{\Theta_i}+O(\|\Delta\boldsymbol{\Theta}\|^2) \tag{3.446}$$

Now, it is clearly seen that the most important parameter is the amount of drug in the oral dose and that as time goes on, the effect of parameter changes on the concentration is more and more difficult to see due to the measurement noise. Interestingly, we see that there are moments at which the sensitivity may vanish (for instance, the sensitivity to the distribution volume vanishes at about 4 hours; this means that if we have to determine the distribution volume using only samples at about 4 hours, we would have a huge variability).

All the sensitivity analysis described so far can be named *linear sensitivity* because it has been developed after linearizing the closed-form expression of the drug concentration. Alternatively, recent advances in sensitvity analysis have introduced the concept of variance based sensitivity analysis (Saltelli et al., 2004) that has been translated into pharmacokinetics in the following way (Kimko and Duffull, 2003)[p. 153]

$$\mathrm{Var}\{C(t)\}=\sum_{i=1}^{p}\left(\left.\frac{\partial C(t,\boldsymbol{\Theta})}{\partial\Theta_i}\right|_{t,\boldsymbol{\Theta}_0}\right)^2\mathrm{Var}\{\Theta_i\} \tag{3.447}$$

However, this decomposition is only an approximation of the real variance because it assumes that the different parameters are independent of each other

(that is, when we estimate one parameter, this estimate is not affected by errors in the estimation of other parameters). But this is not usually the case because the regression process normally compensates errors in one variable by changing its estimate for another variable. This can be done because the Hessian of the concentration is not a diagonal matrix. The Hessian is defined as shown below

$$H(t) = \begin{pmatrix} \frac{\partial^2 C(t)}{\partial \Theta_1^2} & \frac{\partial^2 C(t)}{\partial \Theta_1 \partial \Theta_2} & \cdots & \frac{\partial^2 C(t)}{\partial \Theta_1 \partial \Theta_p} \\ \frac{\partial^2 C(t)}{\partial \Theta_1 \partial \Theta_2} & \frac{\partial^2 C(t)}{\partial^2 \Theta_2} & \cdots & \frac{\partial^2 C(t)}{\partial \Theta_2 \partial \Theta_p} \\ \cdots & \cdots & \cdots & \cdots \\ \frac{\partial^2 C(t)}{\partial \Theta_p \partial \Theta_1} & \frac{\partial^2 C(t)}{\partial \Theta_p \partial \Theta_2} & \cdots & \frac{\partial^2 C(t)}{\partial^2 \Theta_p} \end{pmatrix} \tag{3.448}$$

We may define the normalized Hessian as a matrix such that is ij-th index is $\tilde{H}_{ij}(t) = H_{ij}(t)\Theta_i\Theta_j$. Then, when we consider the second order terms in the Taylor expansion we have

$$\begin{aligned} C(t, \boldsymbol{\Theta}_0 + \Delta\boldsymbol{\Theta}) \approx\ & C(t, \boldsymbol{\Theta}_0) + \sum_{i=1}^{p} \left.\frac{\partial C(t,\boldsymbol{\Theta})}{\partial \Theta_i}\right|_{t,\boldsymbol{\Theta}_0} \Theta_i \frac{\Delta\Theta_i}{\Theta_i} + \\ & \frac{1}{2}\sum_{i=1}^{p}\sum_{j=1}^{p} \left.\frac{\partial^2 C(t,\boldsymbol{\Theta})}{\partial \Theta_i \partial \Theta_j}\right|_{t,\boldsymbol{\Theta}_0} \Theta_i\Theta_j \frac{\Delta\Theta_i}{\Theta_i}\frac{\Delta\Theta_j}{\Theta_j} \end{aligned} \tag{3.449}$$

It is more difficult to show how the Hessian evolves over time. However, let us inspect the normalized Hessian at a given time point, for instance, 1 hour after giving the oral dose:

$$\tilde{H}(60) = \begin{pmatrix} 0.0000 & 0.1482 & -0.1126 & -0.0370 & -0.1488 \\ 0.1482 & -0.0629 & -0.0762 & -0.0318 & -0.1165 \\ -0.1126 & -0.0762 & 0.1090 & 0.0156 & 0.0969 \\ -0.0370 & -0.0318 & 0.0156 & 0.0085 & 0.0654 \\ -0.1488 & -0.1165 & 0.0969 & 0.0654 & 0.2321 \end{pmatrix} \tag{3.450}$$

Off-diagonal, large values in this matrix indicate that the two parameters may compensate each other. For instance, the following parameters can have a strong cross-talk with each other: D_{po} and V (-0.1488), D_{po} and K_a (0.1482), D_{po} and K_d (-0.1126), K_a and V (-0.1165), K_a and K_d (-0.0762), K_d and V (0.0969), and Cl and V (0.0654).

Additionally, there is a key aspect that cannot be overlooked. The sensitivity analysis is only valid in a neighbourhood of the current parameters. As soon as we get away from them, the Taylor expansion is no longer valid. Let us take as an example the sensitivity to the distribution volume of the drug concentration at 1 hour after adminitering the oral dose. At this point, the Taylor expansion gives

$$C(60, V + \Delta V) \approx C(60) - S_V(60)\Delta V \tag{3.451}$$

Since we know that concentration measurements can be accurate up to 15%, we may be interested in realizing which is the ΔV range that produces such a change. Solving for ΔV, we get

$$\Delta V = \frac{C(60)(1-K)}{SV(60)} \tag{3.452}$$

where K is 1.15 or 0.85 depending on whether we are interested in positive or negative deviations from the nominal concentration. In this particular case

$\Delta V = 1.86$ meaning that $V = 10 + 1.86$ L is predicted to result in a change of -15% in the nominal concentration, and $V = 10 - 1.86$ L is predicted to result in a change of +15% in the nominal concentration. However, in reality it results in a change of -13.08% and 17.55%. This is because the relationship between $C(t)$ and V is non-linear, and the linearized Taylor expansion cannot fully account for the non-linear effects of V on $C(t)$. Additionally, we may be interested not only at a particular time point ($t = 60$) but in the whole time range and wonder which is the range of V in which the drug concentration at any time does not depart from its nominal value more than 15% in each direction. For doing so, we simply have to simulate the model with different distribution volumes and check which fulfill this condition. We easily identify that the distribution volume must be between 9.74 and 10.24 L (note that the ΔV range is assymetric, $\Delta V \in [-0.26, 0.24]$, due to the non-linearity).

All this analysis means that a careful prediction of the variance of $C(t)$ or the sensitivity of $C(t)$ to its parameters cannot be oversimplified. The approach that is put forward in this thesis is a Monte-Carlo simulation of the drug concentration curve (Binder and Heermann, 2010). Ideally, the Montecarlo simulation should take parameter values from the distribution observed in a population PKPD study. Additionally, Monte-Carlo simulation solves the problem that all the previous sensitivity analysis needed closed-form formulas, while the methodology defended in this thesis is based on sets of differential equations. In Monte-Carlo simulation, we vary the model parameters in a known region (for instance, $\pm 1\%$ of their nominal value) and see how these variations translate into different $C(t)$ curves. Based on these simulations we may establish all kind of distributional properties. Fig. 3.27 shows an example of some of the simulated curves. Note how the variance differs at different time points (in the peak the concentration varies more than anywhere else).

From measurements to parameters

In this section we wonder how much information is actually present in our measurements in order to estimate the system parameters. During our discussion we will learn that there is a lower bound for the variance of each parameter. Additionally we will learn how to design the data collection strategy so as to maximize the amount of information.

Fisher's information is a way of measuring how much information there is in a random variable X with respect to a set of model parameters Θ. For the moment let us assume that X is a univariate random variable. It is defined in terms of the conditional probability function of X given Θ

$$I = E\left\{ \left(\frac{\partial \log f(x|\Theta)}{\partial \Theta} \right)^2 \middle| \Theta \right\} = \int \left(\frac{\frac{\partial f(x|\Theta)}{\partial \Theta}}{f(x|\Theta)} \right)^2 f(x|\Theta) dx \tag{3.453}$$

which we may understand in the following way. Let's say that we have an observed value x. This value brings a lot of information if x is in a region of low likelihood ($\frac{1}{f(x|\Theta)}$) and there is some variation of likelihood in that area (it does not bring much information in a very flat area close to 0; $\frac{\partial f(x|\Theta)}{\partial \Theta}$). Additionally, we do not care whether this variation is positive or negative, $(\cdot)^2$. It can be proven (Cover and Thomas, 2006) that we may also compute Fisher's

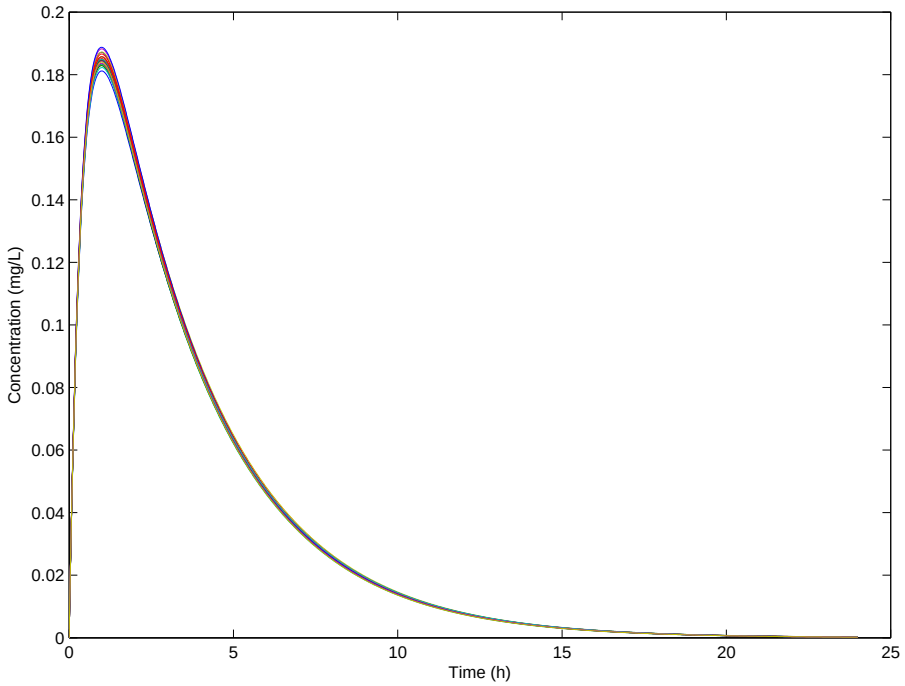

Figure 3.27: Several simulated curves varying the model parameters within a neighbourhood of their nominal values.

information as

$$I = Var\left\{\frac{\partial \log f(x|\Theta)}{\partial \Theta}\right\} = -E\left\{\frac{\partial^2 \log f(x|\Theta)}{\partial \Theta^2}\middle|\Theta\right\} \tag{3.454}$$

The function $\frac{\partial \log f(x|\Theta)}{\partial \Theta}$ receives the name of *score function*, that is, in its turn, another random variable.

Let us consider now n independent and identically distributed (i.i.d.) variables X_1, X_2, ..., X_n. Each one with a marginal distribution $f(x|\Theta)$. The Fisher's information of the whole dataset about Θ is n times the information of a single observation (Cover and Thomas, 2006):

$$I_n = nI \tag{3.455}$$

Crámer-Rao inequality states that the variance of any unbiased estimator of the model parameters $\hat{\Theta}$ based on the n observations has a variance such that

$$Var\{\hat{\Theta}\} \geq \frac{1}{I_n} \tag{3.456}$$

Consequently, Fisher's information has a very important meaning, it sets a lower bound to the variance of any estimate of the model parameter. No matter how "clever" we design our estimation procedure, there is always a lower bound on the variance of our estimate. In fact, an estimator that reaches this lower bound is said to be efficient (e.g., the mean estimate of the mean of a Gaussian distribution, $\hat{\mu} = \frac{1}{n}\sum_{i=1}^{n} x_i$ is an efficient estimator). Another interesting result is that as n goes to infinity, the asymptotic distribution of a Maximum Likelihood Estimate of the parameters Θ is distributed as

$$\hat{\Theta}_{MLE} \sim N\left(\Theta_{true}, \frac{1}{I_n}\right) \tag{3.457}$$

We can extend these results to multiple parameters. Fisher's information becomes a Fisher's information matrix defined as

$$I = E\left\{\frac{\partial \log f(x|\mathbf{\Theta})}{\partial \mathbf{\Theta}}\left(\frac{\partial \log f(x|\mathbf{\Theta})}{\partial \mathbf{\Theta}}\right)^T\middle|\mathbf{\Theta}\right\} \tag{3.458}$$

so that the ij-th element is

$$I_{ij} = E\left\{\frac{\partial^2 \log f(x|\Theta)}{\partial \Theta_i \partial \Theta_j}\middle|\mathbf{\Theta}\right\} \tag{3.459}$$

The Crámer-Rao inequality becomes

$$Var\{\hat{\Theta}_i\} \geq (I^{-1})_{ii} \tag{3.460}$$

that is the lower bound of the variance of each estimator is given by the diagonal of the inverse of the information matrix. And the asymptotic distribution of the maximum likelihood estimate becomes

$$\hat{\mathbf{\Theta}}_{MLE} \sim N\left(\mathbf{\Theta}_{true}, I_n\right) \tag{3.461}$$

With this asymptotic distribution we may estimate confidence intervals for $\boldsymbol{\Theta}_{MLE}$ as ellipsoids centered on $\hat{\boldsymbol{\Theta}}_{MLE}$. In fact, Fisher's information matrix can be interpreted as the expected Hessian of the log-likelihood function at $\hat{\boldsymbol{\Theta}}_{MLE}$ (Komorowski et al., 2011). Let us refer as λ_i to the eigenvalues of this matrix. Then, there exists a change of variable such that the log-likelihood around the true estimate can be calculated as

$$\log f(\hat{\boldsymbol{\Theta}}_{MLE} + \Delta\boldsymbol{\Theta}) \approx \log f(\hat{\boldsymbol{\Theta}}_{MLE}) - \frac{1}{2}\sum_{i=1}^{p} \lambda_i \Delta\Theta_i^2 \tag{3.462}$$

So we see that the higher the eigenvalues of I, the more robust our estimate is; or equivalently, the more information carry the observed samples on the system parameters. In fact, the number of non-zero eigenvalues of I is the number of system parameters that can be identified.

Based on Fisher's information matrix we may define sensitivity in a new way. Let us diagonalize this matrix as

$$I = PDP^{-1} \tag{3.463}$$

The new set of parameters $\boldsymbol{\Theta}' = P(\boldsymbol{\Theta} - \hat{\boldsymbol{\Theta}})$ has D as its Fisher's information matrix (Komorowski et al., 2011). We may regard

$$\mathcal{S}_{ij} = \lambda_i^{\frac{1}{2}} P_{ij} \tag{3.464}$$

as the contribution of parameter Θ_j to the information on Θ_i'. If we sum all the contributions for all i's we have a sensitivity value defined for the j-th parameter:

$$\mathcal{S}_j = \sum_{i=1}^{p} \mathcal{S}_{ij}^2 \tag{3.465}$$

This is a very important result because it defines a sensitivity for a system parameter not based on how the concentration change by varying the parameter, but based on the actual samples we have observed, and this sensitivity increases as we add more samples to our measurements.

Two interesting matrices in the context of pharmaceutical modeling are the Fisher's information matrices of the univariate Gaussian ($N(\mu, \sigma^2)$)

$$I = \begin{pmatrix} \frac{1}{\sigma^2} & 0 \\ 0 & \frac{1}{2\sigma^4} \end{pmatrix} \tag{3.466}$$

and the multivariate Gaussian whose mean depends on a set of parameters but its covariance does not $N(\boldsymbol{\mu}(\boldsymbol{\Theta}), \Sigma)$

$$I_{ij} = \left(\frac{\partial \boldsymbol{\mu}(\boldsymbol{\Theta})}{\partial \Theta_i}\right)^T \Sigma^{-1} \frac{\partial \boldsymbol{\mu}(\boldsymbol{\Theta})}{\partial \Theta_j} \tag{3.467}$$

This is the most common case encountered in Least Squares estimations. In this case the multivariate Gaussian is the presumed distribution of the residuals after performing the fitting. Let us assume that $\hat{\boldsymbol{\Theta}}$ is our estimate of the model parameters. The residual of the fitting for a particular point (t_i, C_i) is

$$\epsilon_i = C_i - C(t_i, \hat{\boldsymbol{\Theta}}) \tag{3.468}$$

Since we have single realization of this random variable, we estimate its mean as

$$\mu_i = C_i - C(t_i, \hat{\mathbf{\Theta}}) \tag{3.469}$$

and its variance as the variance of the fitting

$$\sigma_i^2 = \sigma_\epsilon^2 \tag{3.470}$$

Then we can rewrite Eq. (3.467)

$$\begin{aligned} I_{ij} &= \frac{1}{\sigma_\epsilon^2} \sum_{n=1}^{N} \frac{\partial (C_n - C(t_n, \hat{\mathbf{\Theta}}))}{\partial \Theta_i} \frac{\partial (C_n - C(t_n, \hat{\mathbf{\Theta}}))}{\partial \Theta_j} \\ &= \frac{1}{\sigma_\epsilon^2} \sum_{n=1}^{N} \frac{\partial C(t_n, \hat{\mathbf{\Theta}})}{\partial \Theta_i} \frac{\partial C(t_n, \hat{\mathbf{\Theta}})}{\partial \Theta_j} \end{aligned} \tag{3.471}$$

We need to calculate the terms $\frac{\partial C(t_n, \hat{\mathbf{\Theta}})}{\partial \Theta_i}$, but the dependence of the drug concentration on the system parameters is through the differential equation

$$\frac{dC}{dt} = f(t, C, \mathbf{\Theta}) \tag{3.472}$$

where for simplicity we have dropped the dependence of the drug concentration, C, on t and $\mathbf{\Theta}$, at any moment we may show any of the depedencies again to highlight them. If we now differentiate the left hand side of the equation above with respect to Θ_i we have

$$\frac{\partial}{\partial \Theta_i} \left(\frac{dC}{dt} \right) = \frac{d}{dt} \left(\frac{\partial C}{\partial \Theta_i} \right) \tag{3.473}$$

where we have assumed that $C(t)$ is a C^2 function (that is, it is continuous, its first derivative is continuous, and its second derivative is continuous) when we have interchanged the differentiation order (Clairaut's Theorem). Now, we differentiate the right hand side:

$$\frac{\partial}{\partial \Theta_i} \left(f(t, C, \mathbf{\Theta}) \right) = \frac{\partial f}{\partial C} \frac{\partial C}{\partial \Theta_i} + \frac{\partial f}{\partial \Theta_i} \tag{3.474}$$

Let us define

$$s_{\Theta_i}(t) = \frac{\partial C(t)}{\partial \Theta_i} \tag{3.475}$$

Then, the differentiation of Eq. (3.472) with respect to Θ_i can be written as

$$\frac{d s_{\Theta_i}(t)}{dt} = \frac{\partial f}{\partial C} s_{\Theta_i}(t) + \frac{\partial f}{\partial \Theta_i} \tag{3.476}$$

that is an Ordinary Differential Equation with the initial value $s_{\Theta_i}(t_0) = 0$. Summarizing, all we have to do is:

1. Perform the Least Squares fitting to find $\hat{\mathbf{\Theta}}$ and σ_ϵ^2.
2. Solve numerically the differential equation system

$$\frac{d s_{\Theta_i}(t)}{dt} = \frac{\partial f}{\partial C} s_{\Theta_i}(t) + \frac{\partial f}{\partial \Theta_i} \quad i = 1, 2, ..., p \tag{3.477}$$

presuming that the system is at rest before being excited ($C(t) = s_{\Theta_i}(t) = 0 \quad \forall t < 0, \forall i = 1, 2, ..., p$) particularizing the system parameters with those found during the Least Squares.

3. Calculate Fisher's information matrix as

$$I_{ij} = \frac{1}{\sigma_\epsilon^2} \sum_{n=1}^{N} s_{\Theta_i}(t_n) s_{\Theta_j}(t_n) \tag{3.478}$$

4. Calculate the number of identifiable parameters by analyzing the eigenvalues of I.

5. Calculate the sensitivity for each parameter as in Eq. (3.465).

Interestingly, using Fisher's information matrix we can even design where to perform the concentration measurements to maximize their information content about each one of the parameters. This can be done through the Generalized Sensitivity Function (Thomaseth and Cobelli, 1999; Batzel et al., 2006; Banks et al., 2009). It has been shown that we can reduce the variance associated to each system parameter by placing samples at the time intervals with large generalized sensitivity (Banks et al., 2007). The Generalized Sensitivity Analysis is defined at a given time t_{n_0} as

$$gs_{\Theta_i}(n_0) = \frac{1}{\sigma_\epsilon^2} \sum_{n=0}^{n_0} (I^{-1}\mathbf{s}_\Theta(t_n))_i s_{\Theta_i}(t_n) \tag{3.479}$$

where $\mathbf{s}_\Theta(t_n)$ is a vector with all the sensitivities computed in the previous analysis. It can be easily shown that $gs_{\Theta_i}(N) = 1$, that is, all generalized sensitivities go to 1 at the end of the observed period, this is called the "Forced-to-one" artifact (Banks et al., 2007) and one should be careful of not overinterpreting some high values close to the end of the analyzed period. Note that the generalized sensitivity can be computed recursively as

$$gs_{\Theta_i}(n_0) = gs_{\Theta_i}(n_0 - 1) + \frac{1}{\sigma_\epsilon^2}(I^{-1}\mathbf{s}_\Theta(t_{n_0}))_i s_{\Theta_i}(t_{n_0}) \tag{3.480}$$

Let us illustrate next how to apply all this theory to a specific case in PKPD modelling. Let us use the same example of the drug concentration in plasma following an oral dose. However, we now use the constitutive differential equations (see Section 3.3.3) instead of its closed-form solution:

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & -(K_a + K_d)A_g(t) + D_{po}(t) \\ V\frac{dC(t)}{dt} & = & -ClC(t) + K_a A_g(t) \end{array} \tag{3.481}$$

Fig. 3.28 shows the samples taken and the fitted model.

Let us formalize the previous equations as

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & f_{A_g}(t, A_g(t), C(t), D_{po}(t), \mathbf{\Theta}) \\ \frac{dC(t)}{dt} & = & f_C(t, A_g(t), C(t), D_{po}(t), \mathbf{\Theta}) \end{array} \tag{3.482}$$

Now we have to write the differential equations for the sensitivity. As we showed in Eq. (3.476), we may calculate the differential equation for the sensitivity of $A_g(t)$ to the i-th parameter, $s_{\Theta_i}^{A_g}$ as

$$\frac{ds_{\Theta_i}^{A_g}}{dt} = \frac{\partial f_{A_g}}{\partial A_g} s_{\Theta_i}^{A_g} + \frac{\partial f_{A_g}}{\partial C} s_{\Theta_i}^{C} + \frac{\partial f_{A_g}}{\partial D_{po}} s_{\Theta_i}^{D_{po}} + \frac{\partial f_{A_g}}{\partial \Theta_i} \tag{3.483}$$

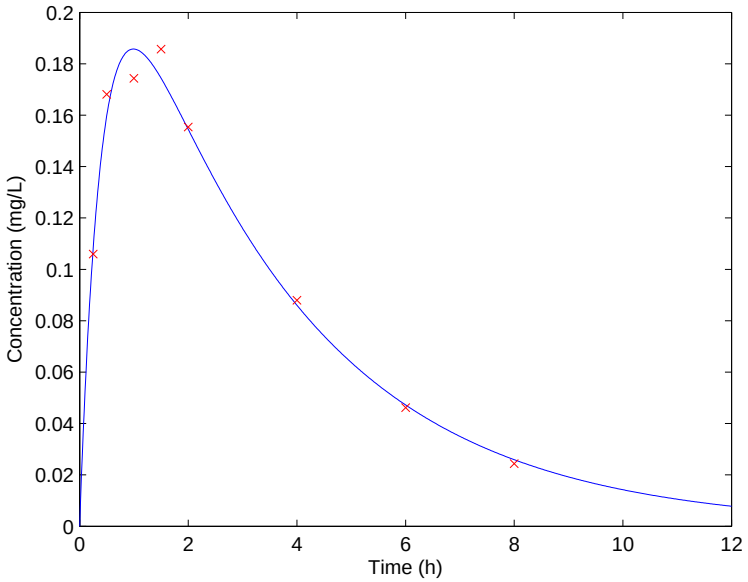

Figure 3.28: Drug concentration over time (samples and fitted model).

Similarly for $C(t)$. In this particular case it gives

$$\begin{array}{rcl}
\frac{ds^{A_g}_{K_a}(t)}{dt} & = & -K_a s^{A_g}_{K_a}(t) - A_g(t) \\
\frac{ds^{A_g}_{K_d}(t)}{dt} & = & -K_d s^{A_g}_{K_d}(t) - A_g(t) \\
\frac{ds^{C}_{K_a}(t)}{dt} & = & \frac{K_a}{V} s^{A_g}_{K_a}(t) - \frac{Cl}{V} s^{C}_{K_a}(t) + \frac{1}{V} A_g(t) \\
\frac{ds^{C}_{K_d}(t)}{dt} & = & \frac{K_a}{V} s^{A_g}_{K_d}(t) - \frac{Cl}{V} s^{C}_{K_d}(t) \\
\frac{ds^{C}_{Cl}(t)}{dt} & = & -\frac{Cl}{V} s^{A_g}_{Cl}(t) - \frac{1}{V} C(t) \\
\frac{ds^{C}_{V}(t)}{dt} & = & -\frac{Cl}{V} s^{C}_{V}(t) + \frac{Cl}{V^2} C(t) - \frac{K_a}{V^2} A_g(t)
\end{array} \tag{3.484}$$

Figures 3.29 and 3.30 show the sensitivity of $A_g(t)$ and $C(t)$ to all parameters. Note that the results in Fig. 3.30 are slightly different to those in Fig. 3.24 because the ones in Fig. 3.24 did not take into account the interplay between the sensitivitis of $A_g(t)$ and $C(t)$.

At this point we can calculate Fisher's information matrix, we will do it only for the drug concentration (the order is K_a, K_d, Cl, V).

$$I = 10^6 \begin{pmatrix} 9.1813 & -2.3869 & -0.3270 & -0.0108 \\ -2.3869 & 1.5674 & 0.4615 & 0.0033 \\ -0.3270 & 0.4615 & 0.1895 & 0.0005 \\ -0.0108 & 0.0033 & 0.0005 & 0.0000 \end{pmatrix} \tag{3.485}$$

Its eigenvalues are $9.89 \cdot 10^6$, $1.03 \cdot 10^6$, $2.41 \cdot 10^4$, $2.34 \cdot 10^{-2}$. It does not have any null eigenvalue but it is very ill-conditioned (the ratio between the largest and the smallest eigenvalues is very large). It would seem that there is a parameter

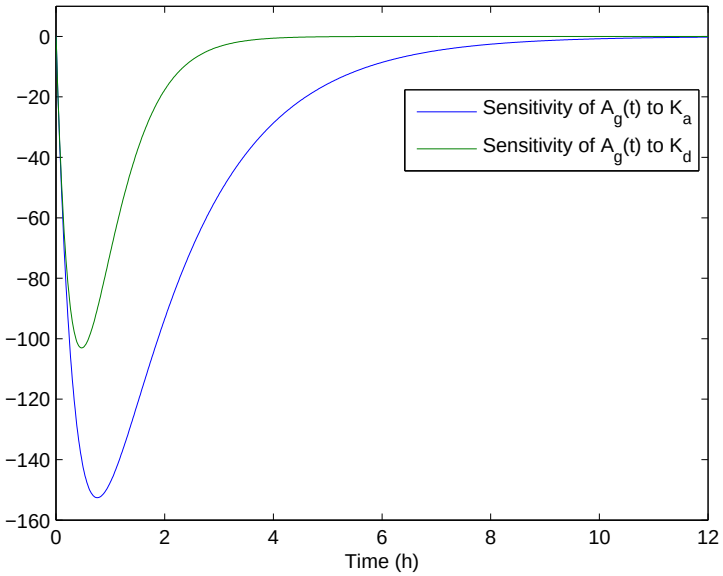

Figure 3.29: Sensitivity of $A_g(t)$ to its parameters.

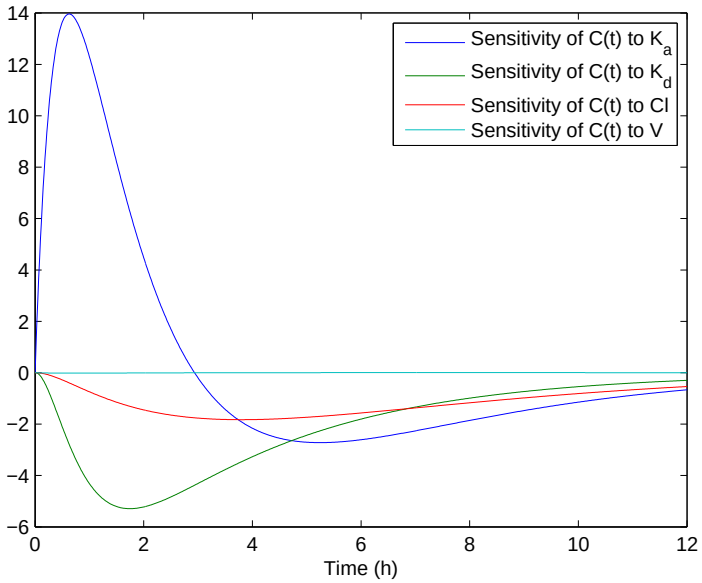

Figure 3.30: Sensitivity of $C(t)$ to its parameters.

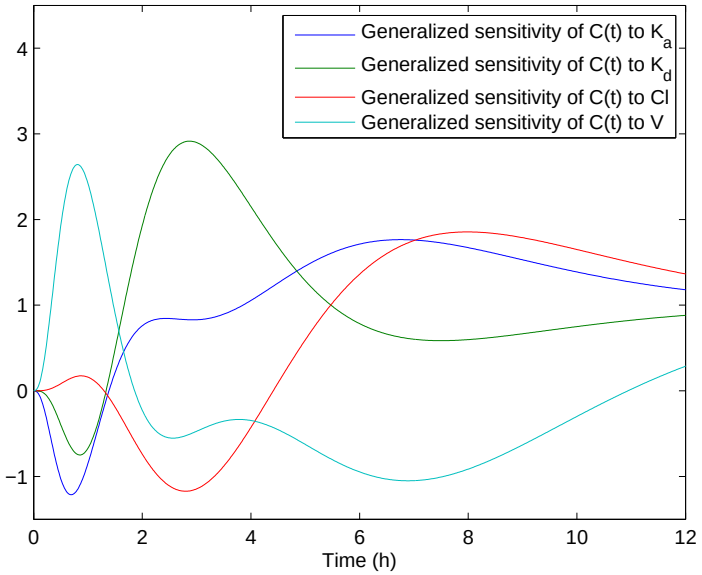

Figure 3.31: Generalized sensitivity of $C(t)$ to its parameters.

(V) that cannot be identified. However, this is not correct due to the very different ranges of the parameters. Let us analyze the Crámer-Rao lower bound for each one of the estimates. For doing so, we calculate the inverse of the matrix above and keep its diagonal. In the following table we report the lower bound as well as its coefficient of variation (standard deviation divided by the nominal value of the parameter)

Parameter	**Variance lower bound**	**Coefficient of variation** (%)
K_a	$1.95 \cdot 10^{-5}$	44.20
K_d	$3.77 \cdot 10^{-5}$	20.46
Cl	$9.00 \cdot 10^{-5}$	18.97
V	22.62	47.56

Finally, we calculate the generalized sensitivity functions to learn where we can put more samples so that they are maximally informative. The idea is to place samples at time points where the slope of the generalized sensitivity is positive and high. Fig. 3.31 shows the generalized sensitivity of $C(t)$ to the different parameters.

Chapter 4

Results

In this chapter we present a number of applications of the methodology developed along the thesis. These are only examples of its power and its applications are not restricted to the examples given in this chapter. Most of the examples are taken from the pharmaceutical literature which, by its traditional approach, are normally rather limited in the complexity they can afford.

4.1 Pharmacokinetics

Griseofulvin is an antifungal drug used to treat fungal infections of the skin. It is produced by the mold *Penicillium griseofulvum* and is administered orally. It binds to keratin in keratin precursor cells of the host and when his hair or skin is replaced by the keratin-griseofulvin complex it is transferred to the infectious fungus. Then, it binds to the microtubule of the fungus cells and interferes with mitosis. A human subject was given 100mg of griseofulvin intravenously and 500mg orally. Table 4.1 shows the plasma concentration values at different times after administration (Rowland et al., 1968). We are interested in the system parameters governing the kinetics of this drug.

Let us follow the methodology developed in this thesis. First, we write the differential equations governing the system. Let us assume a two-compartments model (see Sections 3.3.3 and 3.3.4).

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & -K_a A_g(t) + D_{po}(t) \\ V\frac{dC(t)}{dt} & = & K_a f A_g(t) - ClC(t) - Cl_p(C(t) - C_p(t)) + D_{iv}(t) \\ V_p\frac{dC_p(t)}{dt} & = & Cl_p(C(t) - C_p(t)) \end{array} \tag{4.1}$$

In this model we have integrated both kinds of doses, extravascular and intravascular. This is a feature that cannot be done with the traditional pharmacokinetics approach. The experiment with an intravascular dose (Experiment 1) can be modelled with the double input

$$\begin{array}{rcl} D_{po,1}(t) & = & 0 \\ D_{iv,1}(t) & = & 100\delta(t) \end{array} \tag{4.2}$$

While the experiment with an oral dose (Experiment 2) can be modelled with

Time (h)	Conc. (mg/L) i.v.	Conc. (mg/L) p.o.
0	0	0
1	1.4	0.40
2	1.1	0.95
3	0.98	1.15
4	0.90	1.15
5	0.80	1.05
7	-	1.20
8	0.68	1.20
12	0.55	0.90
24	0.37	1.05
28	-	0.90
32	0.24	0.85
35	-	0.80
48	0.14	0.50

Table 4.1: Concentration in plasma of griseofulvin after an intravenous bolus or an oral dose.

the double input

$$\begin{array}{rcl} D_{po,2}(t) & = & 500\delta(t) \\ D_{iv,2}(t) & = & 0 \end{array} \tag{4.3}$$

We now discretize the differential equations (first, the homogeneous equation and then we add the inhomogeneous term)

$$\begin{array}{rcl} \frac{A_g[n]-A_g[n-1]}{T_s} & = & -K_a A_g[n-1] + D_{po}[n] \\ V\frac{C[n]-C[n-1]}{\Delta t} & = & K_a f A_g[n-1] - ClC[n-1] - Cl_p(C[n]-C_p[n-1]) + D_{iv}[n] \\ V_p\frac{C_p[n]-C_p[n-1]}{\Delta t} & = & Cl_p(C[n-1]-C_p[n-1]) \end{array} \tag{4.4}$$

and reorganize the terms to produce a useful recursion

$$\begin{array}{rcl} A_g[n] & = & (1-K_a\Delta t)A_g[n-1] + D_{po}[n] \\ C[n] & = & \left(1-\frac{Cl\Delta t}{V}\right)C[n-1] + \frac{K_a\Delta t}{V}A_g[n-1] - \frac{Cl_p\Delta t}{V}(C[n-1]-C_p[n-1]) + \\ & & \frac{1}{V}D_{iv}[n] \\ C_p[n] & = & C_p[n-1] + \frac{Cl_p\Delta t}{V_p}(C[n-1]-C_p[n-1]) \end{array} \tag{4.5}$$

Interestingly, we can use both experiments at the same time to estimate all model parameters K_a (absorption constant), f (bioavailability), V (distribution volume in the central compartment), Cl (clearance from the first compartment), V_p (distribution volume in the second compartment), Cl_p (transfer constant between the first and second compartments). This is so because in our setting the optimizer proposes a set of values for the three parameters and checks which is the likelihood of these parameters. Then, using this information proposes a new set of values till the maximum likelihood parameters are found. Note that this is not possible in the traditional pharmacokinetics approach, each experiment is treated separately and a consensus set of parameters is produced from the different parameter estimates. If we fit the parameters to the data

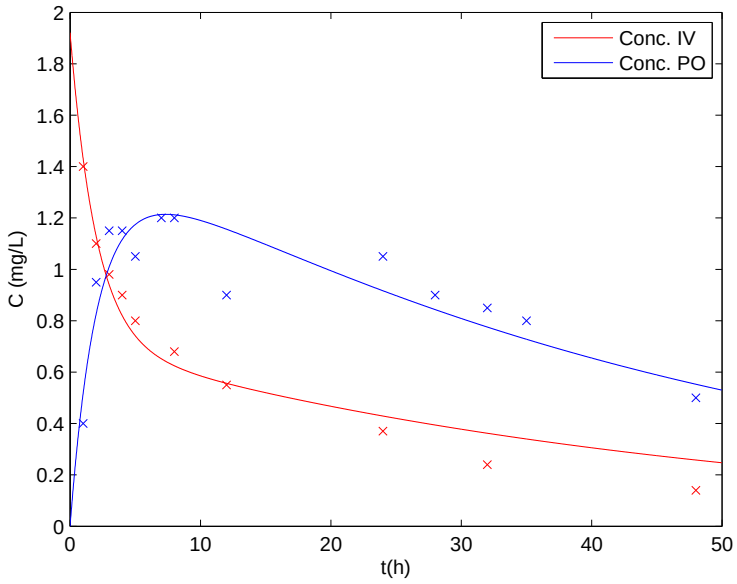

Figure 4.1: Concentration measurements and corresponding fitted values for intravenous (IV) and extravascular (PO) administration.

we get $K_a = 0.18\text{h}^{-1}$, $f = 37.82\%$, $V = 52.06\text{L}$, $Cl = 2.77\text{L/h}$, $V_p = 70.21\text{L}$, $Cl_p = 13.90\text{L/h}$ (see Fig. 4.1).

4.2 Clinical pharmacokinetics

Vancomycin is a glycopeptide antibiotic rather effective for Gram-positive bacteria that can be isolated from the bacteria *Amycolatopsis orientalis*. It is normally used in patients with bacterial infections resistant to other antibiotics. It acts by inhibiting cell wall synthesis by: 1) preventing the polymerization of the N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) in the backbone of the cell wall; and 2) preventing cross-linking of the cell wall polymers (Cho et al., 2007). Since it is a large molecule (its molecular weight is 1448 g/mol) it used to be non-dyalizable. Boehler et al. (1992) studied the dialyzability of vancomycin with high-flux, high molecular weight cutoff membranes. A patient was infused 1g of vancomycin during 1 hour and then was hemodialyzed on days 3, 5 and 7 (periods of hemodialysis: 66-70h, 110-114h, 158-162h). Table 4.2 shows the plasma concentration of vancomycin for a number of time points.

We will develop a three-compartments model (central connected to peripheral 1, and peripheral 1 connected to peripheral 2). This problem is interesting because clearance from the central compartment has two different constants (one when the patient is not being hemodialyzed and another during hemodialysis). Let us define a function, $dial(t)$ that takes the value 1 during hemodialysis and 0 otherwise. If t is expressed in hours, this function can be analytically be

Time (min)	Vancomycin concentration (mg/L)
1	31.3
4	22.0
6	18.7
24	12.5
66	10.0
66.5	8.65
67.5	8.02
70	6.40
71	8.01
73	8.50
76	8.74
110	7.33
114	5.22
120	6.72
158	5.64
162	3.74
167	4.75

Table 4.2: Concentration in plasma of vancomycin after an intravenous infusion with 3 hemodialysis periods.

written as

$$dial(t) = (u(t-66)-u(t-70))+(u(t-110)-u(t-114))+(u(t-158)-u(t-162)) \tag{4.6}$$

With this function we may setup the following continuous system

$$\begin{aligned} V\frac{dC(t)}{dt} &= -(Cl + Cl_{dial}dial(t))C(t) - Cl_{p1}(C(t) - C_{p1}(t)) + D_{iv}(t) \\ V_{p1}\frac{dC_{p1}(t)}{dt} &= Cl_{p1}(C(t) - C_{p1}(t)) - Cl_{p2}(C_{p1}(t) - C_{p2}(t)) \\ V_{p2}\frac{dC_{p2}(t)}{dt} &= Cl_{p2}(C_{p1}(t) - C_{p2}(t)) \end{aligned} \tag{4.7}$$

With this time varying clearance, it is impossible to get a closed-form solution for the concentration in the main compartment.

The discretization of this system yields the following recursion

$$\begin{aligned} C[n] &= C[n-1] - \frac{(Cl+Cl_{dial}dial[n-1])\Delta t}{V}C[n-1] - \frac{Cl_{p1}\Delta t}{V}(C[n-1] - C_{p1}[n-1]) + \\ &\quad \frac{1}{V}D_{iv}[n-1] \\ C_{p1}[n] &= C_{p1}[n-1] + \frac{Cl_{p1}\Delta t}{V_{p1}}(C[n-1] - C_{p1}[n-1]) - \frac{Cl_{p2}\Delta t}{V_{p1}}(C_{p1}[n-1] - C_{p2}[n-1]) \\ C_{p2}[n] &= C_{p2}[n-1] + \frac{Cl_{p2}\Delta t}{V_{p2}}(C_{p1}[n-1] - C_{p2}[n-1]) \end{aligned} \tag{4.8}$$

After fitting the parameters we get $V = 29.55$L, $Cl = 0.2501$L/h, $Cl_{dial} = 2.8774$L/h, $Cl_{p1} = 4.3180$L/h, $V_{p1} = 35.29$L, $Cl_{p2} = 0.6245$L/h, $V_{p2} = 13.08$L. See Fig. 4.2 to see the fitting.

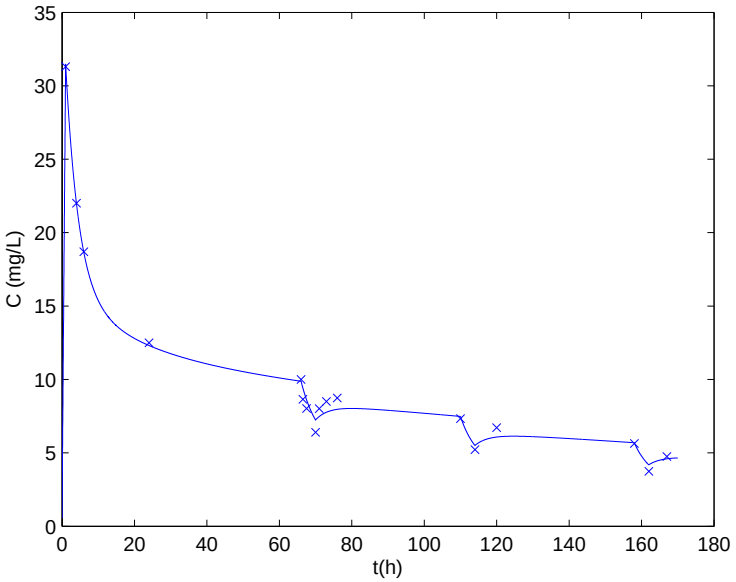

Figure 4.2: Concentration measurements and corresponding fitted values in a problem with hemodialysis.

4.3 Toxicokinetics

Monomethyl Hydrazine (MMH) is a toxic, volatile hydrazine believed to be the main responsible of the toxicity of the fungus *Gyromitra esculenta*. It is also used as rocket propellant and the National Institute of Occupational Safety and Health (NIOSH) has specific recommendations and maximum limits for exposure. In particular, it forbids to be exposed to a concentration higher than $8{\cdot}10^{-5}$ mg/L for more than 2 hours or spills on the skin with a concentration higher than $3.5{\cdot}10^{-4}$ mg/L. MMH is also thought to be carcinogen.

Six groups of pregnant rats were infused MMH with a constant infusion rate of values 0, 0.1, 0.2, 0.3 and 0.6 mg/L. They were infused from day 6 to 13 of gestation, and the offspring were evaluated at day 21. The survival rate of each group is shown in the Table 4.3.

Using this data we may model survival rate as a sigmoid function (see Section 3.5.2)

$$SurvivalRate = S_0 \left(1 - \frac{C_{MMH}^{\alpha}}{LD_{50}^{\alpha} + C_{MMH}^{\alpha}}\right) \tag{4.9}$$

Fitting the data results in $S_0 = 82.25\%$, $\alpha = 6.692$, and $LD_{50} = 0.3489$ mg/L (the fitting has an adjusted coefficient of determination of 0.9927). LD_{50} is the dose at which 50% of the rat offsprings die because of MMH. We may extrapolate this value to the case of humans. For doing so, we would need to calculate (see Section 3.3.7)

$$LD_{50}^{man} = LD_{50}^{rat} \left(\frac{BW_{man}}{BW_{rat}}\right)^{b} \tag{4.10}$$

MMH concentration (mg/L)	Survival rate (%)
0	86.4
0.072	80.0
0.21	77.3
0.31	57.1
0.5	7.00
0.6	0.0

Table 4.3: Survival rate of the offspring of pregnant rats with different levels of MMH.

Time (min)	MMH concentration (mg/L)
5	2.71
30	2.9
60	4.08
90	3.75
120	3.4
150	2.21
180	2.24
210	1.52
240	1.38
270	0.96
300	0.95
330	0.88
360	0.66
390	0.64
420	0.42
450	0.38
480	0.305

Table 4.4: Concentration of MMH in plasma after a fast infusion.

Although we do not have access to any allometric study to correctly estimate b, assuming it is $b = 1$, we would have

$$LD_{50}^{man} = 0.3489\frac{70}{0.25} = 97.7mg/L \tag{4.11}$$

We see that the NIOSH limit is at least 5 orders of magnitude below.

In order to characterize kinetically MMH, 12 rats were infused during 3 minutes a total of 2.19 mg of MMH. 3 or 4 plasma samples were extracted from each rat and the values were averaged to produce the plasma concentration shown in Table 4.4 (each sample is the average of 3 or 4 animals).

For this compound it is known that part of the MMH infused is sequestered in the stomach and then released back into the blood stream at a different rate. Let us call f the fraction of the infused dosis that is sequestered. We may describe the system calculating the MMH concentration in blood, C, and the

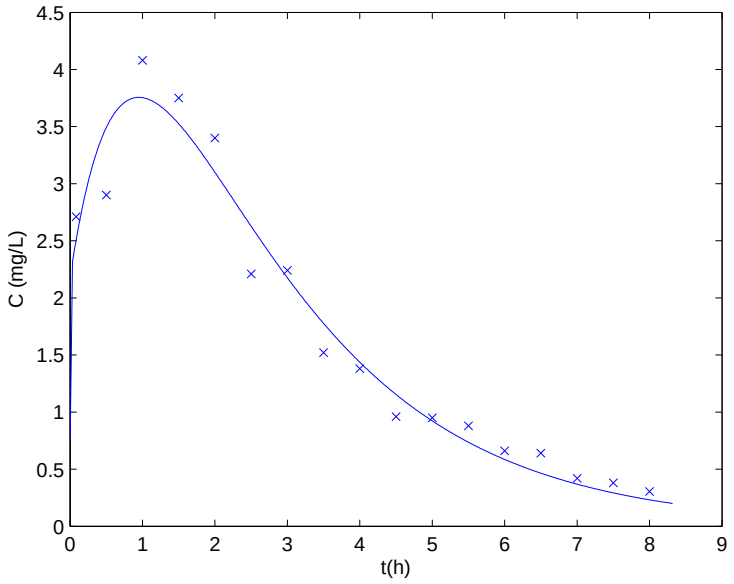

Figure 4.3: Concentration measurements and corresponding fitted values of the MMH concentration in plasma.

sequestered amount A_s using the following differential equations:

$$\begin{array}{rcl} V\frac{dC(t)}{dt} & = & -ClC(t) + K_a A_s(t) + (1-f)D_{iv}(t) \\ \frac{dA_s(t)}{dt} & = & -K_a A_s(t) + fD_{iv}(t) \end{array} \tag{4.12}$$

Note that the methodology developed in this thesis is capable of splitting the input dose in two different fractions undergoing different physiological processes. The corresponding discrete system is given by

$$\begin{array}{rcl} C[n] & = & C[n-1] - \frac{Cl\Delta t}{V}C[n-1] + \frac{K_a\Delta t}{V}A_s[n-1] + \frac{1}{V}(1-f)D_{iv}[n-1] \\ A_s[n] & = & A_s[n-1] - K_a\Delta t A_s[n-1] + fD_{iv}[n-1] \end{array} \tag{4.13}$$

The fitted parameters are $V = 0.326$L, $Cl = 0.15$L/h, $f = 66.46\%$, $K_a = 1.15\text{h}^{-1}$ (see Fig. 4.3).

4.4 Pharmacodynamics

Cortisol is the main steroid hormone secreted by the adrenal cortex in mammalians. Adrenocoricotropic hormone (ACTH) is presumed to stimulate the production of cortisol. However, cortisol secretion is moderated by an endogenous moderator M. Urquhart and Li (1969) performed some experiments to unveil the interplay between cortisol production, ACTH stimulation and the endogeneous moderator. For doing so, they infused ACTH at a constant rate of 1μU/(mL.min) for 1 hour, then 2 μU/(mL.min) during the second hour, and

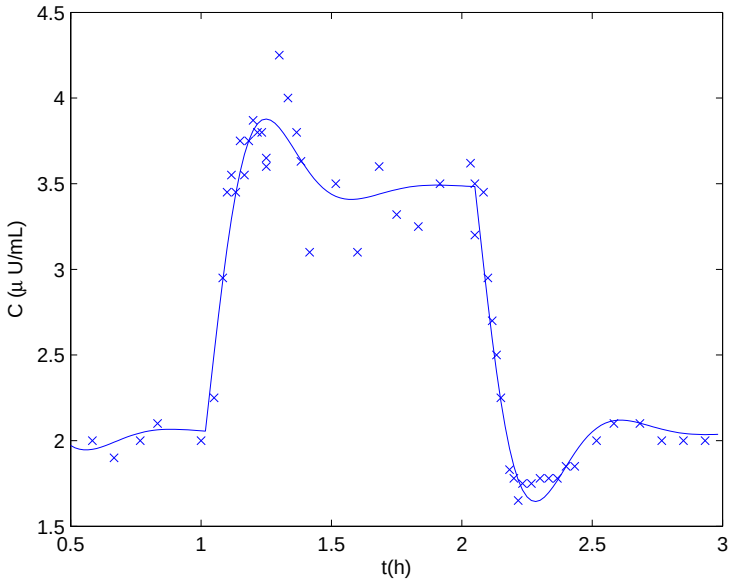

Figure 4.4: Concentration measurements and corresponding fitted values for cortisol under ACTH stimulation.

back to 1 μU/(mL.min) during the third hour. They measured the cortisol concentration at different time points (see Table 4.5)

The time plot of this concentration clearly shows a rebound when the infusion rate is changed. This can be successfully modelled by a negative feedback loop between cortisol and its moderator (see Section 3.5.6). The cortisol and its moderator are supposed to interact according to the following model

$$\begin{array}{rcl} \frac{dC_{cortisol}(t)}{dt} & = & K_{0C}D^N_{ACTH}(t) - K_{1C}C_{moderator}(t) \\ \frac{dC_{moderator}(t)}{dt} & = & K_{0M}C_{cortisol}(t) - K_{1M}C_{moderator}(t) \end{array} \tag{4.14}$$

where $D_{ACTH}(t)$ is the instantaneous ACTH dose. Discretizing the equation and reorganizing the terms, we arrive at

$$\begin{array}{rcl} C_{cortisol}[n] & = & C_{cortisol}[n-1] + K_{0C}\Delta t D^N_{ACTH}[n-1] - K_{1C}\Delta t C_{moderator}[n-1] \\ C_{moderator}[n] & = & C_{moderator}[n-1] + K_{0M}\Delta t C_{cortisol}[n-1] - K_{1M}\Delta t C_{moderator}[n-1] \end{array} \tag{4.15}$$

Figure 4.4 shows the fitted curve. The parameters found are $K_{0C} = 19.55\text{h}^{-1}$, $K_{1C} = 9.88\text{h}^{-1}$, $K_{0M} = 11.11\text{h}^{-1}$, $K_{1M} = 11.50\text{h}^{-1}$, $N = 0.7644$.

4.5 Biopharmaceutics

Tolazamide is an oral drug to lower blood glucose level in Diabetes Type II patients. Its mechanism of action has not yet been clearly established and it belongs to the family of sulfonylurea hypoglycemic drugs. Welling et al. (1982)

Time (min)	Cortisol concentration (μU/mL)
35	2
40	1.9
46	2
50	2.1
60	2
63	2.25
65	2.95
66	3.45
67	3.55
68	3.45
69	3.75
70	3.55
71	3.75
72	3.87
73	3.8
74	3.8
75	3.6
75	3.65
78	4.25
80	4
82	3.8
83	3.63
85	3.1
91	3.5
96	3.1
101	3.6
105	3.32
110	3.25
115	3.5
122	3.62
123	3.5
123	3.2
125	3.45
126	2.95
127	2.7
128	2.5
129	2.25
131	1.83
132	1.78
133	1.65
134	1.75
136	1.75
138	1.78
140	1.78
142	1.78
144	1.85
146	1.85
151	2
155	2.1
161	2.1
166	2
171	2
176	2

Table 4.5: Concentration in plasma of cortisol during a continuous infusion of ACTH.

Time (min)	Tolazamide concentration (mg/L)
0	1.3
1	2.8
2	4.4
3	5.7
4	6.6
5	6.6
6	6.8
8	6.6
12	5.5
16	4.6
24	3.1

Table 4.6: Concentration in plasma of tolazamide after administration of a tolazamide formulation.

studied four different formulations and how it was absorbed using Wagner-Nelson's method. For one of the formulations they found the tolazamide concentration in blood shown in Table 4.6.

Instead of using Wagner-Nelson method, which does not model the absorption process itself, but only the observed amount of absorbed drug, we will use the folllowing description of the continuous system (see Section 3.6.3):

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & -K_a A_g(t) \\ V\frac{dC(t)}{dt} & = & K_a A_g(t) - ClC(t) \end{array} \tag{4.16}$$

whose discretization gives the recursion

$$\begin{array}{rcl} A_g[n] & = & A_g[n-1](1-K_a\Delta t) \\ C[n] & = & C[n-1] - \frac{Cl\Delta t}{V}C[n-1] + K_a\Delta t A_g[n-1] \end{array} \tag{4.17}$$

Note that we do not know the dosis of the formulation (the data source simply does not state it, Shargel et al. (2012)[pp. 447]). However, we can estimate it with our model. The fitted values are $K_a = 0.3640\text{h}^{-1}$, $V = 23.45\text{L}$,$Cl = 1.26\text{L/h}$,$A_0 = 220.9\text{mg}$ (Tolinase is a commercial brand of Tolazamide, and it has a recommended dosage of 250mg/day). Fig. 4.5 shows the measured points as well as the fitted curve.

4.6 Modelling pitfalls

One may incur in many modelling pitfalls unadvertedly. Modelling programs are not fullproof and do not report of all possible situations. It is the insight of the researcher and her posterior interpretation what must provide an overall sense to the numerical results. In the following sections we illustrate some possible pitfalls. They do not cover all possibilities and are shown only as a caution warning on the difficulties of modelling.

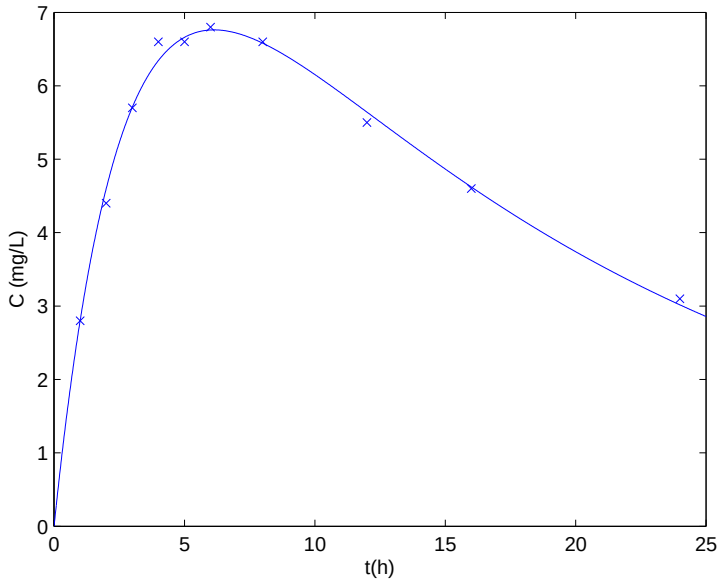

Figure 4.5: Concentration measurements and corresponding fitted values corresponding to the absorption of a tolazamide formulation.

4.6.1 Low-power or underdetermined models

Warfarin is an anticoagulant used to prevent thrombosis and thromboembolism. It acts by inhibiting the synthesis of clotting factors II, VII, IX and X, as well as the regulatory factors protein C, S and Z (Ansell et al., 2008). Warfarin prevents the carboxylation of the glutamic acid residues of their precursors that allow these factors to bind to the phospholipid surface of the vascular endothelium. The enzyme that performs this carboxylation (gamma-glutamil carboxylase) requires oxidizing at the same time the reduced form of vitamin K. The oxidized form of vitamin K is then reduced back by the vitamin K epoxide reductase. Warfarin inhibits this enzyme and, as a consequence, the clotting factors stay in their precursor form. Warfarin effect is measured through the Prothrombin Complex Activity (PCA) measured in seconds(Pitsiu et al., 1993). A single intravenous bolus of 5 mg of warfarin was administered to a person. Table 4.7 shows the PCA at different measurement times.

Let us presume a model for warfarin with a single compartment.

$$V\frac{dC(t)}{dt} = -ClC(t) + D_{iv}(t) \tag{4.18}$$

We may model the inhibitory effect of warfarin as a function of its concentration

$$I(C(t)) = \frac{1}{1 + \left(\frac{C(t)}{IC_{50}}\right)^n} \tag{4.19}$$

Since warfarin is acting on the reduction-oxidation cycle of vitamin K, let us call C_{red} the concentration of vitamin K in its reduced form, and C_{oxi} the

Time (h)	PCA (s)
0	124.40
12	92.00
24	56.77
36	40.01
48	40.78
60	42.98
72	53.00
96	77.49
120	99.22
144	100.80

Table 4.7: Prothrombin Complex Activity after a single intravenous bolus of warfarin.

concentration of vitamin K in its oxidized form.

$$\begin{array}{rcl} \frac{dC_{red}(t)}{dt} & = & k_{1,oxi}I(C(t))C_{oxi}(t) - k_{1,red}C_{red}(t) \\ \frac{dC_{oxi}(t)}{dt} & = & k_{1,red}C_{red}(t) - k_{1,oxi}I(C(t))C_{oxi}(t) \end{array} \tag{4.20}$$

But in fact, these two equations are redundant because

$$C_{red}(t) + C_{oxi}(t) = C_K \tag{4.21}$$

where C_K is the total concentration of vitamin K. In this way we may simplify the previous equation system to

$$\frac{dC_{red}(t)}{dt} = k_{1,oxi}I(C(t))(C_K - C_{red}(t)) - k_{1,red}C_{red}(t) \tag{4.22}$$

Finally, we can relate the PCA to the concentration of reduced vitamin K:

$$PCA(t) = AC_{red}(t) + B \tag{4.23}$$

Although this line of reasoning is correct and with a physiological background we only have 10 measurements to determine 9 parameters (V, Cl, IC_{50}, n, C_K, $k_{1,oxi}$, $k_{1,red}$, A and B). The problem is severely ill-posed (it has low-power in Statistical language). If we had more parameters than measurements, the problem would be undetermined. In multiple regression it is normally recommended to have a sample size in the order of $50 + 8p$ (Newton and Rudestam, 1999)[pp.250], being p the number of parameters to estimate. This number of measurements is seldom encountered in many pharmaceutical studies. A thorough design of the number of samples needed for regression must be based on a power design (Faul et al., 2009). In fact, we can indirectly evaluate the power of the model through the Adjusted Coefficient of Determination (see Section 3.3.2) (Newton and Rudestam, 1999)[pp.251]. When the number of parameters approaches the number of samples, the model loses its generalization power (most likely, this model is overfitting the observed data and it does not explain the underlying physiology).

4.6.2 Incorrect modelling

The case study shown in the previous section was addressed in Gabrielsson and Weiner (2007)[pp. 925]. In their analysis, they identify that warfarin acts by

Time (h)	Plasma concentration (mg/L)
0.25	91
0.5	120.6
1	100.4
2	128
3	81.5
4	51.6
6	25.6

Table 4.8: Plasma concentration of felodipine after an oral dose of 10 mg of the drug.

inhibiting a precursor molecule according to the differential equation

$$\frac{dC_{pre}(t)}{dt} = k_{0,pre}I(C(t)) - k_{1,pre}C_{pre}(t) \tag{4.24}$$

where $I(C(t))$ is the same as in Eq. (4.19). Then, the PCA response is modelled as

$$\frac{dPCA(t)}{dt} = k_{0,PCA}C_{pre}(t) - k_{1,PCA}PCA(t) \tag{4.25}$$

In steady state there is no variation with respect to time, and if we presume there is no medication, we can write

$$\begin{aligned} k_{0,pre} = k_{1,pre}C_{pre}^{(0)} &\Rightarrow C_{pre}^{(0)} = \frac{k_{0,pre}}{k_{1,pre}} \\ k_{0,PCA}C_{pre}^{(0)} = k_{1,PCA}PCA^{(0)} &\Rightarrow PCA^{(0)} = C_{pre}^{(0)}\frac{k_{0,PCA}}{k_{1,PCA}} \end{aligned} \tag{4.26}$$

Then, the model is simplified by setting $k_{0,PCA} = k_{1,PCA}$ arguing that in their fitting there is a unitary correlation between the two. It is important to note that they can only be equal numerically, because their units are different ($k_{0,PCA}$ has units of L/mg, while $k_{1,PCA}$ has units of s^{-1}); and also that a unitary correlation implies a linear relationship between the two and not that they are equal. Additionally, if these two constants are equal, then

$$PCA^{(0)} = C_{pre}^{(0)} \tag{4.27}$$

which only makes sense because PCA is the measure of the complex activity (in seconds) and C_{pre} is the concentration of the precursor (in mg/L). In fact, the modelling error comes because in the WinNonLin code used for fitting the model $k_{0,PCA}$ was missing. However, the last equation $PCA^{(0)} = C_{pre}^{(0)}$ should have raised a warning flag in the interpretation of the results.

4.6.3 Presence of outliers

Felodipine is an antagonist of calcium in calcium channels. It is used to reduce hypertension because it relaxes blood vessels. An experiment was set up in rats to determine its absorption and elimination (data kindly provided by Dr. Pérez de la Cruz Moreno) after administering 10mg of felodipine. Table 4.8 shows the drug concentration in plasma at a number of time points for a single rat.

At this dose we do not expect to see any nonlinear effect. The accepted model is made of linear absorption and elimination processes (the same model

as in Section 4.5):

$$\begin{array}{rcl} \frac{dA_g(t)}{dt} & = & -K_a A_g(t) \\ V\frac{dC(t)}{dt} & = & K_a A_g(t) - ClC(t) \end{array} \tag{4.28}$$

For its discretization see Section 4.5.

The fitting of this data with the model above gives $V = 5.15$L, $Cl = 16.84$L/h, $K_a = 0.264\text{h}^{-1}$. The coefficient of determination is $R^2 = 0.8913$ and the adjusted coefficient of determination $R^2_{adj} = 0.6739$, which is not too good. We suspected that there were outliers in the data. However, with so little time points, it was difficult to identify which ones. The bootstrap method presented in Section 3.3.2 helped us to identify the outliers with so little data. We took 100 bootstrap samples of the original data, and for each sample we fitted the model above. Then, we measured the residuals of each one of the observations and averaged them in absolute value. After computing the mean, we see that the measurements at 1h and 2h have residuals much larger than the rest of time points. If we remove the two outliers and refit the data, we get $V = 7.23L$. $Cl = 18.34$L/h, $K_a = 0.349\text{h}^{-1}$. The coefficient of determination is $R^2 = 0.9934$ and the adjusted coefficient of determination $R^2_{adj} = 0.9803$, confirming our suspicion that these two measurements did not fit with the proposed model. See Fig. 4.6 for the fitting of both models.

However, outliers may also point out an incorrect pharmaceutical modelling instead of an error in the measurements. How to decide whether the model is correct (and the measurements are not) or incorrect (and the measurements are good) requires the revision of the literature related to the drug as well as the whole measurement process.

4.6.4 Incomplete studies

The study in the previous section was aimed at identifying pharmacokinetics differences between taking felodipine with food and without food. For doing so, two rats were given felodipine along with food and two other rats after fasting. The drug concentration in plasma was measured at the same time points as in the previous case. Following the same bootstrapping methodology described in the previous paragraph we were able to compute the covariance matrix of the model parameters:

$$\begin{array}{ccc} 1.0000 & 0.5174 & 0.9910 \\ 0.5174 & 1.0000 & 0.5743 \\ 0.9910 & 0.5743 & 1.0000 \end{array}$$

A naive analysis of this covariance matrix would indicate that there are two parameters that are highly redundant (parameters 1 and 3; V and K_a) so that one of them could be eliminated from the model. However, using the same methodology we can compute the histograms of each one of the parameters (see Fig. 4.7). The allegedly redundant parameters have bimodal distribution due to the fact that we have analyzed two animals, and the high correlation in the covariance matrix is indicating that the two parameters are highly dependent of the animal being analyzed. We draw, thus, the consequence that more animals should have been studied if we wanted to analyze the differences between fasting and not fasting when taking felodipine. A standard procedure to minimize the number of animals to study is to add animals one by one to each one of the

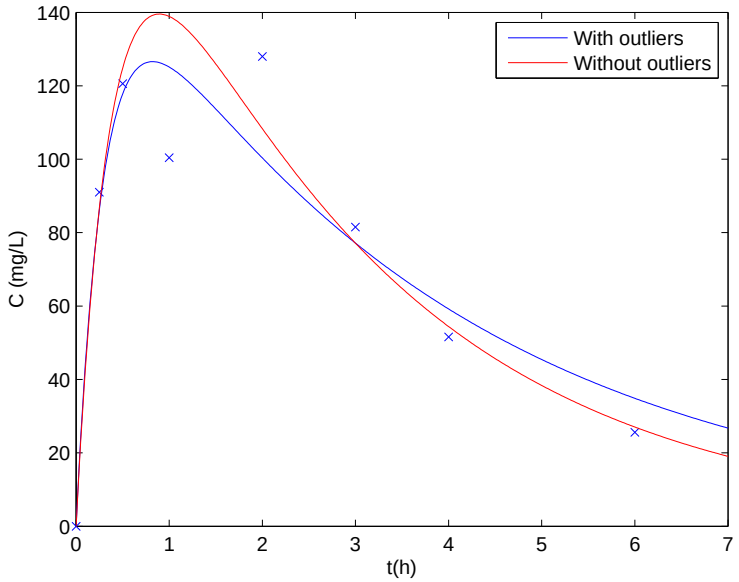

Figure 4.6: Concentration measurements and corresponding fitted values corresponding to the absorption of felodipine.

groups until the population distribution of parameters does not change. The change of the distribution can be detected with a Kolmogorov-Smirnov test (Massey Jr, 1951). The problem of analyzing the differences using only two animals per group is that the conclusions are too tied to the two exemplars being analyzed and that those conclusions cannot be generalized to the whole population.

4.7 Biopharmaceutics+Pharmacokinetics +Pharmacodynamics

4.7.1 Modeling

As an illustration of the methodology developed in this thesis, let us construct a complex model in which we will incorporate many of the building blocks developed along the thesis (dissolution, absorption, compartmental analysis, nonlinear kinetics, clearance, and pharmacodynamics of the therapeutic effect). For doing so we will progressively construct our model to incorporate all effects.

We start assuming a Korsmeyer-Peppas dissolution profile (Eq. (3.341)). For a single dose, the differential equations governing the process are

$$\begin{array}{rcl} \frac{dA_a(t)}{dt} & = & Dose(t) - A_{max} amt^{m-1} \\ \frac{dA_g(t)}{dt} & = & A_{max} amt^{m-1} \end{array} \tag{4.29}$$

Knowing that the integrated curve is $A_g(t) = A_{max} at^m$ and that we are dealing

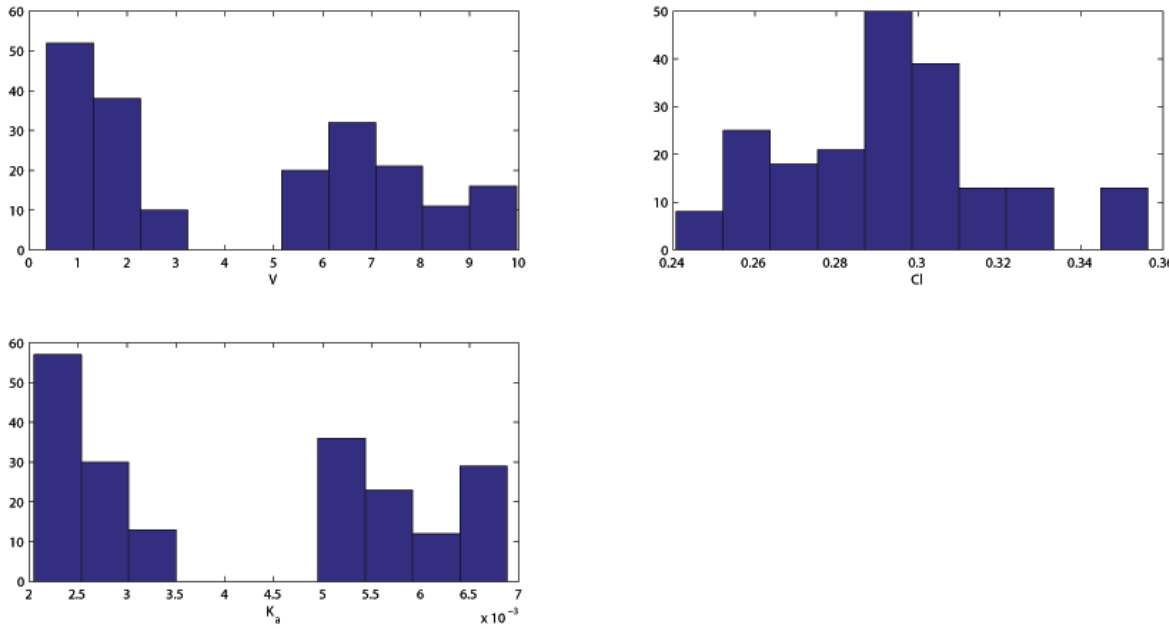

Figure 4.7: Bootstrap histograms for each one of the pharmacokinetics parameters in the two fasting animals.

with a single dose (this is because different doses have different absorption sites and this is not well handled by time systems, we would need spatial-time systems), we may adopt momentarily a different approach to the standard discretization approach defended in this thesis. The amount released between $(n-1)T_s$ and nT_s is $A_g(nT_s) - A_g((n-1)T_s)$, so that the discrete system becomes

$$\begin{array}{rcl} A_a[n] & = & T_s Dose[n] - A_{max} a((nT_s)^m - ((n-1)T_s)^m) \\ A_g[n] & = & A_{max} a((nT_s)^m - ((n-1)T_s)^m) \end{array} \tag{4.30}$$

For illustration purposes, let us assume $A_{max} = 5mmol$, $m = 0.7$ and $a = 0.02min^{-0.7}$. Fig. 4.8 shows the functions $A_a(t)$ and $A_g(t)$. Note that we have not included yet the absorption and that is why the drug does not disappear ($A_g(t)$ does not decrease). It can be seen that the tablet is totally dissolved before 5 hours.

Now, we may calculate the linear density of mass along the small intestine. Let us consider its length $L = 700cm$ and the speed of the fluid inside the intestine $v = 1.76cm \cdot min^{-1}$. The linear density of available mass becomes (see Eq. (3.360))

$$\frac{\partial A_{g,l}(z,t)}{\partial t} = \frac{dA_g(t)}{dt} \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{1}{2}\left(\frac{z-vt}{\sigma}\right)^2\right) \tag{4.31}$$

The local concentration can be calculated as $C_{g,l}(z,t) = \frac{A_{g,l}(z,t)}{S}$. Let us assume $\sigma = 5cm$, and the small intestine to have a diameter of 2.75 cm. Fig. 4.9 shows

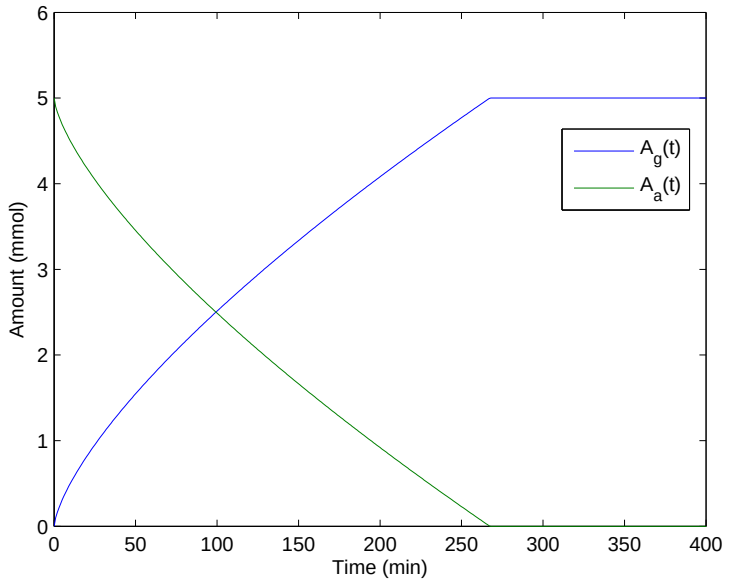

Figure 4.8: Dissolution profile for a Korsmeyer-Peppas model with $A_{max} = 5(mmol)$, $n = 0.7$, $a = 0.02min^{-0.7}$.

the local concentration along time and space due only to the dissolution of the tablet. We see that the tablet is dissolved before 5 hours and that it takes place before 5 m of the small intestine.

At this point, we have to discretize Eq. (3.361). A direct discretization gives

$$\begin{aligned}\frac{C_g[k,n]-C_g[k,n-1])}{T_s} &= D\frac{C_g[k,n]-2C_g[k-1,n]+C_g[k-2,n]}{h^2} \\ &-v\frac{C_g[k,n]-C_g[k-1,n]}{h} \\ &-k_a(C_g[k,n-1]-C[n-1])-k_dC_g[k,n-1]\end{aligned} \tag{4.32}$$

We may reorganize it as

$$\begin{aligned}\left(\frac{1}{T_s}+\frac{v}{h}-\frac{D}{h^2}\right)C_g[k,n] &= \left(\frac{v}{h}-2\frac{D}{h^2}\right)C_g[k-1,n]+\frac{D}{h^2}C_g[k-2,n] \\ &+k_aC[n-1]+\left(\frac{1}{T_s}-k_a-k_d\right)C_g[k,n-1]\end{aligned} \tag{4.33}$$

and, then

$$\begin{aligned}C_g[k,n] &= \frac{1}{\frac{1}{T_s}+\frac{v}{h}-\frac{D}{h^2}}\left(\left(\frac{v}{h}-2\frac{D}{h^2}\right)C_g[k-1,n]+\frac{D}{h^2}C_g[k-2,n]\right. \\ &\left.+k_aC[n-1]+\left(\frac{1}{T_s}-k_a-k_d\right)C_g[k,n-1]\right)\end{aligned} \tag{4.34}$$

We still need to add the contribution from the dosage dissolution

$$\begin{aligned}C_g[k,n] &= \frac{1}{\frac{1}{T_s}+\frac{v}{h}-\frac{D}{h^2}}\left(\left(\frac{v}{h}-2\frac{D}{h^2}\right)C_g[k-1,n]+\frac{D}{h^2}C_g[k-2,n]\right. \\ &\left.+k_aC[n-1]+\left(\frac{1}{T_s}-k_a-k_d\right)C_g[k,n-1]\right)+C_{g,l}[k.n]\end{aligned} \tag{4.35}$$

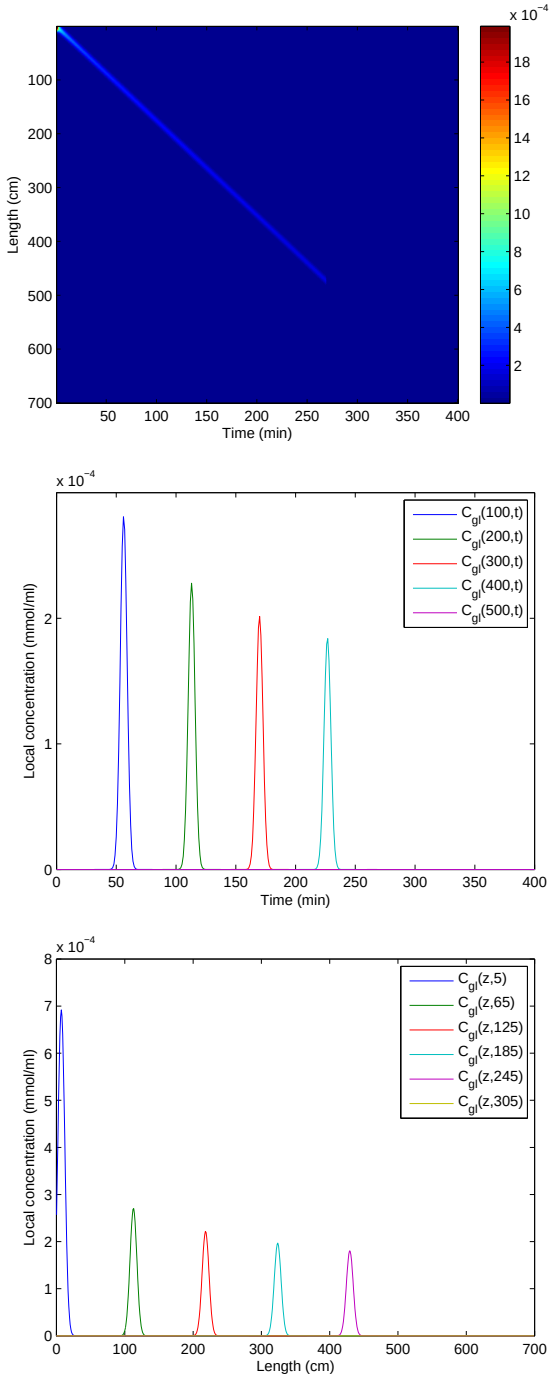

Figure 4.9: Local concentration of released drug along time and space $C_{gl}(z,t)$ and some profiles for $z = 100, 200, 300, 400, 500$ cm and $t = 5, 65, 125, 185, 245 min$.

Fig. 4.10 shows the local concentration along time and space due to the dissolution of the tablet, its transport along the small intestine ($v = 1.76cm \cdot min^{-1}$), its diffusion ($D = 0.01cm^2 \cdot min^{-1}$), its absorption (considering for the moment that the central compartment behaves as a perfect sink; $k_a = 0.025min^{-1}$) and its degradation inside the intestine ($k_d = 0.005min^{-1}$). We see that all profiles widen with respect to the corresponding profiles in Fig. 4.9.

We now consider the total amount of drug entering into the central compartment. This is given by the contribution of all the absorption taking place along the whole tube. It can be calculated as

$$A_{ev}(t) = \int_0^L k_a(C_g(z,t) - C(t))dz \tag{4.36}$$

Assuming that the central compartment behaves as a perfect sink, we will remove this assumption later, the profile of such absorption is shown in Fig. 4.11. We see that absorption ceases after about 6 hours. The total amount of absorbed drug is 1.5033 mmol (which is equal to the integral of the two curves in Fig. 4.11). Biodisponibility can be calculated as this amount divided by the initial given amount (5 mmol)

$$F = \frac{1.5033}{5} = 30.07\% \tag{4.37}$$

In practice, this biodisponibility will decrease when we include the effect that the body is not a perfect sink. Note that if we take the logarithm of the absorbed time (Fig. 4.12), it is not exactly represented by straight lines clearly indicating nonlinear effects.

Let us now model the patient as a three-compartment system: a central compartment and two peripheral compartments attached to the central one. Only one of the peripheral compartments exert a therapeutic effect. Let us presume that the central compartment has a distribution volume of 10 L (V), that the first peripheral compartment has a distribution volume of 3 L (V_{p1}) and the second peripheral compartment 1 L (V_{p2}). Let us also assume that the clearance coefficient from the central compartment to the peripheral is $Cl_p = 50mL/min$ and the hepatic clearance is $Cl_H = 100mL/min$. See Sec. 3.3.4 for a complete description of the model. The equations governing the dynamics of the compartment model are

$$\begin{array}{rcl} V\frac{dC(t)}{dt} & = & -Cl_H C(t) - Cl_p(C(t) - C_{p1}(t)) - Cl_p(C(t) - C_{p2}(t)) + A_{ev}(t) \\ V_{p1}\frac{dC_{p1}(t)}{dt} & = & Cl_p(C(t) - C_{p1}(t)) \\ V_{p2}\frac{dC_{p2}(t)}{dt} & = & Cl_p(C(t) - C_{p2}(t)) \end{array} \tag{4.38}$$

An Euler discretization of this equation system yields

$$\begin{array}{rcl} \frac{C[n]-C[n-1]}{T_s} & = & -\frac{Cl_H}{V}C[n-1] - \frac{Cl_p}{V}(C[n-1] - C_{p1}[n-1]) \\ & & -\frac{Cl_p}{V}(C[n-1] - C_{p2}[n-1]) + \frac{1}{V}A_{ev}[n] \\ \frac{C_{p1}[n]-C_{p1}[n-1]}{T_s} & = & \frac{Cl_p}{V_{p1}}(C[n-1] - C_{p1}[n-1]) \\ \frac{C_{p2}[n]-C_{p2}[n-1]}{T_s} & = & \frac{Cl_p}{V_{p2}}(C[n-1] - C_{p2}[n-1]) \end{array} \tag{4.39}$$

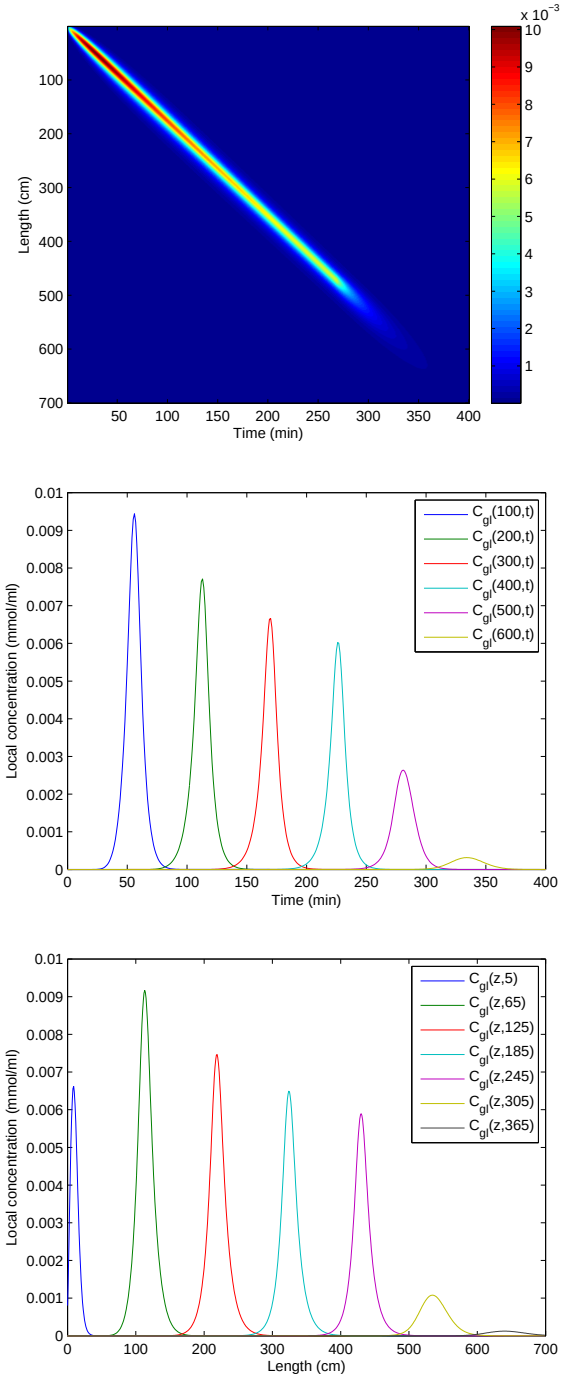

Figure 4.10: Local concentration of drug along time and space $C_{gl}(z,t)$ and some profiles for $z = 100, 200, 300, 400, 500$ cm and $t = 5, 65, 125, 185, 245 min$ considering drug release, transportation, absorption, and degradation.

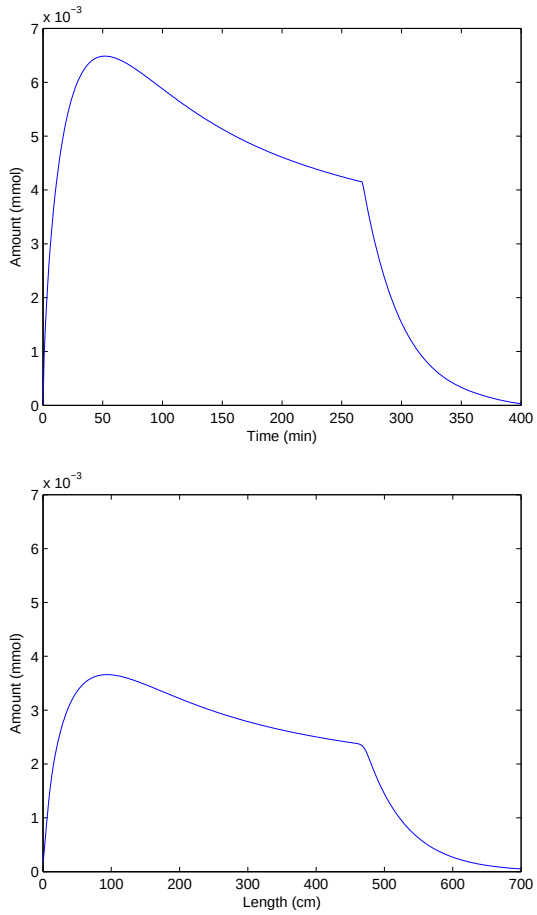

Figure 4.11: Top: Absorption profile along time (amount of drug transferred from the small intestine into the central compartment per time unit). Bottom: Absorption profile along the small intestine.

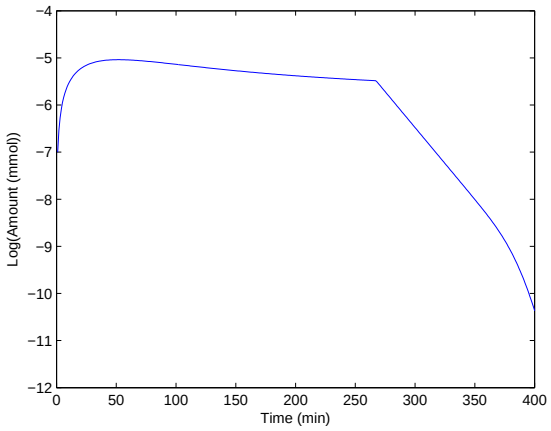

Figure 4.12: Logarithm of the absorption profile along time.

Finally, we may use the following recursive formula

$$\begin{aligned} C[n] &= C[n-1] - \frac{Cl_H T_s}{V} C[n-1] - \frac{Cl_p T_s}{V}(2C[n-1] - (C_{p1}[n-1] + C_{p2}[n-1])) + \frac{T_s}{V} A_{ev}[n] \\ C_{p1}[n] &= C_{p1}[n-1] + \frac{Cl_p T_s}{V_{p1}}(C[n-1] - C_{p1}[n-1]) \\ C_{p2}[n] &= C_{p2}[n-1] + \frac{Cl_p T_s}{V_{p2}}(C[n-1] - C_{p2}[n-1]) \end{aligned} \tag{4.40}$$

The concentration of each of the compartments are shown in Fig. 4.13. Note the nonlinear effects of the absorption, but its linear elimination. In Fig. 4.14 we show how the amount of drug transferred from the intestine to the central compartment is modified due to the concentration increase in the central compartment so that it does not behaves as a perfect sink. In practice, there is not a significant difference and Eq. (4.36) can be approximated by

$$A_{ev}(t) \approx \int_0^L k_a C_g(z,t) dz \tag{4.41}$$

Let us assume that our drug acts in the second peripheral compartment on a receptor with which it has a dissociation constant of 10^{-5}. Let us assume that our drug increases blood flow. According to Eq. (3.189), it exerts a variation of blood flow described by

$$Q_H(C_{p2}(t)) = Q_H^{(0)} + (Q_H^{max} - Q_H^{(0)}) \frac{C_{p2}(t)}{K_D + C_{p2}(t)} \tag{4.42}$$

Let us assume that the basal flow is 1.5 L/min, and that the drug can cause an increase up to 30%. Fig. 4.15 shows the predicted blood flow if the concentration in the second compartment were given by that in Fig. 4.13. However, note that this is not the right concentration because an increase of blood flow would result in an increase of hepatic clearance. This effect is modelled below. Let us assume that the product of the fraction of unbound drug and the intrinsic

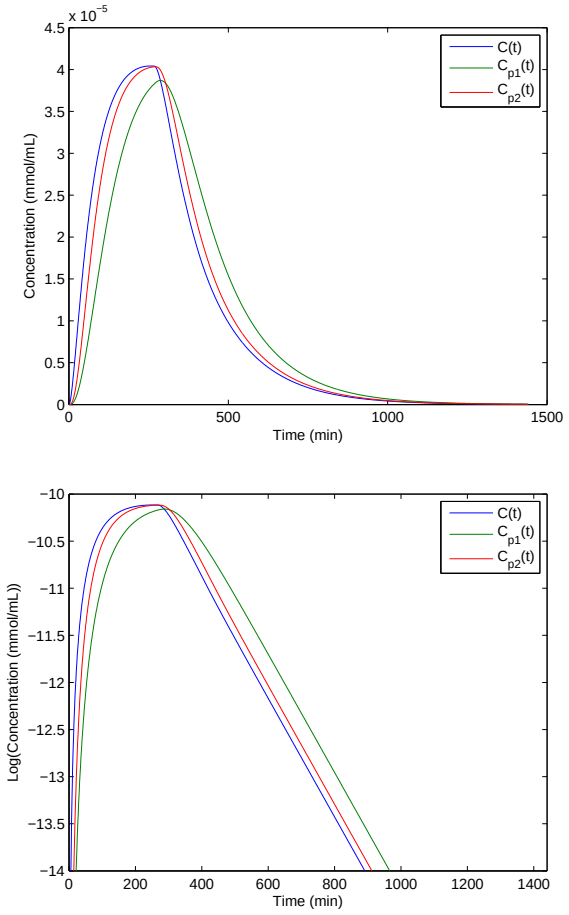

Figure 4.13: Concentration at each of the compartments (top) and its logarithm (bottom).

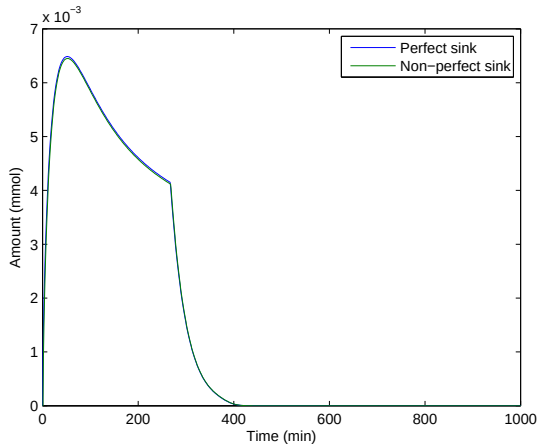

Figure 4.14: Difference between the transferred amount of drug assuming that the central compartment behaves as a perfect sink and when it does not.

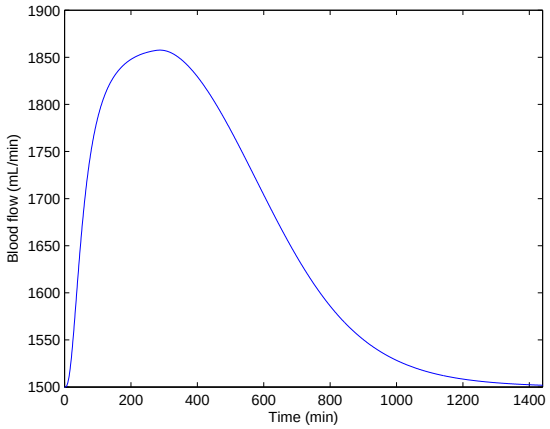

Figure 4.15: Predicted blood flow for the concentration profile in Fig. 4.13.

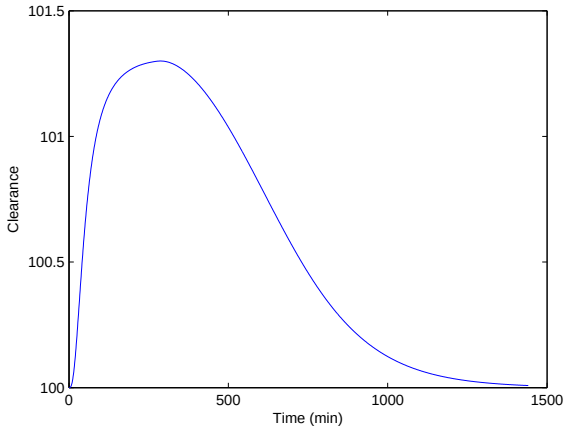

Figure 4.16: Clearance variation for the concentration profile in Fig. 4.13.

clearance is 107 ml/min. With a basal blood flow of 1.5 L/min, this results in a clearance of 100 mL/min (see Eq. (3.147)). However, if the blood flow increases as much as 30%, this results in a small variation of about 1.25% as shown by Fig. (4.16). The peripheral clearance changes accordingly. We may now fully include this clearance variation into the dynamic equations in Eq. 4.39. This small variations do not make any significant difference in the blood concentration (see Fig. 4.17).

4.7.2 Implementation

Although it is not customary to include implementation details in a Ph.D. thesis, we provide here a few implementation details that illustrate the power of the modelling framework developed in this thesis. The whole idea has been based on modelling pharmaceutical and physiological effects through differential equation systems that are discretized later. These difference equations have to be implemented in some computer language. We have chosen Matlab for several reasons:

- It is a wide-spread program with a wide programming community.
- It is specifically focused on numerical algorithms.
- It allows some object-oriented programming features.
- It has optimization libraries well suited to the problem at hand.

We have developed a number of classes that provide basic support to the definition of the kind of models (pharmacokinetics, pharmacodynamics and biopharmaceutics) developed in this thesis. In this way, a new model, like the one developed in this Results section only needs to concentrate on the definition of its specificities. See, for instance, the Matlab code below to define the model. It contains only those lines specific to the model.

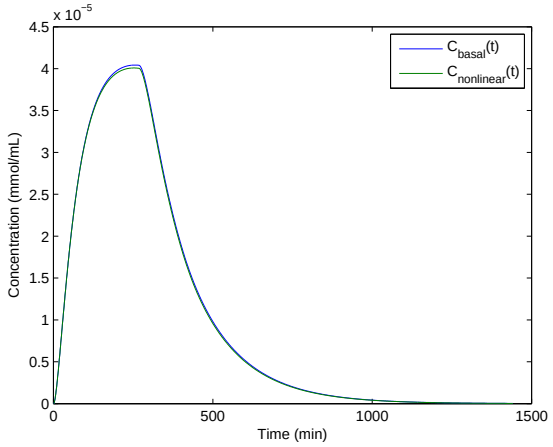

Figure 4.17: Concentration in the central compartment with basal blood flow, and a nonlinear variation of the blood flow.

```
classdef pkpd_modelComplex < pkpd_model
    methods (Static)
        function p=numberOfParameters()
            p=17;
        end
        function str=title()
            str='Complex model';
        end
    end

    methods
        function str=getTemplate(obj)
            str={'Dissolution a (min^-n)=',
                 % a=0.02
                 'Dissolution m=',
                 % m=0.7
                 'Dissolution sigma (cm)=',
                 % sigma=5
                 'Small intestine length L (cm)=',
                 % L=700
                 'Small intestine speed v (cm/min)=',
                 % v=1.76
                 'Small intestine diameter d (cm)=',
                 % d=2.75
                 'Small intestine diffusion D (cm^2/min)=',
                 % D=0.01*60
                 'Absorbtion rate at intestine ka (min^-1)=',
                 % ka=0.025
                 'Degradation rate at intestine kd (min^-1)=',
                 % kd=0.005
                 'Distribution volume central compartment V (mL)=',
                 % V=10e3
                 'Distribution volume peripheral compartment1 Vp1 (mL)=',
                 % Vp1=3e3
                 'Distribution volume peripheral compartment2 Vp2 (mL)=',
                 % Vp2=1e3
                 'Clearance to peripheral compartment Clp (mL/min)=',
```

```
                % Clp=50
                 'Basal hepatic clearance ClH (mL/min)=',
                % ClH=100
                 'Basal hepatic flow (mL/min)=',
                % QH0=1500
                 'Maximum hepatic flow (mL/min)='
                % QHmax=1950
                 'Concentration half effect KD (mmol/mL)='
                % KD=1e-5
                };
        end

        function [C,Agl,Cp1,Cp2,Cgl]=responseToDose(obj,Dose)
            h=1; % Sampling space in intestine length
            [C,Agl,Cp1,Cp2,Cgl]=pkpd_modelComplex_response(Dose,obj.x,obj.Ts,h);
        end
    end
end
```

However, Matlab is relatively slow when implementing loops. Complementarily, we may use C++ code to actually implement the difference equations. We have to bind this code to Matlab, so that Matlab is able to interact with it. This is done through the Mex extension of Matlab. See the C++ code below that actually calculates the time response of each one of the signals involved $(C[n], C_{p1}[n], C_{p2}[n], ...)$. The complexity of the simulation has been shifted from Matlab to C++ gaining a time factor larger than 4 (the C++ code is more than 4 times faster than the equivalent Matlab code). Additionally, we may distribute the C++ compiled code (not its source), so that the "know-how" of the models may remain private to a given institution if desired. In any case, it can also be seen that the C++ code is only related to the specificities of the model at hand, all the support functions to define a model are provided by the Matlab classes from which any model inherits.

```
#include <mex.h>
#include <math.h>

/* Test code
 *
 * path(path,'../../v2')
 * dosePlan=pkpd_doseprescription();
 * dosePlan=dosePlan.load('prescription_delta.txt');
 * [t,tdose]=dosePlan.createInstance(0,0,-1,24);
 * tdose=tdose*5;
 * plot(t,tdose)
 * x=[0.02 0.7 5 700 1.76 2.75 0.01*60 0.025 0.005
 *    10e3 3e3 1e3 50 100 1500 1950 1e-5];
 * [C,Agl,Cp1,Cp2,Cgl]=pkpd_modelComplex_response(tdose,x,1,1);
 */

#ifndef PI
#define PI 3.14159265359
#endif

int myRound(double x) { return (int)floor(x+0.5); }

void pkpd_ComplexModel_computeAgl(double *Agl, double *dose, size_t N,
        size_t Nz, double Ts, double h, double a, double m, double v,
        double sigma)
{
    double K=1.0/sqrt(2*PI*sigma);
```

```
    double Ksigma=-0.5/(sigma*sigma);
    int gaussianLength=(int)ceil(6*sigma/h);
    double *e=new double[2*gaussianLength+1];

    for (size_t n=0; n<N; ++n)
    {
        if (dose[n]!=0) {
            // There is a dose
            double Amax_a=dose[n]*a;
            double n0=1;
            double Aremaining=dose[n];
            for (size_t np=n+1; np<N; ++np, ++n0)
            {
                if (Aremaining==0)
                    break;
                // Pointer to the corresponding column of Agl
                double *Agl_np=Agl+Nz*np;

                // Compute how much is diffused at this sample
                double delta=Amax_a*(pow(n0,m)-pow(n0-1,m));
                            // This is the definite integral
                if (delta>Aremaining)
                    delta=Aremaining;
                Aremaining-=delta;

                // Compute Gaussian around the location of maximal release
                double zmax=v*n0; // where the maximal release is
                int idx_zmax=myRound(zmax/h);
                double sum_e=0.0;
                double z=(idx_zmax-gaussianLength)*h;
                int idx=0;
                for (int k=idx_zmax-gaussianLength;
                     k<=idx_zmax+gaussianLength; ++k, ++z, ++idx)
                {
                    if (k>=0 && k<Nz)
                    {
                        double zdiff=z-zmax;
                        double aux_e=K*exp(Ksigma*zdiff*zdiff);
                        e[idx]=aux_e;
                        sum_e+=aux_e;
                    }
                }

                // Actually distribute
                double Kdistribution=delta/sum_e;
                idx=0;
                for (int k=idx_zmax-gaussianLength;
                     k<=idx_zmax+gaussianLength; ++k, ++idx)
                    if (k>=0 && k<Nz)
                        Agl_np[k]=Kdistribution*e[idx];
            }
        }
    }
    delete e;
}

void pkpd_ComplexModel_actualResponse(double *C, double *Agl, double *Cp1,
        double *Cp2, double *Cgl, double *dose, size_t N, size_t Nz,
        double *x, double Ts, double h)
{
    // Input model parameters
    double a    =x[0];  // Dissolution a (min^-n)
```

```
double m     =x[1];  // Dissolution m
double sigma=x[2];  // Dissolution sigma (cm)
double L     =x[3];  // Small intestine length L (cm)
double v     =x[4];  // Small intestine speed v (cm/min)
double d     =x[5];  // Small intestine diameter d (cm)
double D     =x[6];  // Small intestine diffusion D (cm^2/min)
double ka    =x[7];  // Absorbtion rate at intestine ka (min^-1)
double kd    =x[8];  // Degradation rate at intestine kd (min^-1)
double V     =x[9];  // Distrib vol central compartment V (mL)
double Vp1   =x[10]; // Distrib vol peripheral compartment1 Vp1 (mL)
double Vp2   =x[11]; // Distrib vol peripheral compartment2 Vp2 (mL)
double Clp   =x[12]; // Clearance to peripheral compartment Clp (mL/min)
double ClH   =x[13]; // Basal hepatic clearance ClH (mL/min)
double QH0   =x[14]; // Basal hepatic flow (mL/min)
double QHmax=x[15]; // Maximum hepatic flow (mL/min)
double KD    =x[16]; // Hepatic flow KD (mmol/mL)

// Derived model parameters
double S=PI*(d/2.0)*(d/2.0); // Diameter of the intestine
double iS=1.0/S;
double fuClint=QH0*ClH/(QH0-ClH);
       // Fraction unbound * Intrinsic Clearance (Central)
double fuClintp=QH0*Clp/(QH0-Clp);
       // Fraction unbound * Intrinsic Clearance (Peripheral)
double iV=1.0/V;

// Some useful constants
double h2=h*h;
double a0=1/Ts-D/h2+v/h;
double b0p=(-kd+1/Ts);
double ia0=1.0/a0;
double b0pia0=b0p*ia0;
double a1=(v/h-2*D/h2);
double a2=D/h2;
double TsiV=Ts/V;
double TsiVp1=Ts/Vp1;
double TsiVp2=Ts/Vp2;

// Compute how the tablet dissolves
pkpd_ComplexModel_computeAgl(Agl, dose, N, Nz, Ts, h, a, m, v, sigma);

// Compute the full dynamics
double *Aev=new double[N]; // Extravascular amount
double *Aevk=new double[Nz]; // Extravascular amount along z
double *b=new double[Nz]; // Auxiliary variable for Cgl

// Compute concentrations for n=0;
Aev[0]=0;
for (size_t z=0; z<Nz; ++z)
{
    Cgl[z]=Agl[z]*iS;
    Aev[0]+=Cgl[z];
}

double QHt=QH0;
double ClHt=QHt*fuClint/(QHt+fuClint);
double Clpt=QHt*fuClintp/(QHt+fuClintp);
for (size_t n=1; n<N; ++n)
{
    // Update clearance parameters and blood flow
    size_t n_1=n-1;
    ClHt=QHt*fuClint/(QHt+fuClint);
```

```
        Clpt=QHt*fuClintp/(QHt+fuClintp);
        QHt=QH0+(QHmax-QH0)*Cp1[n_1]/(KD+Cp1[n_1]);
        double ClHTsiV=ClHt*TsiV;
        double ClpTsiVp1=Clpt*TsiVp1;
        double ClpTsiVp2=Clpt*TsiVp2;
        double ClpTsiV=Clpt*TsiV;

        // Calculate b
        double *Cgln_1=Cgl+(n-1)*Nz; // Cgl(:,n-1)
        double *Agln=Agl+n*Nz; // Agl(:,n)
        Aev[n]=0;
        for (size_t z=0; z<Nz; ++z)
        {
            Aevk[z]=ka*Cgln_1[z]-C[n-1];
            if (Aevk[z]<0)
                Aevk[z]=0;
            Aev[n]+=Aevk[z];
            b[z]=-Aevk[z]*ia0+b0pia0*Cgln_1[z]+Agln[z]*iS;
        }

        // Update Cgl
        double *Cgln=Cgl+n*Nz; // Cgl(:,n)
        Cgln[0]=b[0];
        Cgln[1]=(a1*Cgln[0])*ia0+b[1];
        for (size_t z=3; z<Nz; ++z)
        {
            Cgln[z]=(Cgln[z-1]*a1+Cgln[z-2]*a2)*ia0+b[z];
            if (Cgln[z]<0)
                Cgln[z]=0;
        }

        // Update C, Cp1 and Cp2
        C[n]=C[n-1]-ClHTsiV*C[n-1]-ClpTsiV*(2*C[n-1]-Cp1[n-1]-Cp2[n-1])
             +iV*Aev[n];
        Cp1[n]=Cp1[n-1]+ClpTsiVp1*(C[n-1]-Cp1[n-1]);
        Cp2[n]=Cp2[n-1]+ClpTsiVp2*(C[n-1]-Cp2[n-1]);
    }
    delete Aev;
    delete Aevk;
    delete b;
}

/* the gateway function */
void mexFunction( int nlhs, mxArray *plhs[],
                  int nrhs, const mxArray *prhs[])
{
  /* check for proper number of arguments */
  if (nrhs!=4)
    mexErrMsgTxt("4 inputs required.");

  /* Get dose */
  const mxArray *mDose=prhs[0];
  double *dose=mxGetPr(mDose);
  mwSize N=mxGetN(mDose)*mxGetM(mDose);

  /* Get parameters */
  const mxArray *mX=prhs[1];
  double *x=mxGetPr(mX);
  mwSize Nx=mxGetN(mX)*mxGetM(mX);
  if (Nx!=17)
      mexErrMsgTxt("Model needs exactly 17 parameters");
```

```
  /* Get sampling rates */
  double Ts=mxGetScalar(prhs[2]);
  double h=mxGetScalar(prhs[3]);

  /*  set the output pointer to the output matrices */
  plhs[0]=mxCreateDoubleMatrix(N,1, mxREAL);
  double *C=mxGetPr(plhs[0]);
  double L    =x[3];  // Small intestine length L (cm)
  size_t Nz = (size_t)myRound(L/h);
  plhs[1]=mxCreateDoubleMatrix(Nz,N, mxREAL);
  double *Agl=mxGetPr(plhs[1]);
  plhs[2]=mxCreateDoubleMatrix(N,1, mxREAL);
  double *Cp1=mxGetPr(plhs[2]);
  plhs[3]=mxCreateDoubleMatrix(N,1, mxREAL);
  double *Cp2=mxGetPr(plhs[3]);
  plhs[4]=mxCreateDoubleMatrix(Nz,N, mxREAL);
  double *Cgl=mxGetPr(plhs[4]);

  /*  call the C subroutine */
  pkpd_ComplexModel_actualResponse(C, Agl, Cp1, Cp2, Cgl, dose, N,
                                   Nz, x, Ts, h);
}
```

4.7.3 System identification

An interesting fact about simulation is that it allows to easily identifying the model parameters by simulating what would be the response of the system for different system parameters. Then, we can see whether the simulated profile matches or not the observed concentration values as shown in Section 3.3.2. To illustrate this application, let us simulate the blood concentration of the system in the previous section when an intake of 5 mmol is given every 4 hours during 3 days. Fig. 4.18 shows the simulated profile along with samples taken every hour. The error in the samples is presumed to be Gaussianly distributed and to have a maximum coefficient of variation of 15% which is the maximum allowed by Committee for Medicinal Products for Human Use (CHMP) (2011).

Note that profile simulation and regression is the only approach available that can exploit the transient samples at the beginning and at the end of the treatment (non-stationary parts of the signal). Also, there is no closed form for the stationary part for this particular model.

For the sake of illustrating the simulation framework developed in this thesis, consider the Matlab code needed to perform this simulation:

```
path(path,'../../v2')

%% Define prescription
prescription=pkpd_doseprescription();
prescription=prescription.addRepeatedIntake(0,72,4,5);
[t,tdose]=prescription.createInstance(0,0,-1,80);
prescription.save('prescription.txt');

%% Create true model
trueModel=pkpd_modelComplex();
trueModel.x=[0.02  0.7  5  700  1.76  2.75  0.01*60  0.025  0.005  ...
    10e3  3e3  1e3  50  100  1500  1950  1e-5];
trueModel.Ts=1; % min
[C,Agl,Cp1,Cp2,Cgl]=trueModel.responseToDose(tdose);
Ctrue=C;
ttrue=t;
```

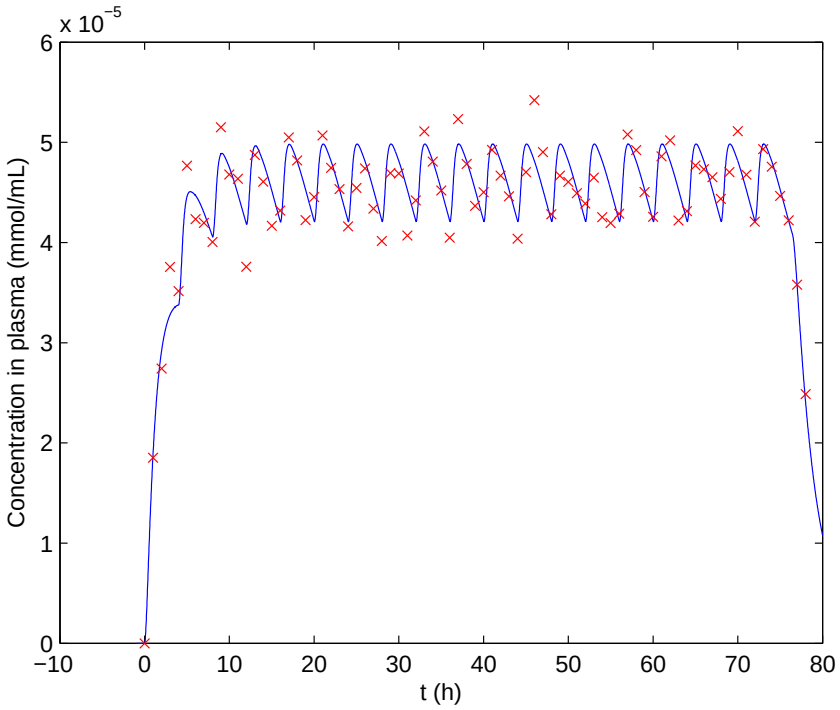

Figure 4.18: Concentration in the central compartment with basal blood flow, and a nonlinear variation of the blood flow.

```
save('trueModel.mat','trueModel','Ctrue','ttrue');

%% Take samples
samples=pkpd_samples();
samples=samples.takeRegularSample(t,C,0,60,0.5,15);
plot(t/60,C)
hold on
samples.show()
xlabel('t (h)')
ylabel('Concentration in plasma (mmol/mL)')
samples.write('samples.mat');
```

It clearly allows the user to concentrate on the task at hand since it hides many "administrative" tasks handled by high-level classes like `pkpd_doseprescription`, `pkpd_model` and `pkpd_samples`.

Let us assume that we are given the concentration samples, and we are asked to estimate the model parameters. This is a system identification problem. We will assume that we know the input to the system (the administered dose) and that we observe its output at given time points. Then, we need to determine the parameters defining the model. Let us say that we are rather certain about the dissolution parameters and that we would like to characterize the distribution and clearance parameters as well as the effect of the drug on the blood flow. In the search limits for the global optimizer we leave no room for optimizing the dissolution parameters and introduce our uncertainty about the rest of parameters. See the Matlab code for the fitting below:

```
path(path,'../../v2')

%% Load prescription and samples
prescription=pkpd_doseprescription();
prescription=prescription.load('prescription.txt');
samples=pkpd_samples();
samples=samples.load('samples.mat');

%% Define model and its limits
model=pkpd_modelComplex();
model.x0=[0.02  0.7  5  700  1.76  2.75  0.01*60  0.025  0.005  ...
           8e3  2e3  0.5e3  40   80  1300  1750  0.5e-5];
model.xF=[0.02  0.7  5  700  1.76  2.75  0.01*60  0.025  0.005  ...
          12e3  4e3  1.5e3  60  120  1700  2150  1.5e-5];
model.Ts=1; % min

%% Fit model
fitter=pkpd_modelFitter(samples,prescription,model)
fitter=fitter.produceSideInfo()
fitter=fitter.globalOptimization()
fitter=fitter.localOptimization()
fitter.report()
fitter.show()
ylabel('C(mmol/mL)')

%% Verify curve
load('trueModel.mat')
hold on
plot(ttrue/60,Ctrue,'k');
legend('Predicted concentration','Observed concentration','True concentration')
```

With this very simple code we get the report below and as can be seen in Fig. 4.19, we can see that the fitting to the true concentration is nearly perfect.

```
Model ================================
```

```
Complex model
Dissolution a (min^-n)= 0.02 Search: [0.02,0.02]
Dissolution m= 0.7 Search: [0.7,0.7]
Dissolution sigma (cm)= 5 Search: [5,5]
Small intestine length L (cm)= 700 Search: [700,700]
Small intestine speed v (cm/min)= 1.76 Search: [1.76,1.76]
Small intestine diameter d (cm)= 2.75 Search: [2.75,2.75]
Small intestine diffusion D (cm^2/min)= 0.6 Search: [0.6,0.6]
Absorbtion rate at intestine ka (min^-1)= 0.025 Search: [0.025,0.025]
Degradation rate at intestine kd (min^-1)= 0.005 Search: [0.005,0.005]
Distribution volume central compartment V (mL)= 10778.5464 Search: [8000,12000]
Distribution volume peripheral compartment1 Vp1 (mL)= 2688.9248 Search: [2000,4000]
Distribution volume peripheral compartment2 Vp2 (mL)= 500.0002 Search: [500,1500]
Clearance to peripheral compartment Clp (mL/min)= 54.497 Search: [40,60]
Basal hepatic clearance ClH (mL/min)= 100.9584 Search: [80,120]
Basal hepatic flow (mL/min)= 1511.8799 Search: [1300,1700]
Maximum hepatic flow (mL/min)= 2031.6893 Search: [1750,2150]
Concentration half effect KD (mmol/mL)= 8.2757e-06 Search: [5e-06,1.5e-05]

Max. Impulse Response(1-day) (mmol/L) = 6.7729e-06
Max. Impulse Response(1-day) (min) = 255

Goodness of fitting ==================
Coefficient of determination R2=          0.91818
Adjusted coefficient of determination R2= 0.89538
Correlation (observed,predicted)=         0.95822
Variance of residuals=                    4.6597e-12
Akaike Information Criterion=             -25.6617
Bayesian Information Criterion=           -25.1518
Final Prediction Error=                   7.215e-12

Fitting ==============================
Time (min) Observed Conc.(mg/L) Predicted Conc. (mg/L) Residue (mg/L) z-score
0 0 0 0 -0.020011
60 1.8527e-05 1.8368e-05 1.5992e-07 0.054073
120 2.7421e-05 2.8855e-05 -1.4334e-06 -0.68405
180 3.756e-05 3.267e-05 4.8895e-06 2.2451
240 3.5154e-05 3.371e-05 1.4444e-06 0.6491
300 4.7661e-05 4.4256e-05 3.4048e-06 1.5573
360 4.2338e-05 4.4578e-05 -2.24e-06 -1.0577
420 4.1965e-05 4.2859e-05 -8.9478e-07 -0.43452
480 4.0058e-05 4.0458e-05 -4.0029e-07 -0.20545
540 5.1511e-05 4.8367e-05 3.1441e-06 1.4365
600 4.6793e-05 4.7215e-05 -4.221e-07 -0.21555
660 4.6368e-05 4.4593e-05 1.7754e-06 0.80244
720 3.7586e-05 4.1609e-05 -4.0237e-06 -1.884
780 4.8742e-05 4.907e-05 -3.2732e-07 -0.17164
840 4.6071e-05 4.7666e-05 -1.5945e-06 -0.75865
900 4.1679e-05 4.4889e-05 -3.2103e-06 -1.5072
960 4.319e-05 4.1806e-05 1.3836e-06 0.62095
1020 5.0482e-05 4.919e-05 1.2927e-06 0.57882
1080 4.8187e-05 4.7743e-05 4.4451e-07 0.18591
1140 4.2243e-05 4.494e-05 -2.6968e-06 -1.2693
1200 4.4541e-05 4.184e-05 2.7016e-06 1.2315
```

```
1260 5.068e-05 4.921e-05 1.4698e-06 0.66087
1320 4.7446e-05 4.7756e-05 -3.1006e-07 -0.16365
1380 4.5325e-05 4.4948e-05 3.7675e-07 0.15452
1440 4.162e-05 4.1845e-05 -2.2533e-07 -0.1244
1500 4.5454e-05 4.9214e-05 -3.7599e-06 -1.7618
1560 4.74e-05 4.7758e-05 -3.5813e-07 -0.18592
1620 4.3393e-05 4.495e-05 -1.5563e-06 -0.74097
1680 4.0158e-05 4.1846e-05 -1.6889e-06 -0.80239
1740 4.6922e-05 4.9214e-05 -2.2921e-06 -1.0818
1800 4.6885e-05 4.7758e-05 -8.7347e-07 -0.42465
1860 4.0696e-05 4.495e-05 -4.254e-06 -1.9907
1920 4.4208e-05 4.1847e-05 2.3611e-06 1.0738
1980 5.1097e-05 4.9214e-05 1.8825e-06 0.85207
2040 4.8081e-05 4.7758e-05 3.2224e-07 0.12927
2100 4.5197e-05 4.495e-05 2.4724e-07 0.094523
2160 4.0491e-05 4.1847e-05 -1.3553e-06 -0.64785
2220 5.2322e-05 4.9214e-05 3.1078e-06 1.4197
2280 4.7849e-05 4.7759e-05 9.0813e-08 0.022059
2340 4.3658e-05 4.495e-05 -1.2916e-06 -0.61835
2400 4.5024e-05 4.1847e-05 3.1775e-06 1.452
2460 4.9242e-05 4.9214e-05 2.7825e-08 -0.007121
2520 4.6671e-05 4.7759e-05 -1.0873e-06 -0.52369
2580 4.4611e-05 4.495e-05 -3.3904e-07 -0.17707
2640 4.0386e-05 4.1847e-05 -1.4602e-06 -0.69646
2700 4.7024e-05 4.9214e-05 -2.1901e-06 -1.0346
2760 5.4208e-05 4.7759e-05 6.4491e-06 2.9676
2820 4.9024e-05 4.495e-05 4.0737e-06 1.8671
2880 4.2823e-05 4.1847e-05 9.7635e-07 0.43229
2940 4.6672e-05 4.9214e-05 -2.5428e-06 -1.198
3000 4.6046e-05 4.7759e-05 -1.7123e-06 -0.81322
3060 4.4927e-05 4.495e-05 -2.2697e-08 -0.030526
3120 4.3896e-05 4.1847e-05 2.0495e-06 0.92944
3180 4.6485e-05 4.9214e-05 -2.7293e-06 -1.2844
3240 4.2522e-05 4.7759e-05 -5.2363e-06 -2.4457
3300 4.1948e-05 4.495e-05 -3.0023e-06 -1.4108
3360 4.2878e-05 4.1847e-05 1.0311e-06 0.45766
3420 5.0776e-05 4.9214e-05 1.562e-06 0.70361
3480 4.9216e-05 4.7759e-05 1.4574e-06 0.65513
3540 4.5032e-05 4.495e-05 8.2121e-08 0.018032
3600 4.2562e-05 4.1847e-05 7.1519e-07 0.3113
3660 4.8601e-05 4.9214e-05 -6.1329e-07 -0.30412
3720 5.0203e-05 4.7759e-05 2.4449e-06 1.1126
3780 4.2193e-05 4.495e-05 -2.7566e-06 -1.297
3840 4.3134e-05 4.1847e-05 1.2874e-06 0.57637
3900 4.7689e-05 4.9214e-05 -1.5258e-06 -0.72686
3960 4.7323e-05 4.7759e-05 -4.3544e-07 -0.22173
4020 4.6527e-05 4.495e-05 1.5774e-06 0.7107
4080 4.4366e-05 4.1847e-05 2.519e-06 1.1469
4140 4.7019e-05 4.9214e-05 -2.1955e-06 -1.0371
4200 5.1119e-05 4.7759e-05 3.3601e-06 1.5366
4260 4.677e-05 4.495e-05 1.8204e-06 0.82329
4320 4.2082e-05 4.1847e-05 2.3534e-07 0.089012
4380 4.9316e-05 4.9214e-05 1.0141e-07 0.026967
4440 4.7564e-05 4.7759e-05 -1.9423e-07 -0.10999
```

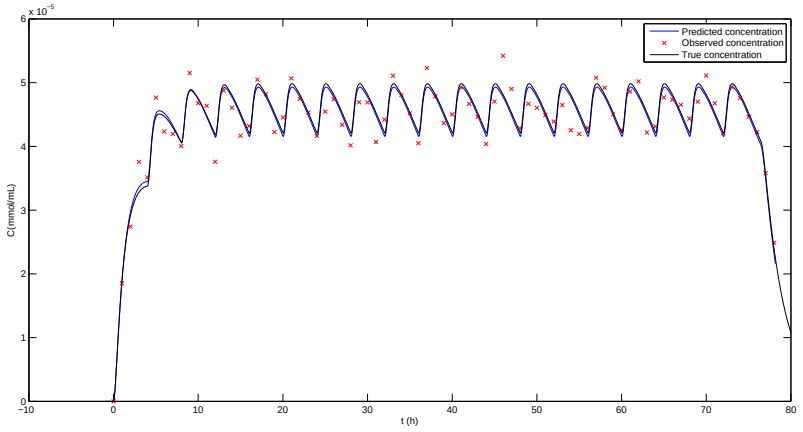

Figure 4.19: Predicted, observed and true concentration in plasma

```
4500 4.464e-05 4.495e-05 -3.0956e-07 -0.16342
4560 4.2223e-05 4.1847e-05 3.7644e-07 0.15437
4620 3.5795e-05 3.5545e-05 2.4994e-07 0.095776
4680 2.4871e-05 2.3971e-05 9.0082e-07 0.3973
```

Although we have opted for a scripting solution to report the results in this thesis, we have also developed a Graphical User Interface (see Fig. 4.20) in which adding a new model is just adding 3 lines of code.

In the following table we compare the estimated and the true parameters for this experiment, we also report the corresponding relative error:

Parameter	**Estimated value**	**True value**	**Relative error (%)**
Distribution volume central compartment	10778.5464	10000	7.79
Distribution volume peripheral compartment1	2688.9248	3000	-10.37
Distribution volume peripheral compartment2	500.0002	1000	-50
Clearance to peripheral compartment	54.497	50	8.99
Basal hepatic clearance	100.9584	100	0.96
Basal hepatic flow	1511.8799	1500	0.79
Maximum hepatic flow (mL/min)	2031.6893	1950	4.19
Concentration half effect	8.2757e-06	1e-05	-17.24

Most parameters have a relative error around or below 10%. V_{p2} has a large relative error, although its absolute error is not so large. Interestingly, those parameters related to body clearance ($Cl_H^{(0)}$ and $Q_H^{(0)}$ have a large precision. To find out whether this is just by chance (due to a specific realization of noise) or not, we construct the confidence intervals for each one of the parameters by performing bootstrapping (see Section 3.3.2). Note that using this method is just as the previous one but changing one line. This is thanks to the object-oriented classes programmed for this thesis.

```
NB=100; % Number of bootstrap samples
alpha=0.05; % For the trim mean
fitterB=pkpd_modelFitterBootstrap(samples,prescription,model,NB,alpha);
fitterB=fitterB.produceSideInfo();
```

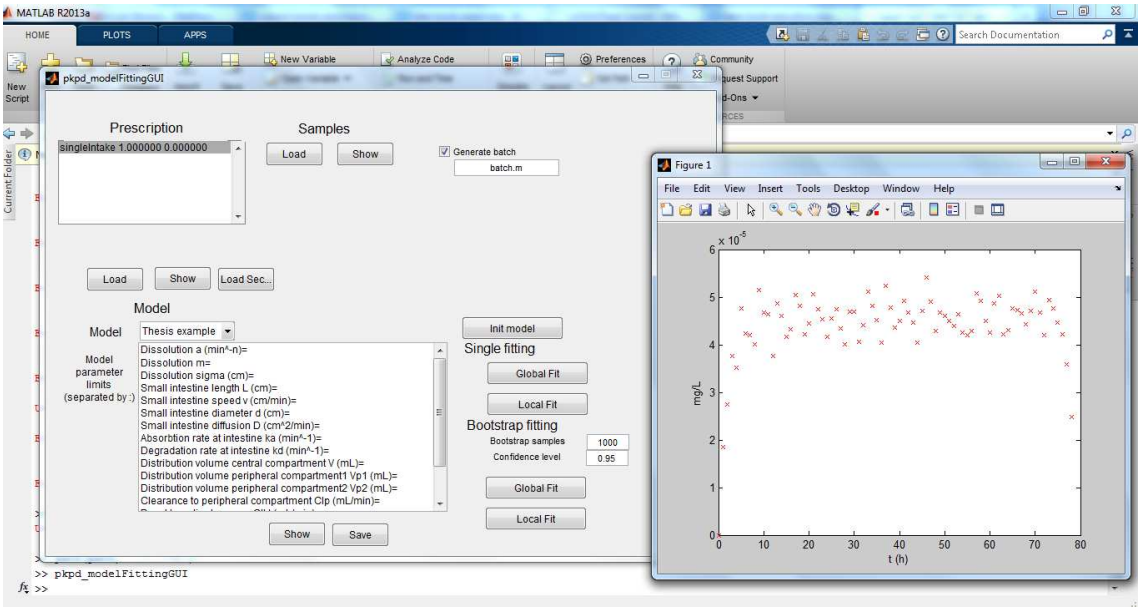

Figure 4.20: Example of Graphical User Iterface to fit a model to a set of samples.

```
fitterB=fitterB.globalOptimization();
fitterB=fitterB.localOptimization();
fitterB.report()
```

```
Model ================================
Complex model
Dissolution a (min^-n)= 0.02 Search: [0.02,0.02]
Dissolution m= 0.7 Search: [0.7,0.7]
Dissolution sigma (cm)= 5 Search: [5,5]
Small intestine length L (cm)= 700 Search: [700,700]
Small intestine speed v (cm/min)= 1.76 Search: [1.76,1.76]
Small intestine diameter d (cm)= 2.75 Search: [2.75,2.75]
Small intestine diffusion D (cm^2/min)= 0.6 Search: [0.6,0.6]
Absorbtion rate at intestine ka (min^-1)= 0.025 Search: [0.025,0.025]
Degradation rate at intestine kd (min^-1)= 0.005 Search: [0.005,0.005]
Distribution volume central compartment V (mL)= 10341.7348 Search: [8000,12000]
Distribution volume peripheral compartment1 Vp1 (mL)= 2570.8181 Search: [2000,4000]
Distribution volume peripheral compartment2 Vp2 (mL)= 925.1495 Search: [500,1500]
Clearance to peripheral compartment Clp (mL/min)= 55.119 Search: [40,60]
Basal hepatic clearance ClH (mL/min)= 100.9712 Search: [80,120]
Basal hepatic flow (mL/min)= 1487.8382 Search: [1300,1700]
Maximum hepatic flow (mL/min)= 1963.8717 Search: [1750,2150]
Concentration half effect KD (mmol/mL)= 1.017e-05 Search: [5e-06,1.5e-05]

Max. Impulse Response(1-day) (mmol/L) = 6.7896e-06
Max. Impulse Response(1-day) (min) = 254

Confidence intervals 95% ========================
```

```
Dissolution a (min^-n)= [0.02,0.02]
Dissolution m= [0.7,0.7]
Dissolution sigma (cm)= [5,5]
Small intestine length L (cm)= [700,700]
Small intestine speed v (cm/min)= [1.76,1.76]
Small intestine diameter d (cm)= [2.75,2.75]
Small intestine diffusion D (cm^2/min)= [0.6,0.6]
Absorbtion rate at intestine ka (min^-1)= [0.025,0.025]
Degradation rate at intestine kd (min^-1)= [0.005,0.005]
Distribution volume central compartment V (mL)= [8602.3957,11829.776]
Distribution volume peripheral compartment1 Vp1 (mL)= [2000.125,3779.4006]
Distribution volume peripheral compartment2 Vp2 (mL)= [505.7916,1472.76]
Clearance to peripheral compartment Clp (mL/min)= [40.4545,59.9921]
Basal hepatic clearance ClH (mL/min)= [99.4016,103.2389]
Basal hepatic flow (mL/min)= [1300.99,1693.7996]
Maximum hepatic flow (mL/min)= [1753.5506,2147.077]
Concentration half effect KD (mmol/mL)= [5.2423e-06,1.4913e-05]
```

We see that the 95% confidence interval always contains the true value. We show below the table of relative error, that has reduced significantly from the previous estimate. This is because a single point estimate is more affected by the random errors of the measurements, while the bootstrap estimate is based in an unbiased procedure involving averaging over multiple estimates.

Parameter	**Estimated value**	**True value**	**Relative error (%)**
Distribution volume central compartment	10341.7348	10000	3.42
Distribution volume peripheral compartment1	2570.3131	3000	-14.32
Distribution volume peripheral compartment2	925.1495	1000	-7.48
Clearance to peripheral compartment	55.119	50	10.23
Basal hepatic clearance	100.9712	100	0.97
Basal hepatic flow	1487.8382	1500	-0.81
Maximum hepatic flow	1963.8717	1950	0.71
Concentration half effect	1.017e-05	1e-05	1.7

Additionally, thanks to the bootstrap approach we may estimate the covariance matrix among the different parameters. We find a significant correlation between the basal hepatic clearance, the basal hepatic flow and the maximum hepatic flow.

1.0000	-0.1378	-0.1362	-0.1880	0.0616	-0.0729	0.0031	-0.0181
-0.1378	1.0000	0.1122	-0.0202	-0.0126	-0.0731	0.1439	-0.2514
-0.1362	0.1122	1.0000	0.0570	0.1741	0.2588	-0.1105	-0.0095
-0.1880	-0.0202	0.0570	1.0000	0.1287	-0.0279	-0.1344	-0.0523
0.0616	-0.0126	0.1741	0.1287	1.0000	0.5881	-0.6022	0.1147
-0.0729	-0.0731	0.2588	-0.0279	0.5881	1.0000	-0.3117	0.2125
0.0031	0.1439	-0.1105	-0.1344	-0.6022	-0.3117	1.0000	-0.0434
-0.0181	-0.2514	-0.0095	-0.0523	0.1147	0.2125	-0.0434	1.0000

We may also use this environment for estimating population parameters. Let us assume that the drug has no effect on 20% of the population (that is $Q_H^{max} = Q_H^{(0)}$). We now measure the concentration in plasma as we did for the previous experiments for 100 individuals and concentrate on the estimate of Q_H^{max}. Figure 4.21 (top) shows the histogram of the estimated values. We do not see the separation between the two poplations. This illustrates the fact that parameters with a relatively small effect cannot be identified when the level of noise and uncertainty about the rest of parameters is high. If we reduce

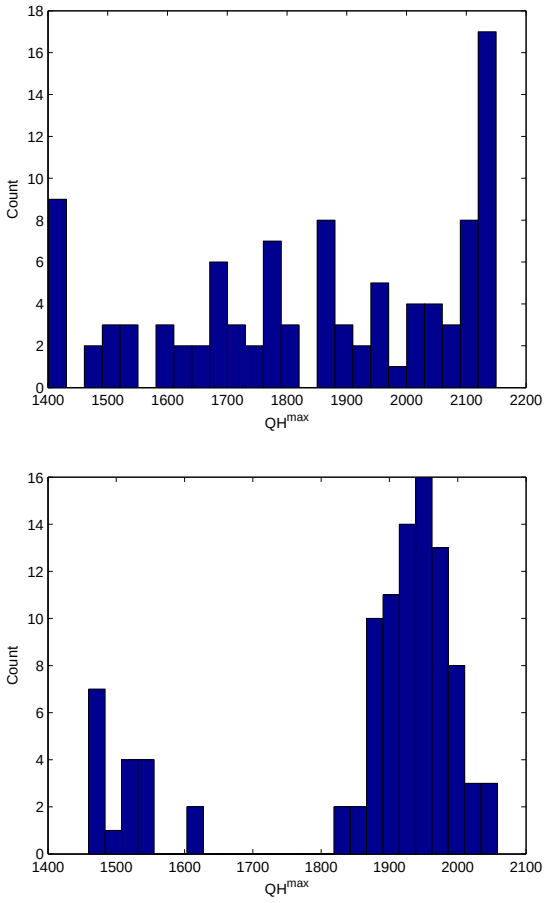

Figure 4.21: Histogram of Q_H^{max} in a population of 100 individuals under large (top) and low (bottom) uncertainty conditions.

the level of measurement noise to 2% and allow no uncertainty about the rest of parameters, then the two populations become clearly visible using the same identification scheme (see Figure 4.21 (bottom)).

4.7.4 Therapeutic planning

We may also use the methodology developed in this thesis to establish a therapeutic prescription. Let us presume that the therapeutic window of our drug in between two concentration limits C_{min} and C_{max}. Below C_{min} the drug has no effect and beyond C_{max} the drug may have toxic effects. We may define a given therapy based on a set of parameters. For instance, "Take X mmol every Y hours". The therapy is described by a two component vector $\Theta = (X, Y)$ and we may optimize this therapy with the goal of reducing the number of intakes and the period of time in which the drug is ineffective. We can achieve this by

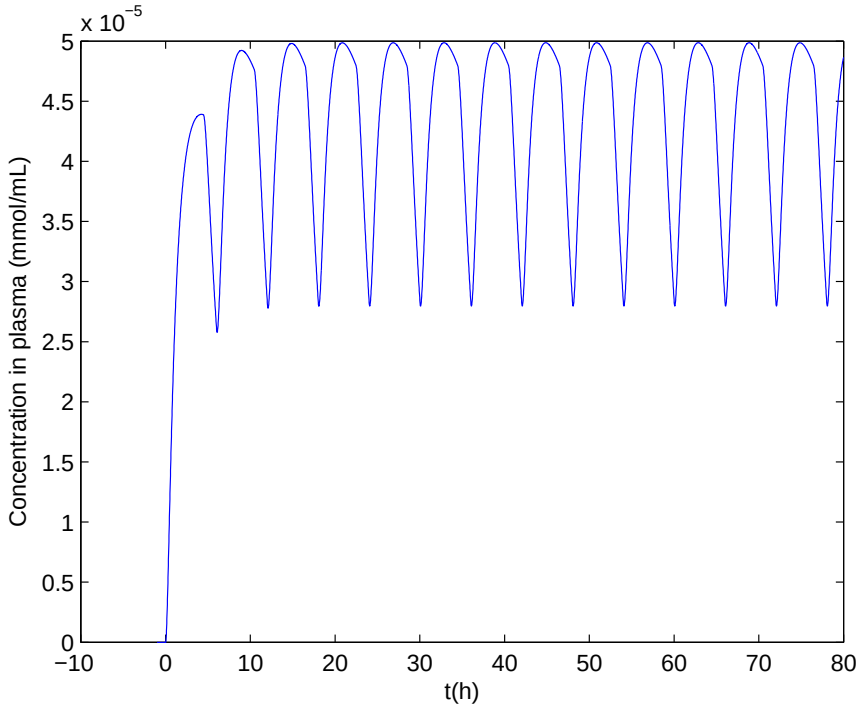

Figure 4.22: Concentration in plasma for the optimal therapeutic plan.

the following optimization problem:

$$\begin{aligned} \Theta^* = \arg\min \quad & \int_{-\infty}^{\infty} (C_{min} - C_\Theta(t)) u(C_{min} - C_\Theta(t)) dt + \lambda n \\ \text{s.t.} \quad & C_\Theta(t) < C_{max} \end{aligned} \tag{4.43}$$

where $u(x)$ is the step (Heaviside) function, n is the number of intakes, and λ is a parameter that balances the relative importance of the two terms being minimized. For instance, let us assume that in our case $C_{min} = 3 \cdot 10^{-5}$ (mmol/mL) and $C_{max} = 5 \cdot 10^{-5}$ (mmol/mL). Let us choose $\lambda = 10^{-5}$. The optimal therapy would be to take 6.45 mmol every 5.84 hours. Since the time interval (5.84 hours) is not realistic, we may set $Y = 6$ (h) and repeat the optimization with $\lambda = 0$. The optimal is found for $X = 6.51$. Fig. 4.22 shows the concentration profile obtained for this plan.

Chapter 5

Discusion

The traditional approach to pharmacokinetics and pharmacodynamics (PKPD) is based on relatively simple linear models with simple dosing regimes. The practitioner is overloaded with tons of equations with different goals (normally, providing initial or final estimates for model parameters or calculating some derived parameter), particular cases (if this parameter is greater than something, then ...) and calculation recipes (fit a line to the first samples, look at the slope of the concentration decay in logarithmic scale). Although, it is true that nonlinear effects are also considered by standard PKPD practitioners, normally they only find an analytical equation for relatively simple dosing regimens. Alternatively, PKPD programs normally offer the possibility of performing nonlinear regression, but starting from a relatively close initial guess of the model parameters. Most PKPD programs are rather fragmented into independent modules implementing specific models and it is not easy to add a physiological effect unforeseen by the program developers. On top of this, we have to add the extremely high price of the program licenses that hinder their widespread use. Biopharmaceutics problems are normally treated separately from PKPD problems, when in practice they are tightly coupled. On the other side, the pharmaceutical industry is increasingly recognizing the importance of mathematical modelling of all these issues and progressively demands more and more professionals with modelling skills. However, given the overall outlook of the current situation given above, these skills are only accessible to a few highly experienced professionals with a wide background education.

In this thesis we have addressed the problems in pharmacokinetics, pharmacodynamics and biopharmaceutics from a holistic, signal processing point of view. The problems are formulated in terms of input signals that are transformed by a system (not necessarily linear) into output signals. Multiple input and output parameters are allowed and they can follow any dosing regimen. The system is first formulated on a continous time basis using differential equations. Then, the system is discretized into a difference equation that is recursively solved using normally previous samples. This is the same approach traditionally followed in numerical analysis for differential equations. The accuracy with which the equation is discretized is variable mostly depending on the complexity of the resulting difference equation. The lost of accuracy comes from the discrete approximation of the derivative. In very sensitive cases in which long simulations are needed, it might be worthy to work with accurate derivative

approximations with which the errors do not accumulate. A brute force approach can fight this effect by reducing the sampling rate (the time difference between two discrete samples). However, as has been shown along the thesis we can control the accuracy of the result by choosing a higher-order discretization method. The complexity obviously increases, but also the payoff in accuracy. In any case, we have shown that it is always possible to construct a discrete, causal system. Additionally, we have shown how the methodology can be applied to a wide variety of physiological situations.

An important issue is the identifiability and sensitivity of the different model parameters. Identifiability depends mostly on four aspects of the problem all of them related to uncertainty: 1) measurement noise: the more accurate our samples are, the easier it is to find the model parameters; 2) the size of the parameter effect: it is difficult to identify the small effects of a parameter, especially, if these small differences are masked by high-levels of noise (in the signal processsing language the relationship between the two is called the Signal-to-Noise Ratio); 3) the search space: small search spaces (tight bounds) on the different parameters reduces the uncertainty on the parameters; 4) parameter independence: dependent parameters are difficult to identify because errors in one of the parameters can be compensated by its related parameters. We have explored several ways of measuring sensitivity. Particularly interesting has been our analysis of Fisher's information matrix because we have learnt that there is a lower bound for the variance of each parameter (no matter how sophisticated is our identification methodology) and because we can design the experiment (when to take blood samples) so that we can collect as much information as possible.

In any case, identification is performed by optimizing some function normally based on some statistical assumption about the distribution of the measurement errors. We may also incorporate *a priori* information about the distribution of the model parameters. We would be, then, under a Bayesian framework. The identification problem is numerically solved by some nonlinear optimization algorithm. The goal function is normally non-convex and for this reason local optimization algorithms normally get trapped in local minima. It is then important to initialize the set of model parameters with values as close as possible to their final values. This can be achieved by spliting the pharmaceutical problem into different subproblems (dissolution, absorption, tissue uptake, ...) and designing laboratory experiments specifically aimed at identifying a small set of parameters. Alternatively, in this thesis we have used global optimization algorithms (in particular, genetic algorithms). Although there exist convergence theorems for some of them, in practice, they may also get trapped in a local minimum, although with a much smaller probability than their local optimizer counterparts.

The methodology developed in the thesis is still compatible with other pharmacokinetics approaches like the non-parametric ones. For those combinations of signals and systems in which it makes sense, we can still report parameters like the Mean-Residence-Time or the Area-Under-the-Curve. However, a parametric methodology supersedes non-parametric approaches by offering a greater explanatory power. Additionally, there are statistical criteria helping to discriminate plausible models from non-plausible or even choosing among a set of different plausible models by likelihood inference tests.

We have implemented a number of classes in Matlab that greatly simplify

the task of adding a new model for testing and provide a graphical interface for it at a very low programming cost. The complex numerical calculations are written in C and bound to Matlab through the Mex capabilities of Matlab. In this way, we can benefit from a fast C code for the heavy numerical calculations while exploiting Matlab libraries for data visualization, graphical interfaces and optimization routines.

Beside system simulation and identification (which we have also illustrated for population PKPD studies), we have shown that there are more applications to the methodology developed along the thesis. In particular, we have shown how to use it to design a therapeutic plan meeting some optimization criteria. Many other applications could be explored like drug-drug interactions, interaction with cellular components, the analysis of biochemical pathways, their control, and systems biology in its more pharmacological aspects.

Summarizing, we have introduced a very general methodology based on an engineering approach capable of handling a wide variety of physiological situations and applications. The methodology lends itself to an easy implementation. The methodology has to be complemented with deep biological and pharmaceutical knowledge that guides its application to specific problems. As in many other scientific fields, once more it is demonstrated that most successful approaches are multidisciplinary.

Conclusions

1. We have developed a general methodology for data analysis in biopharmaceutics, pharmacokinetics and pharmacodynamics.
2. This methodology starts with a differential equation system that is discretized and numerically implemented.
3. We have given special attention to the precision and stability issues of the system.
4. We have studied and proposed solutions for the sensitivity aspects and the selection of the sampling rate within the proposed methodology.
5. We have shown some of the limitations of the traditional approach from the new methodology perspective.
6. The proposed methodology allows the estimation of the parameter uncertainty for any error distribution and any system model.
7. As opposed to the traditional methods, the methodology proposed in this thesis does not require very precise initial guesses of the parameters, it allows handling unobservable parameters and time-varying parameters.
8. It also allows the simultaneous use of multiple administration rputes and measurement methods.
9. We have integrated a wide variety of the existing models in biopharmaceutics, pharmacokinetics and pharmacodynamics into the new framework.
10. The new framework allows the integration of the three disciplines in a single model.
11. We have developed an infrastructure in Matlab and C that allows the rapid development of a new model.
12. We have shown different applications in simulation, system identification, optimal sampling design and posology design.

Conclusiones

1. Se ha desarrollado una metodología general para el análisis de datos en biofarmacia, farmacocinética, y farmacodinamia.
2. Esta metodología parte de un sistema de ecuaciones diferenciales que es discretizado e implementado de forma numérica.
3. Se ha prestado especial atención a la precisión y estabilidad del sistema.
4. Se han estudiado y propuesto soluciones para los aspectos de sensibilidad y selección del periodo de muestreo en el marco propuesto.
5. Se han evidenciado algunas limitaciones de la aproximación tradicional a la luz de la nueva metodología.
6. El marco propuesto permite estimar la incertidumbre para cualquier tipo de distribución de errores y modelo del sistema.
7. A diferencia de los métodos habituales, la solución propuesta en esta tesis no hace necesario estimaciones iniciales de parámetros muy precisas, permite manejar parámetros no observables, y parámetros variables con el tiempo.
8. También permite el uso de diferentes tipos de vías de administración y métodos de medida.
9. Se ha integrado en el nuevo marco una gran variedad de modelos de biofarmacia, farmacocinética y farmacodinamia.
10. La nueva metodología permite integrar en un único problema las tres disciplinas.
11. Se ha desarrollado una infraestructura en Matlab y C que permite desarrollar muy rápidamente un nuevo modelo.
12. Se han mostrado distintas aplicaciones en simulación, identificación de sistemas, diseño de tiempos óptimos de muestreo, y diseño de la posología.

Appendix

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Cost-constrained optimal sampling for system identification in pharmacokinetics applications with population priors and nuisance parameters

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EDICS:	5. BIO-APPL Applications of biomedical signal processing < BIO BIOMEDICAL SIGNAL PROCESSING, 7. BIO-BIOM Biological modeling < BIO BIOMEDICAL SIGNAL PROCESSING, 10. BIO-LSCI Life sciences signal processing < BIO BIOMEDICAL SIGNAL PROCESSING, 11. BIO-MAID Signal processing methods for medical aids < BIO BIOMEDICAL SIGNAL PROCESSING, 30. DSP-SAMP Sampling, extrapolation, and interpolation < DSP DIGITAL AND MULTIRATE SIGNAL PROCESSING

Cost-constrained optimal sampling for system identification in pharmacokinetics applications with population priors and nuisance parameters

C.O.S. Sorzano, M.A. Pérez-de-la-Cruz Moreno, J. Burguet-Castell, C. Montejo, A. Aguilar Ros

***Abstract*—Pharmacokinetics applications can be seen as a special case of non-linear, causal systems with memory. There are cases in which there exists prior knowledge about the distribution of the system parameters in a population. However, for a specific patient in a clinical setting we need to determine her system parameters so that the therapy can be personalized. This system identification is many times performed by measuring drug concentrations in plasma. The objective of this work is to provide an irregular sampling strategy that minimizes the uncertainty about the system parameters with a fixed amount of samples (cost-contrained). We use Monte Carlo simulations to estimate the average Fisher's Information Matrix associated to the pharmacokinetic problem, and then estimate the sampling points that minimize the maximum uncertainty associated to system parameters (a minimax criterion). The minimization is performed employing a genetic algorithm. We show that such a sampling scheme can be designed in a way that is adapted to a particular patient and that it can accommodate any dosing regimen as well as it allows flexible therapeutic strategies.**

***Index Terms*—Irregular sampling, Fisher information matrix, Dynamic systems, Pharmacokinetic time sampling**

I. Introduction

Pharmacokinetics (PK) is the study of the time evolution of the amount of a certain drug in the body as well as its concentration in different tissues and plasma [1]. This evolution is of crucial importance because for many drugs there is a therapeutic window within which the drug is effective (below a certain concentration, the drug has no effect; and above a certain concentration, the drug may become toxic). Following safety recommendations, the therapeutic window is assumed to be the same for all patients. However, each patient has a different response to a certain dose regimen. In fact, drug concentration in plasma can be seen as the output of a non-linear, causal system with memory whose input is the dose applied at each time. In general, it is accepted that the system belongs to a parametric family of systems and that the response of a particular patient corresponds to a particular choice of system parameters. Consequently, personalizing the therapeutic regimen to a particular patient allows to identify here system parameters and particularized dosing regime. Thus, the expected drug concentration in plasma is within the therapeutic window. This is normally done in an intensive care unit for certain pathologies and with drugs whose therapeutic window is relatively tight [2], [3], [4], [5], [6], [7], [8], [9].

In order to determine the patient's parameters, we need to give a first dose (similar to a delta function) and monitor the patient's response (equivalent to her impulse response). This monitorization is performed by extracting blood samples from the patient and analyzing the drug concentration in plasma. For cost reasons and to avoid unnecessary inconveniences to the patient, the number of blood extractions is limited. Additionally, for certain drugs it would be preferrable to be able to administer multiple doses since there are parameters that do not "manifest" their effects at low drug concentration.

The goal of this work is to provide a time sampling basis that, on average over a population, minimizes the maximum uncertainty about any of the system parameters and that can accommodate any dosing regimen. We will presume that the distribution of parameters within the general patient population is known. Then, we will use Monte Carlo simulations to determine which would be the distribution of the Fisher's Information matrix for any sampling scheme. Then, the sampling scheme will be optimized using a global optimization algorithm (in our implementation a genetic algorithm) so that the maximum uncertainty of the worse determined parameter is minimized. If there is a parameter we are particularly interested in, we can minimize instead its uncertainty.

A similar approach has been already proposed [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22] and it is known as D-optimal or C-optimal sampling. Most of these algorithms do differ on the optimization algorithm employed and the use or not of the *a priori* distribution of model parameters. However, our approach differs in a number of points: first, previous approaches presume knowledge of the closed-form solution of the differential equation system being solved, which is not true for any arbitrary dosing regimen; second, our approach easily incorporates random nuisance parameters that do not need to be estimated; third, our goal function is a minimax function which minimizes the maximum variance of any of the parameters, instead of a global measurement of the overall variance. The first two points make an important step forward in the design of the optimal sampling point for highly nonlinear systems. Additionally, our approach can be applied to patient specific parameters instead of providing sampling rules for a general population. This is also an appealing feature of our method since it can be readily used in clinical practice.

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II. METHODS

Most PK models can be described with a first-order linear or non-linear differential equation of the form

$$\frac{d\mathbf{C}}{dt} = \mathbf{f}(t,\mathbf{C},\boldsymbol{\Theta},\boldsymbol{\alpha}) + \mathbf{g}(t,\mathbf{X},\boldsymbol{\Theta},\boldsymbol{\alpha}) \quad (1)$$

where t is the continuous time variable, $\mathbf{C}(t)$ is a vector of concentrations measured at multiple locations (*e.g.*, blood plasma and urine), $\boldsymbol{\Theta}$ is a vector with the model parameters (those that we are interested in determining by the measurement process), $\boldsymbol{\alpha}$ is a vector of nuisance parameters (in which we are not interested but that also affect the concentration levels), and $\mathbf{X}(t)$ is the input driving signal (in our case the dose given to the patient as a function of time; note that this dose is also a vector allowing multiple dosage routes (oral, intravenously, ...)).

The objective of system identification is to find the $\boldsymbol{\Theta}$ parameters from a set of $(t_n, \mathbf{C}_n)$ measurements. This is done by least-squares regression of the model above evaluated at the sampling times, producing the predicted observations $(t_n, \mathbf{C}(t_n))$, and comparing these predictions to the actual measurements $(t_n, \mathbf{C}_n)$. Measurements are supposed to be independent and normally distributed with zero mean and a variance σ_C^2. The variance on its turn depends on the concentration being measured [23]. Concisely, it depends on the assay sensitivity, AS, and the coefficient of variation, CV_{assay},

$$\sigma_C^2 = (AS + CV_{assay}C)^2 \quad (2)$$

It can be proven [24] that the asymptotic Maximum Likelihood Estimate of the system parameters is unbiased and distributed as a Gaussian

$$\hat{\boldsymbol{\Theta}}_{MLE} \sim N\left(\boldsymbol{\Theta}_{true}, I_{\mathcal{T}}^{-1}\right) \quad (3)$$

where $I_{\mathcal{T}}$ is Fisher's information matrix calculated on the N measurements performed at the time points in the set $\mathcal{T}$. Obviously, N must be larger than the number of $\boldsymbol{\Theta}$ parameters, otherwise there would not be any spare degree of freedom to perform the regression, and the fitting would become an interpolation problem highly exposed to measurement errors.

The ij-th element of Fisher's information matrix can be calculated as

$$\begin{aligned} I_{\mathcal{T},ij} &= \sum_{n=1}^{N} \left(\frac{\partial(\mathbf{C}_n - \mathbf{C}(t_n))}{\partial\Theta_i}\right)^T \Sigma_{\mathbf{C}_n}^{-1} \frac{\partial(\mathbf{C}_n - \mathbf{C}(t_n))}{\partial\Theta_j} \\ &= \sum_{n=1}^{N} \left(\frac{\partial\mathbf{C}(t_n)}{\partial\Theta_i}\right)^T \Sigma_{\mathbf{C}_n}^{-1} \frac{\partial\mathbf{C}(t_n)}{\partial\Theta_j} \end{aligned} \quad (4)$$

where $\Sigma_{\mathbf{C}_n}$ is a diagonal matrix whose ii-th entry is the variance associated to the i-th concentration measurement at the n-th time point (Eq. (2)). If we have some *a priori* distribution for the system parameters, as is the case in the problem addressed in this article, we should incorporate this information into the Fisher's Information matrix. For instance, it can be shown [25] that assuming that the parameters are independent and normally distributed amounts to add in the diagonal terms the inverse of the variance of each one of the prior distributions. In this way the diagonal terms become

$$I_{\mathcal{T},ii} = \frac{1}{\sigma_{\Theta_i}^2} + \sum_{n=1}^{N} \left(\frac{\partial\mathbf{C}(t_n)}{\partial\Theta_i}\right)^T \Sigma_{\mathbf{C}_n}^{-1} \frac{\partial\mathbf{C}(t_n)}{\partial\Theta_j}. \quad (5)$$

We need to calculate the terms $\frac{\partial\mathbf{C}(t_n)}{\partial\Theta_i}$. For doing so, let us define the sensitivity with respect to the parameter Θ_i as

$$\mathbf{s}_{\Theta_i} = \frac{\partial\mathbf{C}}{\partial\Theta_i} \quad (6)$$

Obviously, this sensitivity is a vector that depends on t. In [26], [27] a similar derivation was performed for the case of scalar, instead of vector, functions. Let us find a differential equation that the sensitivity must satisfy in order to be able to solve for the sensitivity at any time and, in particular, at the time points t_n. For doing so, we differentiate the previous equation with respect to time

$$\frac{d\mathbf{s}_{\Theta_i}}{dt} = \frac{d}{dt}\left(\frac{\partial\mathbf{C}}{\partial\Theta_i}\right) \quad (7)$$

Assuming that $\mathbf{C}(t)$ is a $\mathcal{C}^2$ function, we can interchange the differentiation order (Clairaut's Theorem) to get

$$\begin{aligned} \frac{d\mathbf{s}_{\Theta_i}}{dt} &= \frac{\partial}{\partial\Theta_i}\left(\frac{d\mathbf{C}}{dt}\right) \\ &= \frac{\partial}{\partial\Theta_i}(\mathbf{f}+\mathbf{g}) \\ &= \frac{\partial\mathbf{f}}{\partial\mathbf{C}}\frac{\partial\mathbf{C}}{\partial\Theta_i} + \frac{\partial\mathbf{f}}{\partial\Theta_i} + \frac{\partial\mathbf{g}}{\partial\Theta_i} \\ &= \frac{\partial\mathbf{f}}{\partial\mathbf{C}}\mathbf{s}_{\Theta_i} + \frac{\partial\mathbf{f}}{\partial\Theta_i} + \frac{\partial\mathbf{g}}{\partial\Theta_i} \end{aligned} \quad (8)$$

Note that the term $\frac{\partial\mathbf{f}}{\partial\mathbf{C}}$ is a full matrix, not a vector. This is an Ordinary Differential Equation with the initial value $\mathbf{s}_{\Theta_i}(t_0) = 0$ [26]. We may use this equation to determine the vectors $\frac{\partial\mathbf{C}(t_n)}{\partial\Theta_i}$ needed by Fisher's Information matrix above. Note that these vectors depend on our estimate of the system parameters, $\hat{\boldsymbol{\Theta}}$, and the nuisance parameters $\boldsymbol{\alpha}$, as well as the time sampling points t_n $(n = 1, 2, ..., N)$. Since these two sets of parameters are random vectors, the sensitivity vectors are also random with a distribution that, in principle, may not be assumed to follow any known distribution (*e.g.*, Gaussian).

As shown in Eq. (3), the uncertainty on the system parameters estimate depend on Fisher's information matrix, which in its turn is also random (since it is calculated using random vectors). So we propose to minimize this uncertainty by choosing a set of N time points, $\mathcal{T}^*$ that minimizes the maximum expected coefficient of variation of the system parameters

$$\begin{aligned} \mathcal{T}^* &= \arg\min_{\mathcal{T}} \max_k \mathrm{E}\{CV_k\} \\ &= \arg\min_{\mathcal{T}} \max_k \mathrm{E}\left\{\frac{\sqrt{\left(I_{\mathcal{T}}^{-1}\right)_{kk}}}{\Theta_k}\right\} \end{aligned} \quad (9)$$

where $(A)_{kk}$ represents the kk-th element of the matrix A. If we are particularly interested in minimizing the uncertainty associated to a particular parameter Θ_k, then we could minimize

$$\mathcal{T}^* = \arg\min_{\mathcal{T}} \mathrm{E}\left\{\frac{\sqrt{\left(I_{\mathcal{T}}^{-1}\right)_{kk}}}{\Theta_k}\right\} \quad (10)$$

In the absence of any *a priori* preference, in the rest of the article we will stick to the first goal function instead of the second.

We propose to estimate this expected value through a Monte Carlo process by which we estimate the distribution of these random variables. For doing so, for each time set $\mathcal{T}$ we simply need to randomly sample the distributions of the vectors $\boldsymbol{\Theta}$ and $\boldsymbol{\alpha}$, estimate $I_{\mathcal{T}}$ and calculate the coefficient of variation

for each system parameter. After repeating this process many times (in our example below, 100 times), we can estimate the mean of the different coefficients of variation and its maximum expected value. Then, we can use a global optimization algorithm to choose the best time set $\mathcal{T}$. In our example below, we use a genetic algorithm [28] as implemented in Matlab Optimization Toolbox, but any other global optimizer may be employed.

III. Results

In order to show the validity of our methodology, we illustrate it with the design of sampling time points for a patient needing phenytoin. Phenytoin is a drug with antiepileptic activity [29], [30]. Its therapeutic window is relatively narrow: a concentration of free drug in plasma below 1 mg/L is ineffective (although it takes several days to reach this level because the maximum intake per day is limited to 15 mg/kg) and above 2 mg/L is toxic [31][Chap. 20]. Additionally, it has a non-linear pharmacokinetics in the therapeutic range. For this reason, it is very important to measure the patient system parameters so that the therapy can be carefully adapted. We must distinguish between free drug in plasma and total drug in plasma. The reason is that a fraction of the total amount of drug is bound to plasma proteins, while another fraction is freely dissolved in plasma. It is the fraction of free drug the one that has a therapeutic effect. Additionally, the measurement assays for free drug are much more accurate that those for the total drug ($AS_{free} = 0.1$ mg/L, $AS_{total} = 1$ mg/L, see Eq. (2); $CV_{assay} = 0.15$ in both cases) [23].

The system dynamics is defined by a constant rate absorption of the drug in the intestine and an enzymatically mediated degradation [23]. The following first order differential equation represents this process

$$V_d \frac{dC(t)}{dt} = -\frac{V_{max} C(t)}{K_m + C(t)} + K_0 \left[u(t) - u\left(t - \frac{Dsb}{K_0}\right)\right] \quad (11)$$

where $C(t)$ is the total concentration of drug in plasma, V_d is the apparent distribution volume (which is normally larger than the volume of plasma due to the binding effect), V_{max} is the maximum degradation rate, K_m is the drug concentration at which half of the maximum degradation rate is attained, K_0 represents the constant rate absorption of the drug, $u(t)$ is the Heaviside step function, D is the administered dose (in mg), s is the tablet salt factor (drugs are many times given in a salt form due to its better dissolution and storage properties), and b is the bioavailability (not all the administered drug is capable of crossing the intestine and hepatic first pass barriers to reach the blood stream). Note that K_0 refers to the amount of drug effectively reaching the blood stream, and $\frac{Dsb}{K_0}$ is the time to exhaust the tablet content.

Since the assay sensitivity for the free drug is much more accurate than that for the total amount of drug (free and bound to plasma proteins), the measurements are aimed to the free drug. The relationship between the concentration of free drug and total concentration of drug is given by [23]

$$C_{free}(t) = F(t) C(t) \quad (12)$$

where

$$F(t) = \frac{1}{1 + f(Cl_{Creatinine}(t)) C_{Albumin}(t)} \quad (13)$$

being $Cl_{Creatinine}(t)$ the clearance of creatinine over time and $C_{Albumin}(t)$ the serum concentration in albumin over time. The clearance of creatinine is, on its turn, estimated to be [32]

$$Cl_{Creatinine} = (0.85)^{female} \frac{(140 - age) LBW}{72 C_{Creatinine}(t)} \quad (14)$$

where $female$ is a variable that takes the value 1 (if the patient is female) or 0 (if it is male), age is the patient's age in years, LBW is the Lean Body Weight (this the total body weight minus the fat weight, because phenytoin does not dissolve in fatty tissues; the lean body weight can be measured using some scales that estimate it by using a current; otherwise it is between 0.9 and 0.7 the total body weight for a non-obese person), and $C_{Creatinine}(t)$ is the serum concentration in creatinine over time. The factor $f(Cl_{Creatinine}(t))$ in Eq. (13) can be calculated as

$$f(x) = \begin{cases} 10^{-4} & 0 \leq x \leq 10 \\ 1.5 \cdot 10^{-4} & 10 < x \leq 24 \\ 1.6 \cdot 10^{-4} & 24 < x \leq 80 \\ 1.9 \cdot 10^{-4} & 80 < x \end{cases} \quad (15)$$

Summarizing, the model on which we will apply our methodology will be

$$\frac{dC_{free}(t)}{dt} = -\frac{1}{V_d} \frac{F V_{max} C_{free}(t)}{F K_m + C_{free}(t)} + \frac{F K_0}{V_d} \left[u(t) - u\left(t - \frac{Dsb}{K_0}\right)\right] \quad (16)$$

where F is a nuisance parameter (the fraction of free drug) calculated using two nuisance parameters (the concentrations of albumin and creatinine).

The whole model has a relatively large number of parameters. Some of them can be accurately measured in a not too invasive way ($female$, age and LBW). Some others like K_0, s and b are assumed to be fixed (with values $K_0 = 0.833$ mg/min, $s = 0.92$ and $b = 0.84$). Finally, the measurement of parameters like $C_{Albumin}(t)$ and $C_{Creatinine}(t)$ would increase the cost of the blood tests required to determine the free drug concentration. They will be treated in this example as nuisance parameters for which an *a priori* distribution will be assumed. This leaves V_d, V_{max} and K_m as the only patient parameters that need to be measured. Consequently, we need to perform four blood tests in order to find these three parameters by weighted least squares regression (the weights are given by the concentration dependent variance of each measurement).

The distribution of the nuisance parameters can be found in the medical literature. For example, the albumin concentration is expected to be between 34 and 54 g/L [33], [34], while the creatinine concentration is expected to be between 8.8 and 11.0 mg/L for women and between 10.0 and 12.9 mg/L for men [35]. In the following, we will assume that the albumin and creatinine serum concentration of a given patient does not change over time.

The *a priori* distribution of the kinetic parameters is also known [23]. For instance, the distribution volume can be

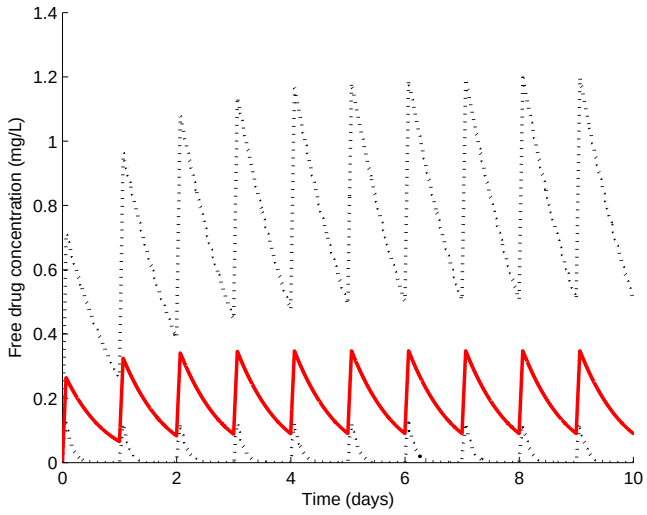

Fig. 1. Solid red line: average response of a 40-years old, male patient of 80 kg and 20% of fat weight to 100 mg. of phenytoin daily. Dashed lines: minimum and maximum responses according to the distribution of nuisance and system parameters.

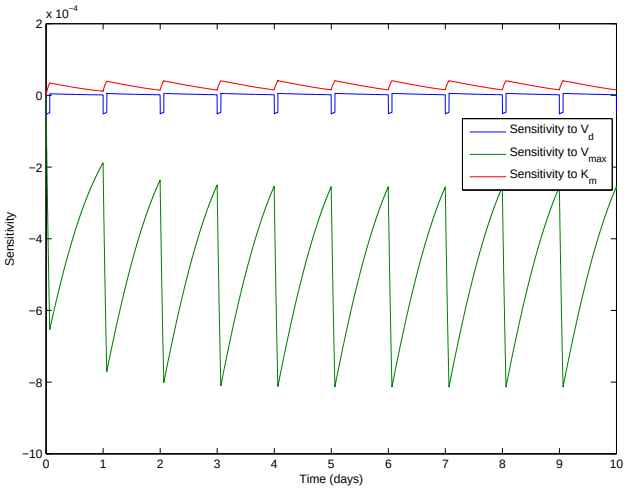

Fig. 2. Sensitivity values to each one of the parameters to be determined for an averagely responding person.

calculated as

$$V_d = BW v_d \quad (17)$$

where BW is the patient's body weight and v_d is the normalized distribution volume between 0.3 and 1.4 L/kg, with a mean of 0.8 L/kg and a standard deviation of 0.16 L/kg. Similarly, the maximum degradation rate can be calculated as

$$V_{max} = BW v_{max} \quad (18)$$

where v_{max} is between 2.48 and 19.84 μg/(kg·min), with a mean of 5.46 μg/(kg·min) and a standard deviation of 1.63 μg/(kg·min). Finally, the concentration at half degradation rate K_m is between 2 and 9 mg/L, with a mean of 5.89 mg/L and a standard deviation of 2.95 mg/L [23].

We will exemplify our methodology with a 40-years old, male patient of 70 kg and 20% of fat weight. Typical responses to a tablet of 100 mg. of phenytoin are depicted in Fig. 1. For the sake of the example, let us say that our plan is to give a patient during 10 days a dose of 100 mg of phenytoin. We have chosen this dosis because in the worse case it does not go above the toxic concentration after 10 days of treatment and it does not exceed the maximal daily dose of 15 mg/kg. However, this dose has to be adjusted to each patient taking into account his gender, age, weight and body fat.

At this point we pose the differential equations of the sensitivity functions

$$\begin{aligned}
\frac{ds_{V_{max}}(t)}{dt} &= \frac{\partial f}{\partial C_{free}}(t) s_{V_{max}}(t) - \frac{1}{V_d}\frac{FC_{free}(t)}{FK_m + C_{free}(t)} \\
\frac{ds_{V_d}(t)}{dt} &= \frac{\partial f}{\partial C_{free}}(t) s_{V_d}(t) + \frac{1}{V_d^2}\frac{FV_{max}C_{free}(t)}{FK_m + C_{free}(t)} \\
&\quad - \frac{FK_0}{V_d^2}\left[u(t) - u\left(t - \frac{Dsb}{K_0}\right)\right] \\
\frac{ds_{K_m}(t)}{dt} &= \frac{\partial f}{\partial C_{free}}(t) s_{K_m}(t) + \frac{1}{V_d}\frac{F^2 V_{max} C_{free}(t)}{(FK_m + C_{free}(t))^2}
\end{aligned} \quad (19)$$

where

$$\frac{\partial f}{\partial C_{free}}(t) = -\frac{F^2 K_m V_{max}}{V_d (FK_m + C_{free}(t))^2}. \quad (20)$$

Fig. 2 shows the sensitivity values for the three parameters V_{max}, V_d and K_m and the average response of the patient in Fig. 1.

We now follow the methodology developed in this article and minimize the maximum coefficient of variation of any of the three system parameters to be determined V_d, V_{max}, and K_m. The methodology suggests to take samples after 7880, 9307, 9439 and 13894 minutes after starting the treatment, or what is the same 5.5, 6.5, 6.6 and 9.6 days. The volume of distribution, V_d, can be determined with an average coefficient of variation of 0.2%, K_m with an average coefficient of variation of 7.1%, and V_{max} with an average coefficient of variation of 1233.3%. The reason why V_{max} is so badly determined is that the administered dose (100 mg, daily) causes a plasma concentration of free drug after 10 days of treatment that is far below the concentration needed to induce the maximum degradation.

This fact suggests a two stage approach to the system identification: in the first stage, of a week of duration, we determine V_d and K_m with a low dose (as we have already done); in the second stage, we increase the dose, in order to faster reach the therapeutic window, and we take extra samples to better determine V_{max}. We illustrate this second phase in this example. In the second phase, of another 10 days, we increase the dose to 300 mg of phenytoin in the morning and 200 mg 12 hours later (the daily maximum for a patient of 70 kg is 1.05 g) so that the therapeutic window can be reached (see Fig. 3). During this second phase our method suggests to take one sample on day 14.5 (minute 20987) so that the coefficient of variation of V_{max} drops to 24.5%;The coefficient of variation of V_d drops to about 1.6% and the one of K_m to about 5.8%. Obviously these optimal sampling minutes can be modified to fit the clinical needs.

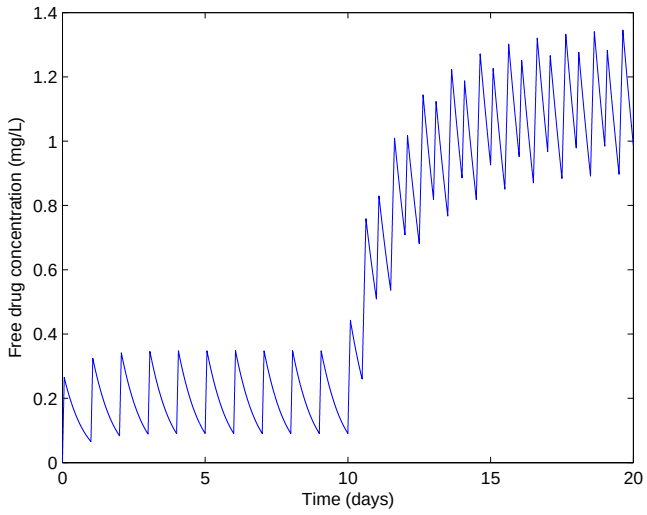

Fig. 3. Free drug concentration after 10 days with a daily dose of 100 mg and 300 mg (morning) and 200mg (evening) after that period.

IV. Conclusions

In this article we have presented a methodology to adapt the sampling time points to any patient taking into account all the *a priori* information available (*a priori* distribution of system and nuisance parameters). The methodology is rather flexible and can deal with any nonlinear pharmacokinetic model, as long as it can be expressed in the form of a differential equation, and any dosing regimen. In this way, we avoid the need of a close-form solution of the model. Notwithstanding, the selection of the time points is perfomed based on a solid theory using Fisher's Information Matrix to maximize the information carried by the measurements on the system parameters. Additionally, we can handle nuisance parameters (parameters that affect the concentration but that cannot be measured) through their distribution in a population. Our theory is valid for multiple simultaneous measurements (plasma, urine, ...), although in our example we have only used plasma concentration. It is our hope that in the future the use of algorithms such as the one presented in this article will help medicine towards a more personalized therapy.

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