Structural Identifiability and Indistinguishability in Mixed-Effects Models

by

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Thesis

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To the memory of Evy and Erik Janzén

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David Janzén

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Declarations

This thesis is the own work of the author. The following articles, poster presentations and oral presentations have been published, are under review or are being prepared to be submitted as a result of the work included in this thesis.

Journal articles

- **D. L. I. Janzén**, M. Jirstrand, M. J. Chappell, N. D. Evans. Structural identifiability of mixed-effects models is dependent on the statistical sub-model. **In preparation.**
- **D. L. I. Janzén**, M. Jirstrand, M. J. Chappell, N. D. Evans. Extending existing structural identifiability analysis methods to mixed-effects models. **Under review.**
- **D. L. I. Janzén**, L. Bergenholm, M. Jirstrand, J. Parkinson, J. Yates, N. D. Evans, M. J. Chappell. Parameter identifiability of fundemental pharmacodynamic models. *Frontiers in Physiology.* 7:590, 2016
- **D. L. I. Janzén**, M. Jirstrand, M. J. Chappell, N. D. Evans. Three novel Approaches to Structural Identifiability Analysis in Mixed-Effects Models *Computer Methods and Programs in Biomedicine.* doi.org/10.1016/j.cmpb.2016.04.024, 2016. In press.
- R. Hendrickx, E. Lamm Bergström, **D. L. I. Janzén**, M. Fridén, U. Eriksson, K. Grime, D. Ferguson. Translational Semi-Physiological Model to Predict

Human Plasma and Lung Pharmacokinetics and Pulmonary Efficacy after Oral Drug Inhalation of Bronchodilators. **In preparation.**

- M. Trägårdh, M. J. Chappell, J. E. Palm, N. D. Evans, **D. L. I. Janzén**, P. Gennemark. Input estimation for extended release formulations of exenatide. *Frontiers in Bioengineering and Biotechnology.* 5:24, 2017

Peer-reviewed conference proceedings

- **D. Janzén**, M. Jirstrand, N.D. Evans, M.J. Chappell, Structural Identifiability in Mixed-Effects Models: Two different approaches Presented at: *9th IFAC Symposium on Biological and Medical Systems* 2015, Berlin, Germany, 31 August - 2 September 2015

Conference oral presentations

- Structural identifiability of mixed-effects models: Methods and insights. *UK Quantitative Systems Pharmacology Network.* Guildford, United Kingdom September 2016.
- Structural Identifiability in Mixed-Effects Models: Two different approaches, *9th IFAC Symposium on Biological and Medical Systems*, Berlin, Germany, September 2015

Conference poster presentations

- Two approaches to study structural identifiability in mixed-effects models, PKUK Chester, United Kingdom November 2015
- Structural Identifiability in Mixed-Effects Models, PKUK Bath, United Kingdom November 2014
- Input Estimation, Dose-Response-Time Modelling and Structural Identifiability as tools for Systems Pharmacology, Life Science Engineering, Chalmers University, Sweden, May 2014

- Input Estimation, Dose-Response-Time Modelling and Structural Identifiability as tools for Systems Pharmacology, 7th Symposium on Pharmacokinetics, Pharmacodynamics and Systems Pharmacology, Noordwijkerhout,The Netherlands. April 2014

- Structural Identifiability and Indistinguishability in Quantitative Systems Pharmacology, Modelling and Simulation Symposium, AstraZeneca Alderley Park, United Kingdom, December 2013

- Structural Identifiability and Indistinguishability in Systems Pharmacology, PKUK Harrogate United Kingdom, October 2013

Abstract

The inverse problem, i.e., estimating parameters in an assumed model structure representing the system of interest, is central in mathematical modelling. Structural identifiability is a prerequisite to successful parameter estimation. If a model is structurally globally identifiable then there exists a unique solution to the inverse problem. Structural indistinguishability relates to the uniqueness of the structures in a set of candidate models. These two closely related concepts are of particular importance in the modelling of biological systems where conclusions are often drawn from the parameter estimates following parameter estimation and where candidate models are used to understand the underlying mechanisms of the biological system.

In this thesis two new definitions of structural identifiability and indistinguishability are presented in which the two concepts have been generalised to now also include the mixed-effects modelling framework which is frequently used in pharmaceutical applications. Several analytical methods applicable to study these concepts in mixed-effects models are presented. These are applicable to any arbitrary mixed-effects models written in state-space form. The developed methods can be used to determine whether the distribution of the set of output functions uniquely, or otherwise, determine the parameter/model structure.

Interesting results have followed from the application of these established techniques to mixed-effects models. It is shown using examples that result from either structural identifiability or indistinguishability analyses of non-mixed-effects models no longer necessarily hold for the corresponding mixed-effects model formulation. This is due to the random effects in the statistical sub-model in three different ways *i)* where the random effects enter into the structural model *ii)* the form of the random effects *iii)* the structure of the covariance matrix related to the random effects. These insights are collected in a set of conjectures.

Several such examples are provided including the well-known unidentifiable one-compartment absorption model whose mixed-effects version is shown to be identifiable depending on the choice of the statistical sub-model.

The contributions from this thesis are thus theoretical, but with direct practical use in a mixed-effects modelling context.

Abbreviations

SGI	Structurally globally identifiable
SLI	Structurally locally identifiable
SU	Structurally unidentifiable
RSE	Relative Standard Error
ODE	Ordinary differential equation
RDE	Random differential equation
θ	Population parameter
η	Random effect
ω	Variance parameter
Ω	Covariance matrix
IV	Intravenous
SC	Subcutaneous
IT	Intratracheal

Chapter 1

Introduction

Mathematical modelling is today an integral part of the process of optimising the development of new pharmaceutical drugs. Such mathematical models are not only used to analyse already gathered experimental data, but also to help design of new, optimal experiments. Since mathematical modelling now plays such a central role, it is important that the models used are reliable. To ensure this, much effort is spent on producing experimental data with high quality, e.g., dense sampling, many subjects, different dose levels etc. However, despite the fact that having a lot of experimental data is necessary for reliable model predictions, it is not sufficient.

The theoretical concept, and subject of this thesis, of structural identifiability is a prerequisite for reliable model predictions. In a structurally identifiable model there exist a unique set of model parameters for every trajectory of the model output function under ideal experimental observations. The related concept of structural indistinguishability, also the subject of this thesis, concerns the uniqueness of the model structure itself for a particular input-output relation. In other words, these two concepts are related to the model structure itself, and not the quality of the experimental data. If a model is structurally unidentifiable, it means that there is a subset of the model parameters that can take on any arbitrary numerical values while the model output function remains unchanged. This in turn means that any biological interpretation of those model parameters is effectively meaningless. If a set of candidate models is structurally indistinguishable it is not possible to conclude

which one of the models is the correct one for application since a generic parameter relation between all models can be established in such a way that the outputs from each candidate model are identical. Details of these two concepts including their mathematical definition, and why they are so important, will be covered in more detail in Chapter 2.

Analytical methods that can determine whether a model is structurally identifiable, or whether a set/pair of models (in the linear/nonlinear case) are structurally indistinguishable exist for models defined in a particular mathematical framework, namely ordinary differential equations in a state-space form. This type of model is referred to as non-mixed-effects model in this thesis to contrast this model type to another modelling framework also written on a state-space form but with a statistical component, as explaned below.

However, a common modelling framework in the pharmaceutical industry is that of mixed-effects models for which no analytical method to study either structural identifiability or structural indistinguishability exist in the literature. In a mixed-effects model, random variables called random effects are associated with the model parameters, resulting in different parameter values for each subject. A form of the distribution of the random effects is postulated in mixed-effects models. Both variance parameters and population parameters are included in the inference problem. Details on the mixed-effects modelling framework are covered in Chapter 2. The lack of methods applicable to study structural identifiability and structural indistinguishability of mixed-effects models is an issue which should be taken seriously, mainly because the results and predictions generated by mixed-effects models are often used as a part of the decision making process as to which direction to take the pharmaceutical drug research development project when moving forward. By using structurally unidentifiable models or structurally indistinguishable models, there is a potential risk of drawing erroneous conclusions about some of the characteristic properties of the pharmaceutical drug under development and potentially also about the biological sub-system in which the drug acts. Therefore, developing analytical methods which can analyse mixed-effects models is of great importance in

order to increase the reliability of the predictions drawn from mixed-effects models in pharmaceutical drug research development projects.

1.1 Aims, objectives

The aims of this thesis are thus to expand the concept of structural identifiability and structural indistinguishability from non-mixed-effects models to mixed-effects models and to develop new methods and approaches that can perform such analysis. For this purpose, the following objectives were set

- Develop formal mathematical definitions of what structural identifiability and structural indistinguishability mean in a mixed-effects framework.
- With respect to the developed mathematical definitions, develop analytical methods applicable to study structural identifiability and structural indistinguishability of mixed-effects models.
- Apply the developed methods to commonly used mixed-effects model structures in a pharmaceutical context.
- Explore how structural identifiability and structural indistinguishability analysis results from non-mixed-effects models translates to the mixed-effects case using the developed methods in this thesis.
- Investigate how random effects associated with different parameters, different covariance structures, and different forms of the distribution of the random effects affect the structural identifiability and structural indistinguishability of a mixed-effects model.
- In addition to considering mixed-effects models, a set of non-mixed-effects models will analysed from a structural identifiability perspective.

1.2 Thesis outline

In Chapter 2, a review of the relevant background literature is provided. The results and insights concluding the main outcomes from this thesis work are presented in Chapters 3–8.

In Chapter 2, an introduction as to why mathematical modelling is a particularly useful tool while developing new pharmaceutical drugs will be provided. Both the advantages and limitations of mathematical modelling in this context will be discussed which will lead into the main topic of the thesis. The mathematical definitions of structural identifiability and structural indistinguishability will be given as well as previously published methods on how to analyse non-mixed-effects models with respect to these two concepts.

In Chapter 3, a set of non-mixed-effects models will be analysed in a structural identifiability context. These models come from four different collaborative projects in which structural identifiability analysis was performed. This analysis was also performed to demonstrate existing methodologies and their application to other models in the IMPACT EU project of which this thesis forms a component.

In Chapter 4, the concept of structural identifiability and structural indistinguishability is generalised to also include mixed-effects models. Two more general mathematical definitions of structural identifiability and structural indistinguishability of mixed-effects models are presented. The new definitions are more general in the sense that identifiability of non-mixed effects models is a special case of identifiabiltiy, i.e., when all variance parameters are set to zero. Five methods applicable to the study of structural identifiability and indistinguishability in this framework are presented.

In Chapter 5, the developed methods for the study of the structural identifiability of mixed-effects models presented in Chapter 4 will be applied to a set of mixed-effects models to illustrate how they can be applied in practice. Examples of structurally globally, locally and unidentifiable mixed-effects models are given. Each method developed is applied to at least one model.

In Chapter 6, the question whether structural identifiability analysis results

translate from the non-mixed-effects case to the mixed-effects case is addressed, i.e., whether a locally identifiable/unidentifiable non-mixed-effects model implies a locally identifiable/unidentifiable mixed-effects model. To answer this question, the methods presented in Chapter 4 are applied to a set of mixed-effects models where the non-mixed-effects versions are known to be structurally globally/locally identifiable and unidentifiable. The effects of how different covariance structures, different forms of distributions of the random effects and where the random effects enter the structural model affect the structural identifiability of the model are all investigated and explored. In particular, it is shown that unidentifiable or locally identifiable non-mixed-effects models may become locally or even globally identifiable in a mixed-effects framework. It is also shown that under certain special conditions, otherwise unidentifiable variance parameters become globally identifiable with a non-zero covariance parameter with some other random effect whose variance parameter is globally identifiable. The insights presented in this chapter are collected in a set of conjectures.

In Chapter 7, a structural indistinguishability analysis of a set of mixed-effects models is presented. Again, the methods developed for structural indistinguishability analysis presented in Chapter 4 are applied to show how they work in practice. The question regarding whether structural indistinguishability results of non-mixed-effects models translates directly or otherwise to the mixed-effects case will be addressed with these examples.

In Chapter 8, general conclusions and discussion about the presented work are given. Suggestions for future potential research projects extending the results presented are also given. The focus in this thesis has been on mixed-effects models with application in the pharmaceutical industry. However, the developed novel methods are generic in nature and can therefore be applied to analyse mixed-effects models of any system, e.g., ecology. This generic nature of the developed methods will in this last chapter also be discussed to some extent.

Chapter 2

Background

2.1 Mathematical modelling in the pharmaceutical industry

Mathematical modelling is a very useful tool when characterizing properties of any system. In the pharmaceutical industry, the system of interests is the interaction between pharmaceutical drugs and the human body. This interaction is typically divided into two parts: *i)* Pharmacokinetics (PK), which is about what the body does to the drug, i.e., the distribution of the drug in different organs and tissues over time. *ii)* Pharmacodynamics (PD), which is about what the drug does to the body, e.g., lowered blood pressure, lowering of glucose levels in the blood. Mathematical modelling of these two types is called PKPD-modelling and is routinely applied at all stages of the model-based drug development process (Milligan et al. [2013]).

In contrast to engineering systems such as, e.g., electric circuits or aircraft systems, the main challenge when modelling a biological system is often the limitation of available experimental data. While time-continuous measurements are often possible in a technical system, experimental data from animals or humans are often quite sparse. This is partly due to practical reasons, and partly because of restrictions due to ethical guidelines. In addition to the challenge of having sparse data is the problem of variability, something which is always present in biological systems.

A modelling framework developed specifically to handle these types of challenges is called mixed-effects modelling, which will be presented in Section 2.3. Despite the challenges of experimental data, mathematical modelling as a tool to optimise the development process of new pharmaceutical drugs is useful.

There are a several advantages of using mathematical modelling when developing new pharmaceutical drugs. Firstly, modelling can be used to *characterise* the properties of a drug. With the use of modelling and experimental data the PKPD behaviour of a drug can be represented by the parameter estimates. These parameter estimates can then be compared and contrasted to other parameters from different compounds. Secondly, a model can be used for the *prediction* of scenarios which may not have been tested experimentally. This would typically include simulating the model for different dosing schedules to fully characterise the dose-response relationship. Simulation can also be used for optimal experimental design, i.e., designing experiments that will yield maximal information with respect to cost. Thirdly, modelling can be used to *understand* how the underlying biological mechanisms work. If there are several competing hypotheses regarding the nature of the mechanisms of the biological system of interest, those hypotheses can be formalised into different candidate models. If a candidate model can be shown through simulation to not be able to describe the available experimental data, then the corresponding hypothesis has been incorrect and thus a deeper understanding of the system has been gained. From this standpoint, additional experiments may be designed which can further eliminate candidate models, something which can be repeated until ideally only one candidate model remains.

A model in its most general form can be summarised as depicted in Figure 2.1, where there is an input function $\boldsymbol{u}(t)$ and an output function $\boldsymbol{y}(t)$ to and from a system $\mathcal{S}$ respectively. In PKPD-modelling, a common scenario is that the input function $\boldsymbol{u}(t)$ is an intravenous (IV) infusion of a pharmaceutical drug A, $\mathcal{S}$ is the human body, and $\boldsymbol{y}(t)$ is measurement of the concentration of the drug A in the blood plasma. The system $\mathcal{S}$ can be represented by a postulated parameterised model written in a state-space form, which will be defined in Section 2.2. The

different parts of the system are represented by the states $\boldsymbol{x}$. Together with the model parameters, they make up the representation of the system. By observing the input-output relation between $\boldsymbol{u}(t)$ and $\boldsymbol{y}(t)$ from the experimental data, numerical values of the model parameters can be inferred in the parameter estimation step using different optimization algorithms.

An important part of the modelling process is to assess the reliability and usefulness of the model after the parameter estimation. Having a model that can describe the available experimental data does not necessarily make the model reliable in the sense that it may not be able to predict the system behaviour under non-tested experimental conditions. A common way of model validation is to divide up the available experimental data into a training data set and a validation data set, often with the ratio two thirds to one third, (Cock et al. [2010]), although this may vary across different disciplines. First the training data set is used in the parameter estimation. Then the predictability of the model is checked by evaluating if the model can describe the validation data set. If so then the model is often considered sufficiently reliable for its purpose. Related to model reliability and model validation is parameter identifiability, which is the main topic of this thesis. Parameter identifiability in the structural sense is a prerequisite to parameter estimation. An introduction to parameter identifiability will be given in Section 2.4.

2.2 State-space modelling

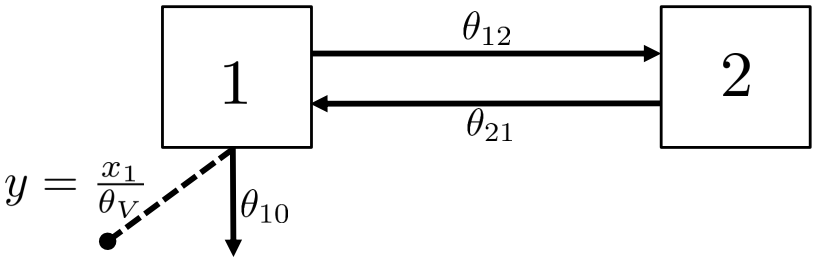
In this section an overview will be given of state-space models as well as the mathematical notation for such models that will be used throughout this thesis.

2.2.1 Introduction

A *state-space model* is a system of first order differential equations (ODE's) where the model states $\boldsymbol{x}(t)$ represent and describe the evolution of the system, with or without some perturbation of the system from an input function $\boldsymbol{u}(t)$. Typically only a subset of the model states are observed from the output functions denoted

Figure 2.1: A system with an input function $\boldsymbol{u}(t)$ and an output function $\boldsymbol{y}(t)$ represented in its most general form.

Figure 2.2: Two-compartment model with an observation of and a linear elimination from compartment 1.



by $\boldsymbol{y}(t)$.

As an example of a model written in a state-space form, consider the two-compartment model in Figure 2.2 with the following structure

$$\begin{aligned} \dot{x}_1 &= -(\theta_{10} + \theta_{12})x_1 + \theta_{21}x_2 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ x_1(0) &= x_0 \\ x_2(0) &= 0 \end{aligned} \tag{2.1}$$

with observation

$$y = \frac{x_1}{\theta_V} \tag{2.2}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (\theta_{10}, \theta_{12}, \theta_{21}, \theta_V). \tag{2.3}$$

By measuring the concentration of the drug in the blood plasma, i.e., y, knowledge can be inferred about the profile of both the observed state x_1 and the unobserved state x_2, as well as the model parameters $\boldsymbol{\theta}$. In a PKPD setting the state x_1 represents the amount of drug in the plasma while x_2 represents the amount of drug in the rest of the body. The model parameters consists of four rate constants $\{\theta_{10}, \theta_{12}, \theta_{21}\}$ and one volume scaling parameter θ_V.

2.2.2 Mathematical definition

A state-space model is written in the following form

$$\begin{aligned} \dot{\boldsymbol{x}}(t) &= \boldsymbol{f}(\boldsymbol{x}(t), \boldsymbol{u}(t), \boldsymbol{\theta}) \\ \boldsymbol{x}(t_0) &= \boldsymbol{x}_0(\boldsymbol{\theta}) \\ \boldsymbol{y}(t) &= \boldsymbol{h}(\boldsymbol{x}(t), \boldsymbol{u}(t), \boldsymbol{\theta}) \end{aligned} \tag{2.4}$$

where $\boldsymbol{x}(t) \in \mathbb{R}^n$ is the state, $\boldsymbol{u}(t) \in \mathbb{R}^q$ is the input, $\boldsymbol{\theta} \in \mathbb{R}^p$ is the vector of the model parameters, $\boldsymbol{y}(t) \in \mathbb{R}^m$ is the output and $\boldsymbol{f}$ and $\boldsymbol{h}$ are smooth rational functions.

2.3 Mixed-effects state-space model

In this section an overview of the mixed-effects mathematical modelling framework will be given.

2.3.1 Introduction

A typical data set involving experiments with a new pharmaceutical drug contains repeated measurements from different subjects. A naive way of analysing such data

is by the so called standard two-stage approach (STS) (Sheiner [1984]). In the STS approach, data from each subject are considered, using a state-space model of the form (2.4), as a separate inference problem. Once individual parameter estimates are obtained from each subject the mean and variability in the population of the model parameters is computed. This approach works reasonably well when the experimental data is densely sampled with relative low signal-to-noise ratio (Karlsson et al. [2015]). However, if the data on an individual level are sparsely sampled then the individual parameters are in general not well-determined. This will in turn lead to erroneous conclusions regarding the population mean and variability.

In a mixed-effects modelling framework (2.5), both the individual parameters from all subjects and the statistical population parameters are estimated in a joint inference problem, (Davidian [1995]). By doing so, the problem of sparsely sampled data on an individual level is minimised, since such a framework allows, in some sense, a sharing of information between all individuals. The framework also allows for estimation of different sources of variability, such as between-subject variability (BSV), inter-study variability (ISV) and intra-occasion variability (IOV), (Karlsson and Sheiner [1993], Bonate [2011]). In addition, by using mixed-effects modelling it is possible to detect and quantify covariates, i.e., subgroups in the population with some common characteristic, such as sex, age etc., whose response following administration of a pharmaceutical drug is statistically significantly different from the rest of the population (Hennig and Karlsson [2014]). Due to the reasons mentioned above, mixed-effects modelling is often considered to be the most advantageous modelling approach when developing new pharmaceutical drugs.

2.3.2 Mathematical definition of a mixed-effects model

By a mixed-effects model we mean a system written in the following form

$$\begin{aligned} \dot{\boldsymbol{x}}_i(t) &= \boldsymbol{f}(\boldsymbol{x}_i(t), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \\ \boldsymbol{x}_i(t_0) &= \boldsymbol{x}_0(\boldsymbol{\phi}_i) \\ \boldsymbol{y}_i(t) &= \boldsymbol{h}(\boldsymbol{x}_i(t), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \end{aligned} \tag{2.5}$$

where

$$\boldsymbol{\phi}_i = \boldsymbol{g}(\boldsymbol{\theta}, \boldsymbol{\eta}_i, \mathbf{C}_i) \tag{2.6}$$

are the parameters for the i:th subject, where $i = 1, 2, \ldots$ and

$$\boldsymbol{\eta}_i \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{2.7}$$

are the random effects where $\boldsymbol{\Omega}$ is the covariance matrix of the random effects $\boldsymbol{\eta}_i$, $\boldsymbol{\theta}$ are the population parameters and $\mathbf{C}_i$ are the covariates for the different subjects in the population.

An example of a simple one-compartment mixed-effects model with a lognormal distribution of the elimination parameter, Figure 2.3, with the initial condition D has the following structure

$$\begin{aligned} \dot{x}_1 &= -\theta e^{\eta} x_1 \\ x_1(0) &= D \end{aligned} \tag{2.8}$$

with observation

$$y = x_1 \tag{2.9}$$

and normally distributed random effect

$$\eta \sim N(0, \sqrt{\omega}). \tag{2.10}$$

2.4 Structural Identifiability

In this section the concept of structural identifiability will be introduced. The mathematical definition of what structural identifiability means for a non-mixed-effects state-space system written in the form (2.4) will be given. Previously established methods applicable to study structural identifiability of models written in such a form will be presented.

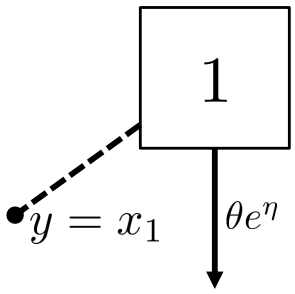


Figure 2.3: The one-compartment mixed-effects model with linear elimination.

2.4.1 Introduction

The parameters in a model often have a biological meaning within the system such as rate constants or saturation levels. Since these parameters most often can not be measured directly, an indirect measurement is instead used by applying a model to experimental data to infer what those parameter values are. When the parameter estimation step is done, biological interpretations of the estimates can be made.

However, in order for the biological interpretations of the parameter estimates to be reliable the concept of *parameter identifiability* must be taken into consideration. There are two types of parameter identifiability: *Practical identifiability* (Raue et al. [2009]; Galvanin et al. [2013]) and *structural identifiability* (Villaverde et al. [2016]), also referred to as *a priori* identifiability (Audoly et al. [2001]). Practical identifiability is sometimes also discussed using the term sloppiness (Chis et al. [2016]; Gutenkunst et al. [2007]) or *a posteriori* identifiability (Balsa-Canto et al. [2010]). The two concepts are related in the sense that they both affect the reliability of the model parameters, but they do this from different perspectives and there are therefore certain important key differences between them.

To study practical identifiability experimental data are required. This is because practical identifiability considers how the amount and quality of the experimental data are translated into the uncertainty of both the parameter estimates and the subsequent model predictions. If the model parameters after parameter estimation have sufficiently small standard deviations the model is said to be practically

identifiable (S. Hengl and Maiwald [2007]). However, there are issues related to only rely on the estimates of the standard deviations of the parameters, (Aoki et al. [2014, 2015]; Janzén et al. [2016]). It is important to quantify how well-determined the parameter estimates are following the parameter estimation as this will influence the weight of insights from the modelling efforts. If the uncertainty in the model parameters is considered to be too high, the most common approach to solve this problem is to perform additional experiments, for instance with more frequent sampling or using a larger group of subjects. Although including additional experimental data in the inference problem will often reduce the parameter uncertainty, there are cases where the parameter uncertainty remains unacceptable despite the addition of new experimental data.

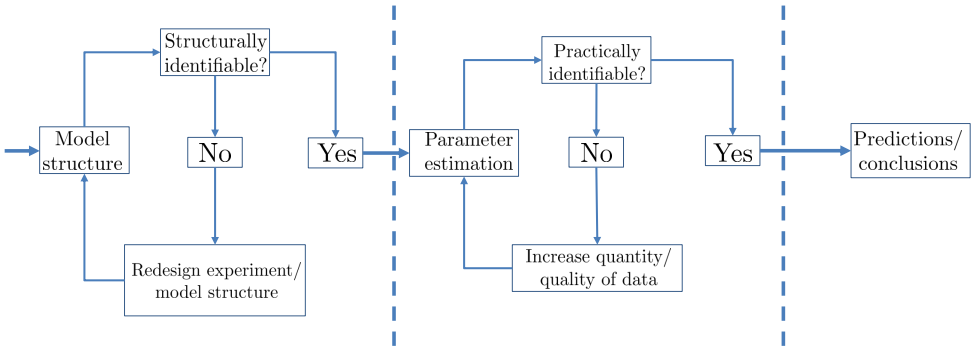
Structural identifiability considers the parameter identifiability under ideal experimental conditions, i.e., with noise-free and time-continuous data (Godfrey [1985]). Under such conditions the determinability of the model parameters is then only dependent on the model structure itself along with the inputs and outputs for the model. For some combinations of input and output functions the model parameters may be determined, where for other combinations some of the model parameters cannot be determined. If the model parameters cannot be determined under ideal experimental conditions it follows that they cannot be determined under less ideal conditions either, i.e., real experimental conditions. Structural identifiability is therefore a prerequisite for successful parameter estimation and for practical identifiability.

If a model is *structurally unidentifiable*, a subset of the model parameters $\boldsymbol{\theta}$ can take on an uncountable number of different numerical values for the experiments(s) while the model output $\boldsymbol{y}(t)$ remains unchanged. A structurally unidentifiable parameter is therefore effectively meaningless in a biological sense as it can take on an uncountable number of different numerical values while the model is still able to describe the experimental data. It is therefore crucial to know whether a model parameter is identifiable or unidentifiable before drawing any biological conclusions from its numerical estimates. Furthermore, if a model is structurally identifiable

it can be either *structurally globally identifiable* or *structurally locally identifiable*. In a structurally globally identifiable model, the model parameters can be determined uniquely, meaning that for every input-output map there exist a unique set of parameters. Having a structurally globally identifiable model is the best case scenario. The second best case scenario, but often sufficient, is a locally identifiable model. In such a model, there exist a countable number of distinct parameter sets for every input-output map and is thus a necessary condition for global identifiability (Rothenburg [1971]). In addition, it is sometimes possible to exclude some of the solutions from a structurally locally identifiable model by setting up parameter constraints derived from prior biological knowledge, e.g., parameter $\theta_1 > \theta_2$. If a model is structurally unidentifiable then a structural identifiability analysis can inform on what parameter combinations, i.e., the functional forms, are identifiable (Eisenberg and Hayashi [2014]; Meshkat et al. [2009, 2011, 2014]; Chappell and Gunn [1998]; Evans and Chappell [2000]; Gunn et al. [1997]; Denis-Vidal and Joly-Blanchard [2004]; Cheung et al. [2012]). Such an analysis can also inform on what additional measurements and/or inputs are required in order to achieve structural identifiability (Chapman et al. [2003]; Godfrey et al. [1980]).

Unfortunately, structural identifiability is an often overlooked concept despite its importance. As depicted in Figure 2.4, the structural identifiability of a model needs to be determined before moving on to parameter estimation, practical identifiability analysis, sensitivity analysis, robustness analysis, model predictions and conclusions. The reason for this is because if no structural identifiability analysis has been performed, there is no way of knowing whether the source of parameter uncertainty comes from a lack of information in the experimental data or from the structure itself. Theoretically, having a practically identifiable model implies that the model is also structurally identifiable. However, because of how a practical identifiability analysis is performed this is not true in practice. For instance, in the inference problem either a global or local optimisation routine is used. If a local optimisation routine is used, then by definition, only a subset of the parameter space is explored. Since no true global optimisation routine exists, since this would re-

Figure 2.4: Workflow of model development with a structural identifiability analysis as a prerequisite to the parameter estimation step.



quire exploring the entire parameter space, which is infinite, using a global routine does not prove structural identifiability either. Other problems with using practical identifiability techniques as a way of proving structural identifiability are numerics, how things are implemented etc. Also, by definition, it is dependent on a particular data set whereas a structural identifiability analysis deals with the generic case.

To illustrate the issue of structural identifiability with a simple example, consider a one-compartment model with the following model structure

$$\begin{aligned} \dot{x}(t) &= -\theta_1 x(t) + \theta_2 u(t) \\ x(0) &= 0 \end{aligned} \tag{2.11}$$

with observation

$$y(t) = \theta_3 x(t) \tag{2.12}$$

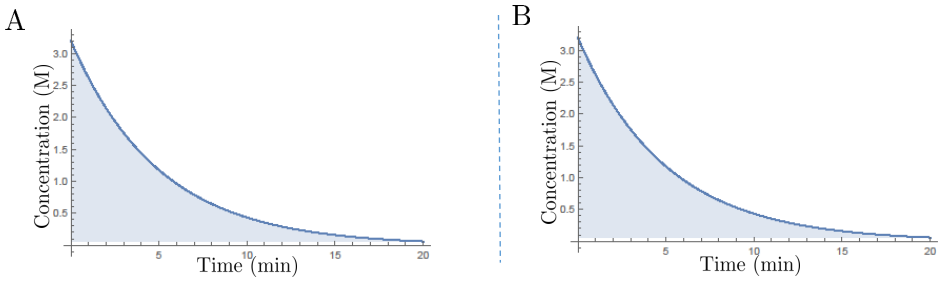
with the unknown model parameters

$$\boldsymbol{\theta} = (\theta_1, \theta_2, \theta_3) \tag{2.13}$$

and input function $u(t)$ and output function $y(t)$. Differentiating the model output $y(t)$ with respect to time t yields the following expression

$$\dot{y}(t) = \theta_3 \dot{x}(t) = -\theta_1 \theta_3 x(t) + \theta_2 \theta_3 u(t). \tag{2.14}$$

Figure 2.5: Two simulations of the model with different parameter values. The used parameter values are listed in Table 2.1. The outputs are identical and thus illustrating the identifiability problem.



The state variable $x(t)$ can be eliminated from (2.14) by substituting $\theta_3 x(t)$ with $y(t)$. The input-output relation is therefore

$$\dot{y}(t) + \theta_1 y(t) - \theta_2 \theta_3 u(t) = 0. \tag{2.15}$$

From the coefficients of the terms in the input-output relation it is clear that parameter θ_1 can be determined but only the product of $\theta_2\theta_3$ can be determined. The model is therefore structurally unidentifiable. This is examplified with two simulations with $u(t) = \delta(t)$ in Figure 2.5 where the parameter values for θ_2 and θ_3 are different in Figure 2.5A and Figure 2.5B, but the numerical value of θ_1 and the product $\theta_2\theta_3$ is still the same. To render the model structurally identifiable, some prior knowledge about either θ_2 or θ_3 must be used so that one of them can be fixed.

Table 2.1: Parameter values that was used for model (2.11) when simulating the model, Figure 2.5. The product $\theta_1\theta_2$ is the same for parameter set A and B which results in identical outputs from the model.

Parameter set	θ_1	θ_2	θ_3
A	0.2	0.8	0.2
B	0.2	0.2	0.8

As can be seen, the example model above is very simple and the subsequent structural identifiability analysis required minimal computation where simply a visual inspection of the input-output relation was sufficient to determine identifiabil-

ity of the model parameters. Even for only slightly more complex model structures the analytical analysis becomes non-trivial. There are several different approaches available that can be used to study structural identifiability of models written in the state-space form as in equation (2.4). Some of the available structural identifiability analysis methods can be used to include the whole (feasible) parameter space and can therefore be used to show structural global identifiability. Other methods can be used only to show whether a model is structurally locally identifiable or not. Obviously, showing that a model is structural locally identifiable does not rule out that the model is structurally globally identifiable if a local identifiability analysis methods has been used. In addition, some methods are only applicable to linear models where others can be applied to nonlinear models as well.

The *Laplace transform approach* was first introduced in Bellman and Åström [1970] and is only applicable to linear models. The *Taylor series expansion approach* was first introduced in Pohjanpalo [1978] and is applicable to both linear and nonlinear models but suffers from expensive computations for already relatively simple model structures. The *Similarity transformation approach*, Vajda et al. [1989], has different versions for linear and nonlinear systems. There are also various approaches to generate the input-output form (Bearup et al. [2013]; Meshkat et al. [2012, 2011]; Bellu et al. [2007]; Ljung and Glad [1994]; Meshkat et al. [2014]) from which the structural identifiability of the model can be studied.

If a model turns out to be structurally unidentifiable a structural identifiability analysis can also be used to determine what is required to make the model structurally identifiable. A model can change from being structurally unidentifiable to being structurally identifiable by either reparametrisation or including additional output functions by assuming additional measurements of something else in the system. Sometimes the lack of identifiability may come from an inadequate input function, which is also something that a structural identifiability analysis will show. A structural identifiability analysis should therefore also be regarded both as a helpful and necessary tool when designing experiments, see again Figure 2.4. However, it is worth reiterating that structural identifiability is a prerequisite for successful

parameter estimation, but not a guarantee.

Unfortunately, there is no straight forward way of deciding what identifiability analysis approach is in general the best. It is also often not possible to predict what method is the most appropiate one for a particular model. Using methods that can generate answer to whether a model is structurally globally or otherwise is the prefered choice. However, such an analysis may result in inconclusive results due to the analytical expressions growing exponentially in size. In such cases it should be attempted to apply methods that can at best prove local identifiability, for instance the EAR approach (Karlsson et al. [2012]) presented later in this chapter. In Chis et al. [2011a] a comprehensive review of some of the most common identifiability approaches is presented and compared. In that publication, a combination of the generating series approach (Walter and Lecourtier [1982]) and the identifiability tableaus (Balsa-Canto et al. [2010]) is concluded to, on average, be the best approach to study structural identifiability with respect to applicability range, computational demand and concluding results.

2.4.2 Definition of Structural Identifiability for Non-Mixed-Effects Model

Definition 1. Let the generic parameter vector $\boldsymbol{\theta}$ belong to a feasible parameter space $\boldsymbol{\Theta}$ such that $\boldsymbol{\theta} \in \boldsymbol{\Theta}$. Let $y(t, \boldsymbol{\theta})$ be the output function from a state-space model (2.4). Further, consider a parameter vector $\bar{\boldsymbol{\theta}}$ where $y(t, \boldsymbol{\theta}) = y(t, \bar{\boldsymbol{\theta}})$ for all t. If this equality, in a neighbourhood $N \in \boldsymbol{\Theta}$ of $\boldsymbol{\theta}$, implies that $\boldsymbol{\theta} = \bar{\boldsymbol{\theta}}$ then the model is *structurally locally identifiable*. If $N = \boldsymbol{\Theta}$ then the model is *structurally globally identifiable*. For a structurally unidentifiable parameter θ_i, every neighbourhood N around θ_i has a parameter vector $\bar{\boldsymbol{\theta}}$ where $\theta_i \neq \bar{\theta}_i$ that gives rise to identical input-output relations.

2.4.3 Methods

In this section structural identifiability methods applicable to models written in the state-space form (2.4) will be outlined.

Taylor Series Expansion

The Taylor series expansion as an approach to study structural identifiability was first introduced by Pohjanpalo [1978]. As mentioned above, ideal experimental conditions are assumed in a structural identifiability analysis. This means that both the output function $\boldsymbol{y}(t)$ and all the higher order derivatives of the output function $\boldsymbol{y}(t)$ are theoretically measurable and known. They are all unique for a particular output which is utilised in the Taylor series approach. For a component i, the Taylor series expansion of $y_i(t, \boldsymbol{\theta})$ around a known time point, typically the initial condition $t = 0$, is

$$y_i(t, \boldsymbol{\theta}) = y_i(0, \boldsymbol{\theta}) + y_i^{(1)}(0, \boldsymbol{\theta})\frac{t}{1!} + y_i^{(2)}(0, \boldsymbol{\theta})\frac{t^2}{2!} + \cdots + y_i^{(k)}(0, \boldsymbol{\theta})\frac{t^k}{k!} + \ldots \tag{2.16}$$

By equating the coefficients in equation (4.68) as

$$\begin{aligned} y_i(0, \boldsymbol{\theta}) &= y_i(0, \bar{\boldsymbol{\theta}}) \\ &\vdots \\ y_i^{(k)}(0, \boldsymbol{\theta}) &= y_i^{(k)}(0, \bar{\boldsymbol{\theta}}) \end{aligned} \tag{2.17}$$

gives an equation system with $k + 1$ equations where $\bar{\boldsymbol{\theta}}$ is an alternative parameter vector. If there is only one solution for the model parameters in this system (2.17), the model is structurally globally identifiable. If there are several, but finite number of solutions the model is structurally locally identifiable. If the system has no solution the model is structurally unidentifiable. However, proving that a model is locally identifiable or unidentifible with the Taylor series expansion approach is often difficult. This is because the upper bounds, i.e., the number of nessecary coefficients in the Taylor series expansion in order to prove local identifiability or unidentifiability, must be reached. Even for relatively simple model structures, the computations often proves to be intractable.

For linear systems the upper bound is $2n-1$ Vajda [1982], for bilinear systems

$2^{2n}-1$ Vajda [1987], for homogeneous polynomial systems $\frac{s^{2n}-1}{s-1}$ Vajda [1987] and for a generic state-space model on the form (2.4) the upper bound is $n+p$ Margaria et al. [2001] where n, p, and s is the number of states, number of parameters and the degree of the polynomial respectively.

Laplace Transform approach

Using the Laplace transform approach, also called the transfer function approach, to study structural identifiability of systems written in a state-space form was first introduced in Bellman and Åström [1970]. The method is limited to the study of structural identifiability of linear systems in the state-space form

$$\begin{aligned} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) &= \boldsymbol{A}(\boldsymbol{\theta})\boldsymbol{x}(t) + \boldsymbol{B}(\boldsymbol{\theta})\boldsymbol{u}(t) \\ \boldsymbol{x}(0,\boldsymbol{\theta}) &= \boldsymbol{x}_0(\boldsymbol{\theta}) \\ \boldsymbol{y}(t,\boldsymbol{\theta}) &= \boldsymbol{C}(\boldsymbol{\theta})\boldsymbol{x}(t) \end{aligned} \tag{2.18}$$

where $\boldsymbol{A}(\boldsymbol{\theta})$, $\boldsymbol{B}(\boldsymbol{\theta})$ and $\boldsymbol{C}(\boldsymbol{\theta})$ are the system matrices. Assuming for simplicity, without loss of generality, a system where the initial conditions of the model states are set to zero, i.e., $\boldsymbol{x}(0,\boldsymbol{\theta}) = 0$, and instead are incorporated into $\boldsymbol{B}(\boldsymbol{\theta})\boldsymbol{u}(t)$. The Laplace transformation of the system is then given by the input-output relation

$$\boldsymbol{Y}(s) = \boldsymbol{G}(s)\boldsymbol{U}(s) \tag{2.19}$$

where

$$\boldsymbol{G}(s) = \boldsymbol{C}(\boldsymbol{\theta})(s\boldsymbol{I} - \boldsymbol{A}(\boldsymbol{\theta}))^{-1}\boldsymbol{B}(\boldsymbol{\theta}) \tag{2.20}$$

is the transfer function matrix of the system. Assuming, again without loss of generality, a single output system. The elements of the transfer function matrix can then be written in the following form

$$G(s) = \frac{b_1(\boldsymbol{\theta})s^{n-1} + \cdots + b_{n-1}(\boldsymbol{\theta})s + b_n(\boldsymbol{\theta})}{s^n + a_1(\boldsymbol{\theta})s^{n-1} + \cdots + a_{n-1}(\boldsymbol{\theta})s + a_n(\boldsymbol{\theta})}. \tag{2.21}$$

The coefficients of the powers of s in the numerator and denominator are called moment invariants (Godfrey and Chapman [1989]) and are unique with respect to the input-output relationship. Let

$$\boldsymbol{\sigma}(\boldsymbol{\theta}) = (a_1(\boldsymbol{\theta}), \ldots, a_n(\boldsymbol{\theta}), b_1(\boldsymbol{\theta}), \ldots, b_n(\boldsymbol{\theta})) \tag{2.22}$$

be the *exhaustive summary* (Walter [1982]) of a model written in the form (2.18) and where $\sigma_k(\boldsymbol{\theta})$ denotes the k:th element in $\boldsymbol{\sigma}(\boldsymbol{\theta})$. If a model is structurally identifiable then the model parameters can be deduced from the exhaustive summary $\boldsymbol{\sigma}(\boldsymbol{\theta})$. By equating and solving

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \ldots, N \tag{2.23}$$

the structural identifiability of a linear state-space system can be studied. If the equation system (2.23) only has one solution for the model parameters $\boldsymbol{\theta}$ the model is structurally globally identifiable. If the equation system has a countable number of solutions it is structurally locally identifiable. If there exists an uncountable number of solutions the model is structurally unidentifiable.

Similarity transformation/exhaustive modelling approach for linear models

The similarity transformation approach, also called the exhaustive modelling approach, provides a way of finding a transformation between two systems while still preserving the input-output relation (Cheung et al. [2013]). Given a linear system $\mathcal{S}$, the similarity transformation approach (Walter [1982]) can be used to generate all linear models that have the same input-output relation as $\mathcal{S}$.

However, in order for the similarity transformation to be applicable the model needs to be *locally reduced*, also called *minimal*. For a model to be minimal it needs to be both *observable* and *controllable*. A sufficient and necessary condition respectively that needs to be satisfied is the *Observability Rank Criterion* (ORC) and the *Controllability Rank Criterion* (CRC).

Therefore, checking whether these two conditions are satisfied provides a means to check whether the model is minimal. The ORC for a linear model written in the form as in 2.18 is satisfied if the observability matrix $\mathcal{O}$ has full rank, i.e.,

$$\mathcal{O} = Rank \begin{pmatrix} \mathbf{C}(\boldsymbol{\theta}) \\ \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta}) \\ \vdots \\ \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta})^{n-1} \end{pmatrix} = n \tag{2.24}$$

where n is the number of model states. The CRC is satisfied if the controllability matrix $\mathcal{C}$ has full rank, i.e.,

$$\mathcal{C} = Rank \begin{pmatrix} \mathbf{B}(\boldsymbol{\theta}) & \mathbf{A}(\boldsymbol{\theta})\mathbf{B}(\boldsymbol{\theta}) & \cdots & \mathbf{A}(\boldsymbol{\theta})^{n-1}\mathbf{B}(\boldsymbol{\theta}) \end{pmatrix} = n \tag{2.25}$$

For a minimal system there exists a transformation $\boldsymbol{T}$ between two systems $(\boldsymbol{A}(\boldsymbol{\theta}), \boldsymbol{B}(\boldsymbol{\theta}), \boldsymbol{C}(\boldsymbol{\theta}))$ and $(\boldsymbol{A}(\bar{\boldsymbol{\theta}}), \boldsymbol{B}(\bar{\boldsymbol{\theta}}), \boldsymbol{C}(\bar{\boldsymbol{\theta}}))$ with the same input-output relation which satisfies

$$\begin{aligned} \boldsymbol{T}\boldsymbol{A}(\boldsymbol{\theta}) &= \boldsymbol{A}(\bar{\boldsymbol{\theta}})\boldsymbol{T} \\ \boldsymbol{T}\boldsymbol{B}(\boldsymbol{\theta}) &= \boldsymbol{B}(\bar{\boldsymbol{\theta}}) \\ \boldsymbol{C}(\boldsymbol{\theta}) &= \boldsymbol{C}(\bar{\boldsymbol{\theta}})\boldsymbol{T} \end{aligned} \tag{2.26}$$

where $\mathbf{T}$ is a nonsingular $n \times n$ matrix. If the only solution of (2.26) is $\boldsymbol{T}$ being the identity matrix $\boldsymbol{I}_n$ then no transform of the system $\boldsymbol{A}(\boldsymbol{\theta}), \boldsymbol{B}(\boldsymbol{\theta}), \boldsymbol{C}(\boldsymbol{\theta})$ can be performed without changing the input-output relation and the system is therefore structurally globally identifiable. If there exists a finite set of element vectors that $\boldsymbol{T}$ can take then the model is structurally locally identifiable, otherwise the model is structurally unidentifiable.

Similarity transformation/exhaustive modelling approach for nonlinear models

The similarity transformation approach was extended in Vajda et al. [1989] to include non-linear systems as well. The method was developed and presented in Vajda

et al. [1989] and is based on the *local state isomorphism theorem* (Sussman [1977], Vajda and Rabitz [1989]).

Under the similarity transformation approach, all state-variable transformations of a particular system that leave both the input-output relation and the model structure invariant are sought (Chis et al. [2011b]). Consider a nonlinear system written in the following form

$$\begin{aligned} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) &= f(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) + \boldsymbol{u}(t)g(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{y}(t,\boldsymbol{\theta}) &= h(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{x}(0,\boldsymbol{\theta}) &= \boldsymbol{x}_0(\boldsymbol{\theta}) \end{aligned} \tag{2.27}$$

where $\boldsymbol{x}(t,\boldsymbol{\theta}) \in \mathbb{R}^n$, $\boldsymbol{y}(t,\boldsymbol{\theta}) \in \mathbb{R}^m$ and $\boldsymbol{\theta} \in \mathbb{R}^q$. By using the local state isomorphism theorem, a set of first order inhomogeneous partial differential equations can be derived which in turn are used to construct the functional form of the transformations which leave both the input-output relation and the model structure the same. The utilisation of the local state isomorphism theorem is presented in Proposition 2 in Vajda and Rabitz [1989] which will here be restated.

Theorem 2. *Consider $\boldsymbol{\theta}$, $\bar{\boldsymbol{\theta}} \in \Omega$, an open neighbourhood V of $\boldsymbol{x}_0(\bar{\boldsymbol{\theta}})$ in R^n, and any analytical map $\boldsymbol{\lambda}$: $V \to R^n$ defined on V such that*

$$\boldsymbol{\lambda}(\boldsymbol{x}_0(\bar{\boldsymbol{\theta}})) = \boldsymbol{x}_0(\boldsymbol{\theta}) \tag{2.28}$$

$$rank\frac{\partial \boldsymbol{\lambda}(\bar{\boldsymbol{x}})}{\partial \bar{\boldsymbol{x}}} = n \tag{2.29}$$

$$\boldsymbol{f}(\lambda(\bar{\boldsymbol{x}}),\boldsymbol{\theta}) = \frac{\partial \boldsymbol{\lambda}(\bar{\boldsymbol{x}})}{\partial \bar{x}} \boldsymbol{f}(\bar{\boldsymbol{x}},\bar{\boldsymbol{\theta}}) \tag{2.30}$$

$$\boldsymbol{g}(\boldsymbol{\lambda}(\bar{\boldsymbol{x}}),\boldsymbol{\theta}) = \frac{\partial \boldsymbol{\lambda}(\bar{\boldsymbol{x}})}{\partial \bar{\boldsymbol{x}}} \boldsymbol{g}(\bar{\boldsymbol{x}},\bar{\boldsymbol{\theta}}) \tag{2.31}$$

$$\boldsymbol{h}(\boldsymbol{\lambda}(\bar{\boldsymbol{x}}),\boldsymbol{\theta}) = \boldsymbol{h}(\bar{\boldsymbol{x}},\bar{\boldsymbol{\theta}}) \tag{2.32}$$

for all $\bar{x} \in V$. If the model is structurally globally identifiable the only solution that fulfils the conditions (2.28)–(2.32) *is $\boldsymbol{\theta} = \bar{\boldsymbol{\theta}}$ for which $\boldsymbol{\lambda}(\bar{\boldsymbol{x}}) = \bar{\boldsymbol{x}}$. If there exists a countable number of solutions to* (2.28)–(2.32) *then the system is structurally locally identifiable. If no solutions exist then the system is structurally unidentifiable.*

An additional property of the similarity transformation approach for nonlinear systems is that if $\boldsymbol{\lambda}(\tilde{\boldsymbol{x}})$ cannot be expressed explicitly, it cannot be determined via the similarity transformation approach whether the system is identifiable or not, (Chappell and Godfrey [1990]).

Similarily as for the linear case, nonlinear models also must be locally reduced in order for the similarity transformation approach to be applicable. To determine if a nonlinear model is minimal the ORC and the CRC for nonlinear models can be used. Checking the ORC and the CRC requires the application of Lie algebra and Lie derivatives which may become quite computationally demanding.

Determining whether a nonlinear system is observable or not can be done by computing the rank of the observability rank matrix $\mathcal{O}$. If the rank of the observability matrix $\mathcal{O}$ is full the system is observable. For a nonlinear system, the application of Lie derivatives is necessary to compute both the observability matrix and the controllability matrix (August and Papachristodoulou [2009]).

Input-Output approach: Characteristic sets

Consider a system written in the following form

$$\begin{aligned} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) &= f(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{y}(t,\boldsymbol{\theta}) &= h(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{x}(0,\boldsymbol{\theta}) &= \boldsymbol{x}_0(\boldsymbol{\theta}). \end{aligned} \tag{2.33}$$

A model of the form as in (2.33) can be rewritten in an input-output form, meaning that the model states $\boldsymbol{x}$ can be substituted for the output function $\boldsymbol{y}$ and its higher derivatives resulting in an expression as

$$g(\boldsymbol{y}(\boldsymbol{\theta}),\dot{\boldsymbol{y}}(\boldsymbol{\theta}),\ldots,\boldsymbol{y}^{(n-1)}(\boldsymbol{\theta})). \tag{2.34}$$

One way of deriving this expression is to compute the characteristic sets of the model, see Ljung and Glad [1994], with some suitable ranking of the variables. One

member of the characteristic sets is the input-output relation. By introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$ and equating two input-output relations as

$$g(\boldsymbol{y}(\boldsymbol{\theta}), \dot{\boldsymbol{y}}(\boldsymbol{\theta}), \ldots, \boldsymbol{y}^{(n-1)}(\boldsymbol{\theta})) = g(\boldsymbol{y}(\bar{\boldsymbol{\theta}}), \dot{\boldsymbol{y}}(\bar{\boldsymbol{\theta}}), \ldots, \boldsymbol{y}^{(n-1)}(\bar{\boldsymbol{\theta}})) \tag{2.35}$$

and solve for $\boldsymbol{\theta}$, the structural identifiability of the model can be determined. If (2.35) has a unique solution for $\boldsymbol{\theta}$ then the model is structurally globally identifiable. If there exist a countable number of solutions for $\boldsymbol{\theta}$ the model is structurally locally identifiable. If an uncountable number of solutions exists the model is structurally unidentifiable. However, it is noted in Saccomani et al. [2003] that identifiability analysis of models using differential algebra approaches may fail, i.e., give an incorrect answer, when the model is analysed at specific initial conditions. This is because in such cases the wrong exhaustive summary is derived.

Input-Output approach: Algebraic relation

An alternative approach to generating the input-output relation of a model written in the form (2.33) has been developed in Evans et al. [2013]. By computing a set of Lie derivatives of the output function $\boldsymbol{y}$ and using them as inputs in the Groebner Bases algorithms in Maple, see Grandjean [2013] and Forsman [1991], the input-output relation can be generated. Again by introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$, equating the two input-output relations as in (2.35), and solve for $\boldsymbol{\theta}$ the structural identifiability of the model can be determined.

Input-Output approach: Observable Normal Form

A third way of generating the input-output relation of a model written in the form (2.33) is by a co-ordinate transformation of the system to a form called Observable Normal Form (Evans et al. [2013]).

The outline of the Observable Normal Form is given in Evans et al. [2013]

and will be restated here. Let $\boldsymbol{f^p} = \boldsymbol{f}(\cdot, \boldsymbol{\theta})$ and define the vector field

$$\boldsymbol{H_\theta}(\boldsymbol{x}) = (\mu_1(\boldsymbol{x}, \boldsymbol{\theta}), \ldots, \mu_n(\boldsymbol{x}, \boldsymbol{\theta}))^T \tag{2.36}$$

where

$$\mu_1(\boldsymbol{x}, \boldsymbol{\theta}) = h(\boldsymbol{x}, \boldsymbol{\theta}) \tag{2.37}$$

$$\mu_{i+1}(\boldsymbol{x}, \boldsymbol{\theta}) = L_{f^p}\mu_i(\boldsymbol{x}, \boldsymbol{\theta}) \qquad i = 1, \ldots, n. \tag{2.38}$$

Then we have that

$$z = \boldsymbol{H_\theta}(\boldsymbol{x}) \tag{2.39}$$

is a co-ordinate transformation of the system (2.33) into the Observable Normal Form

$$\dot{z}(t, \boldsymbol{\theta}) = z_{i+1}(t, \boldsymbol{\theta}) \quad i = 1, \ldots, n-1 \tag{2.40}$$

$$\dot{z}_n(t, \boldsymbol{\theta}) = \mu_{n+1}(\boldsymbol{H_\theta^{-1}}(\boldsymbol{z}(\boldsymbol{\theta}, t)), \boldsymbol{\theta}). \tag{2.41}$$

where the input-output relation can be found in the final state equation $\dot{z}_i(\boldsymbol{\theta}, t)$. By introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$, equating the two input-output relations as

$$\dot{z}_n(t, \boldsymbol{\theta}) = \dot{z}_n(t, \bar{\boldsymbol{\theta}}) \tag{2.42}$$

and solving for $\boldsymbol{\theta}$ the structural identifiability of the system can be determined.

Exact Arithmetic Rank

The Exact Arithmetic Rank (EAR) approach was presented in Karlsson et al. [2012] and is based on a method for local observability by Sedoglavic [2002]. The output function of the model is iteratively differentiated with respect to time. It has been shown in Anguelova [2007] that the number of necessary derivatives is $n + d - 1$, as

higher order derivatives are algebraically dependent on the lower order derivatives. By applying the *inverse function theorem* (Krantz and Parks [2013]) to the system of equations, the rank of the Jacobian of the system of the equations informs on the identifiability of the model. If the rank of the Jacobian is full, the model is at least structurally locally identifiable. If the Jacobian matrix is rank deficient, the model is structurally unidentifiable.

The EAR approach is implemented as a Mathematica function where the user simply defines the model structure, unknown parameters and potentially parameterised initial conditions as arguments to a function which will return whether the model is at least locally identifiable. The EAR approach is a hybrid generic/numeric approach and as such it is very computationally efficient and is therefore suitable for large systems as shown in Raue et al. [2014]. The computational efficiency is due to the randomly instantiation of integers for the model parameters and initial states. The input function is a truncated integer coefficient power series. In the Mathematica implementation there is also an option, based on the work in Anguelova et al. [2012], to compute minimal output sets necessary and sufficient for identifiability.

2.5 Structural Indistinguishability

In this section the problem of structural indistinguishability will be presented along with methods applicable to study it.

2.5.1 Introduction

If a set of candidate models represents different hypotheses on the possible structure of the underlying biological mechanisms can take, then it is important to know whether the available measurements are sufficient to distinguish between different candidate models.

This problem is called *structural indistinguishability* (Godfrey and Chapman [1989]; Godfrey et al. [1994]; Collins and King [1991]; Evans et al. [2004]; Godfrey

and Chapman [1990]). Structural identifiability is neither a necessary nor sufficient condition for indistinguishability (Walter et al. [1984]). While structural identifiability concerns whether the model parameters can be globally/locally identified given a model structure and experiments with perfect input-output data, a structural indistinguishability analysis studies whether two mathematical models with different structures can produce the same input-output map.

It is possible to generate all structurally indistinguishable linear models with the same number of compartments (Collins and King [1991]; Walter and Lecourtier [1981]). However, for the nonlinear case this is not possible. Instead, only pairwise comparisons between nonlinear models can be performed (Godfrey et al. [1994]).

2.5.2 Definition

Definition 3. Consider the following two nonlinear systems

$$\boldsymbol{\Sigma}(\boldsymbol{\theta}) = \begin{cases} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) = \boldsymbol{f}(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{u}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{y}(t,\boldsymbol{\theta}) = \boldsymbol{h}(\boldsymbol{x}(t,\boldsymbol{\theta}),\boldsymbol{\theta}) \\ \boldsymbol{x}(0,\boldsymbol{\theta}) = \boldsymbol{x}_0(\boldsymbol{\theta}) \end{cases} \tag{2.43}$$

$$\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}}) = \begin{cases} \dot{\bar{\boldsymbol{x}}}(t,\bar{\boldsymbol{\theta}}) = \bar{\boldsymbol{f}}(\bar{\boldsymbol{x}}(t,\bar{\boldsymbol{\theta}}),\bar{\boldsymbol{u}}(t,\bar{\boldsymbol{\theta}}),\bar{\boldsymbol{\theta}}) \\ \bar{\boldsymbol{y}}(t,\bar{\boldsymbol{\theta}}) = \bar{\boldsymbol{h}}(\bar{\boldsymbol{x}}(t,\bar{\boldsymbol{\theta}}),\bar{\boldsymbol{\theta}}) \\ \bar{\boldsymbol{x}}(0,\bar{\boldsymbol{\theta}}) = \bar{\boldsymbol{x}}_0(\bar{\boldsymbol{\theta}}) \end{cases} \tag{2.44}$$

where $\boldsymbol{x}(t,\boldsymbol{\theta}) \in \mathbb{R}^n$ and $\bar{\boldsymbol{x}}(t,\bar{\boldsymbol{\theta}}) \in \mathbb{R}^n$ is the state, $\boldsymbol{u}(t,\boldsymbol{\theta}) \in \mathbb{R}^r$ and $\bar{\boldsymbol{u}}(t,\bar{\boldsymbol{\theta}}) \in \mathbb{R}^r$ is the input, $\boldsymbol{y}(t,\boldsymbol{\theta}) \in \mathbb{R}^m$ and $\bar{\boldsymbol{y}}(t,\bar{\boldsymbol{\theta}}) \in \mathbb{R}^m$ is the output, $\boldsymbol{\theta} \in \boldsymbol{\Theta} \subset \mathbb{R}^q$ and $\bar{\boldsymbol{\theta}} \in \bar{\boldsymbol{\Theta}} \subset \mathbb{R}^d$ where $\boldsymbol{\Theta} \subset \mathbb{R}^q$ and $\bar{\boldsymbol{\Theta}} \subset \mathbb{R}^d$ is the set of possible parameter values in a feasible parameter space. The two systems $\boldsymbol{\Sigma}(\boldsymbol{\theta})$ and $\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}})$ are said to be *output indistinguishable*, denoted $\boldsymbol{\Sigma}(\boldsymbol{\theta}) \sim \bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}})$, if $\boldsymbol{y}(t,\boldsymbol{\theta}) = \bar{\boldsymbol{y}}(t,\bar{\boldsymbol{\theta}})$. If for generic $\boldsymbol{\theta}$ there exists a $\bar{\boldsymbol{\theta}}$ so that $\boldsymbol{\Sigma}(\boldsymbol{\theta}) \sim \bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}})$ and at the same time for some generic $\bar{\boldsymbol{\theta}}$ there exists a $\boldsymbol{\theta}$ so that $\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}}) \sim \Sigma(\boldsymbol{\theta})$ then the two systems are *structurally indistinguishable*.

2.5.3 Methods

Since structural identifiability and indistinguishability are related, i.e., structural identifiability is a special case of indistinguishability (Evans et al. [2004]; Walter and Pronzato [1996]; Walter et al. [1984]), the methods to study indistinguishability are modifications of the methods used to study identifiability.

Laplace Transform approach

The Laplace transform approach to study structural indistinguishability involves computing and equating the moment invariants from the two (or more) models and solve for $\boldsymbol{\theta}$. If a generic relation between the model parameters from the investigated models can be found from equating the moment invariants without any contradictions, e.g., some model parameters needed to be equal to zero, then the two models are structurally indistinguishable (Bonate [2011]).

Taylor series expansion

The Taylor series expansion can be used to study structural indistinguishability by equating the coefficients in the Taylor series expansion of the output function $\boldsymbol{y}(t, \boldsymbol{\theta})$ and $\bar{\boldsymbol{y}}(t, \bar{\boldsymbol{\theta}})$ from the two models, see Hattersley et al. [2011]. The number of coefficients included in the comparison between the models is often less than the number of parameters as either relations between the parameters from the two models are established, or contradictions are found, e.g., some parameter from one of the investigated models needs to be equal to zero.

Input-Output approach

By rewriting two models to an input-output form, using either the characteristic sets, algebraic relation or co-ordinate transform, structural indistinguishability can be studied. By equating the corresponding coefficients in the two input-output relations from the two models, and attempting to derive generic relations between the model parameters from the two models it can be shown whether the two are structurally indistinguishable. Similarly, if equality between the coefficients from

the input-output relation from the two models implies some contradiction, then the models are structurally distinguishable.

Chapter 3

Structural identifiability analysis of non-mixed-effects models

3.1 Introduction

As introduced in Chapter 2, structural identifiability is a prerequisite to successful parameter estimation. Although the main focus within this thesis is on the development of new methods and techniques to study structural identifiability of mixed-effects models, structural identifiability of non-mixed-effects models has also been considered to a certain extent. However, for non-mixed-effects models no work has been done on method development in this thesis. Instead, structural identifiability analysis has been performed in a number of collaborative projects. The contribution from this work in these collaborations has been the structural identifiability analysis and is presented below. In these four collaborative projects structural identifiability of the following were considered

- A set of routinely used pharmacodynamic models
- A lung slice model

- A five-compartment lung PK-model
- Estimation of an unknown input function for nonlinear system.

More details of each of these collaborative projects will be given below as well as the main results from the structural identifiability analysis in each project.

In addition to presenting new structural identifiability analysis results of model structures that have never been analysed before, this chapter also serves the purpose as a bridge to the main results of this thesis: structural identifiability and structural indistinguishability in mixed-effects models. By presenting how a structural identifiability analysis of a non-mixed-effects model is undertaken the author hopes that the addition of the statistical sub-model in the mixed-effects case and the presented methods applicable to mixed-effects models will be easier to understand.

3.2 Structural identifiability analysis of state-space models

3.2.1 Pharmacodynamic models

As has been described briefly in Chapter 2, pharmacodynamic models, or PD-models, aims to capture the effect a pharmaceutical drug has on the body, e.g., lowering heart rate, killing cancer cells etc. Often it is the characteristics of the dose-response relation that is sought using a PD-model, i.e., the amount of drug that is required to produce a particular level of response as well as finding the saturation level. This relation often involves capturing the temporal aspects, or delays, of drug effects and for this reason several different models may exist. However, the issue of structural identifiability is often overlooked when such models are used. In Janzén et al. [2016], a total of 16 models, Table 3.2, was analysed from a structural identifiability perspective. These investigated models have three different sources of time delays: biophase distribution, receptor binding and signal transduction. Although often used in practice for parameter estimation, no structural identifiability

analysis of these models has ever been published, as far as the author is aware. A consequence of this is that it is not known whether it is theoretically possible to distinguish the source of the time delay between drug concentration and drug effect. The 16 analysed models are different combinations of the models shown in Figure 3.1 and their explicit model structure is listed in Table 3.2.

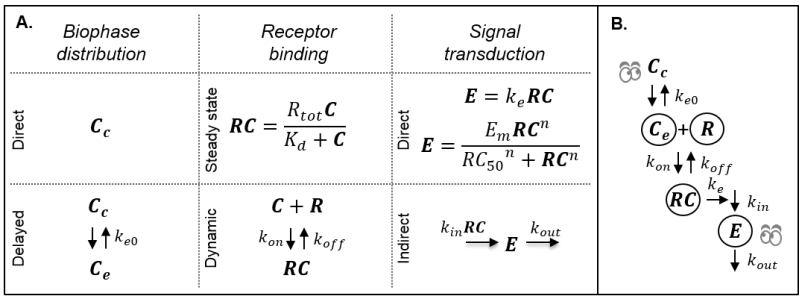


Figure 3.1: Combinations of the different sub-models of biophase distribution, receptor binding and signal transduction analysed from a structural identifiability perspective.

Structural identifiability analysis example 1

All of the 16 PD-models considered were analysed using the input-output approach, Bearup et al. [2013], except for models 6, 10 and 14 where it was not possible to generate an input-output relation. For these three models, the EAR approach, Karlsson et al. [2012], was used instead. Including the details of all of the analysis for each model would take up too much space. Instead, for brevity two examples of an analysis of the models is here instead presented. Model 16 from Table 3.2 was chosen since the analysis itself is relatively straightforward and should be easy to follow. Model 2 from Table 3.2 was chosen since an augmentation of the system was necessary in order to apply the input-output approach and thus exemplifying that structural identifiability analysis often requires problem specific hands-on solutions.

The first example is model 13 from Table 3.2 which has the following structure

$$\begin{aligned} \dot{x}_1 &= k_{e0}(u - x_1) \\ \dot{x}_2 &= k_{on}(R_{tot} - x_2)x_1 - k_{off}x_2 \\ x_1(0) &= 0 \\ x_2(0) &= 0 \end{aligned} \tag{3.1}$$

with observation

$$y = k_e x_2 \tag{3.2}$$

with the unknown parameter vector

$$\boldsymbol{\theta} = (k_{e0}, k_e, k_{on}, k_{off}, R_{tot}) \tag{3.3}$$

where u is the PK-profile assumed to be known, x_1 is the state representing the artificial effect compartment, x_2 is the state representing the receptor complex and R_{tot} is the total number of receptors. By iteratively differentiating the output function y and eliminating the state variables x_1 and x_2 the model can via substitution be rewritten in the following input-output form

$$\begin{aligned} &y\ddot{y} - {R_{tot}}^2 u k_e^2 k_{e0} k_{on} - 2\, R_{tot}\, u k_e k_{e0} k_{on} y + R_{tot}\, k_e k_{e0} k_{off} y - \\ &u k_{e0} k_{on} y^2 + R_{tot}\, k_e k_{e0} \dot{y} + R_{tot}\, k_e k_{off} \dot{y} + k_{e0} k_{off} y^2 + \\ &R_{tot}\, k_e \ddot{y} + k_{e0} y \dot{y} - \dot{y}^2 = 0. \end{aligned} \tag{3.4}$$

The structural identifiability of such a model can be studied by considering the coefficients in the input-output form of the model. By introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$ and collecting the coefficients in the input-output form as

$$\sum_{k=1}^{l} c_k(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}}) \phi_k(y(t, \boldsymbol{\theta}), \dot{y}(t, \boldsymbol{\theta}), \ddot{y}(t, \boldsymbol{\theta}), \dots) = 0 \tag{3.5}$$

the structural identifiability of model (3.1) can be analysed by solving the following

system of coefficient equations:

$$c_1(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}}) = k_{e0} - \bar{k}_{e0} = 0 \tag{3.6}$$

$$c_2(\boldsymbol{\theta}, \boldsymbol{\theta}) = R_{tot}k_e - R_{tot}^2 u k_e^2 k_{e0} k_{on} - (\bar{R}_{tot}\bar{k}_e - \bar{R}_{tot}^2 u \bar{k}_e^2 \bar{k}_{e0}\bar{k}_{on}) = 0 \tag{3.7}$$

$$c_3(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}}) = R_{tot}(k_e k_{e0} + k_e k_{off}) - \bar{R}_{tot}(\bar{k}_e\bar{k}_{e0} + \bar{k}_e\bar{k}_{off}) = 0 \tag{3.8}$$

$$c_4(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}}) = -u k_{e0} k_{on} + k_{e0} k_{off} - (-u\bar{k}_{e0}\bar{k}_{on} + \bar{k}_{e0}\bar{k}_{off}) = 0 \tag{3.9}$$

$$\begin{aligned} c_5(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}}) = &-R_{tot} u k_e k_{e0} k_{on} + R_{tot} k_e k_{e0} k_{off} - \\ &(-\bar{R}_{tot} u \bar{k}_e \bar{k}_{e0} \bar{k}_{on} + \bar{R}_{tot} \bar{k}_e \bar{k}_{e0} \bar{k}_{off}) = 0. \end{aligned} \tag{3.10}$$

In a structural identifiability analysis a visual inspection can sometimes be enough in order to be able to determine that a model is structurally unidentifiable. Model 13 from Table 3.2 is a good example of this. In the equation system (3.6)–(3.10) above it can seen that the two parameters R_{tot} and k_e allways appear together either as a product $R_{tot}k_e$ or as a squared product $(R_{tot}k_e)^2$. From this observation it can be concluded that model 13 is structurally unidentifiable. In order to see whether the product $R_{tot}k_e$ can be determined the R_{tot} parameter was fixed, i.e.,

$$R_{tot} = \bar{R}_{tot}. \tag{3.11}$$

and the subsequent analysis was performed using this assumption. Equation (3.6) has only one solution, namely

$$k_{e0} = \bar{k}_{e0}. \tag{3.12}$$

Equation (3.9) can then be rewritten as

$$-u k_{e0} k_{on} + k_{e0} k_{off} = (-u k_{e0}\bar{k}_{on} + k_{e0}\bar{k}_{off}) \tag{3.13}$$

$$k_{e0}(-u k_{on} + k_{off}) = k_{e0}(-u\bar{k}_{on} + \bar{k}_{off}) \tag{3.14}$$

$$-u k_{on} + k_{off} = -u\bar{k}_{on} + \bar{k}_{off}. \tag{3.15}$$

Again, by using (3.12) equation (3.10) can be rewritten as

$$-uk_e k_{e0} k_{on} + k_e k_{e0} k_{off} = -u\bar{k}_e k_{e0} \bar{k}_{on} + \bar{k}_e k_{e0} \bar{k}_{off} \tag{3.16}$$

$$k_e k_{e0}(-uk_{on} + k_{off}) = \bar{k}_e k_{e0}(-u\bar{k}_{on} + \bar{k}_{off}) \tag{3.17}$$

$$k_e(-uk_{on} + k_{off}) = \bar{k}_e(-u\bar{k}_{on} + \bar{k}_{off}). \tag{3.18}$$

Combining (3.15) and (3.18) gives only one solution, namely

$$k_e = \bar{k}_e. \tag{3.19}$$

From here it is straightforward to see, using the previous results from (3.7) and (3.8) that only one solution exists, namely

$$k_{on} = \bar{k}_{on} \tag{3.20}$$

$$k_{off} = \bar{k}_{off}. \tag{3.21}$$

In other words, this particular system has only one solution which is

$$\boldsymbol{\theta} = \bar{\boldsymbol{\theta}} \tag{3.22}$$

which means that model (3.1) is structurally globally identifiable under the assumption that R_{tot} is fixed. If R_{tot} is not fixed the model is instead structurally unidentifiable. Since the exact total number of receptors in a system is unknown the most obvious numerical value that R_{tot} should be assigned is 100, i.e., the total percentage (instead a number of) of receptors is 100 %.

The result from the structural identifiability of all 16 models, Table 3.2, is summarised in Table 3.1. It was found that the two parameters R_{tot} and k_e can not be determined uniquely which makes all of the models structurally unidentifiable. However, the analysis also found that the product $R_{tot}k_e$ can be determined uniquely. Therefore, by either fixing the parameter R_{tot} or k_e renders all of the 16 models structurally globally identifiable since the other model parameters can be uniquely

Table 3.1: Results of the structural identifiability analysis of the models in Table 3.2. Identifiable and unidentifiable parameters are presented.

Model description		Structural identifiability results	
N	Distr. | Binding | Transd.	Unidentifiable	Globally identifiable
1	Direct | SS | Linear	R_{tot}, k_e	K_d
2	Direct | SS | Sigmoid	R_{tot}, RC_{50}	n, K_d, E_m
3	Direct | SS | Indirect	R_{tot}, k_e	k_{in}, k_{out}, K_d
4	Direct | SS | Indirect	R_{tot}, k_e	k_{in}, k_{out}, K_d
5	Direct | Dynamic | Linear	R_{tot}, k_e	k_{on}, k_{off}
6	Direct | Dynamic | Sigmoid	R_{tot}, RC_{50}	n, k_{on}, k_{off}, E_m
7	Direct | Dynamic | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{on}, k_{off}, k_{in}, k_{out}$
8	Direct | Dynamic | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{on}, k_{off}, k_{in}, k_{out}$
9	Delay | SS | Linear	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, K_d$
10	Delay | SS | Sigmoid	R_{tot}, RC_{50}	$n, k_{e0}, k_{on}, k_{off}, E_m$
11	Delay | SS | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, k_{in}, k_{out}, K_d$
12	Delay | SS | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, k_{in}, k_{out}, K_d$
13	Delay | Dynamic | Linear	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, k_{on}, k_{off}$
14	Delay | Dynamic | Sigmoid	R_{tot}, RC_{50}	$n, k_{e0,}k_{on}, k_{off}, E_m$
15	Delay | Dynamic | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, k_{on}, k_{off}, k_{in}, k_{out}$
16	Delay | Dynamic | Indirect	R_{tot}, k_e	$R_{tot}k_e, k_{e0}, k_{on}, k_{off}, k_{in}, k_{out}$

determined. By fixing the parameter R_{tot} to 100 the parameter would represent a percentage of total number of receptors in the system.

Structural identifiability analysis example 2

Now follows a second example analysis where model 2 from Table (3.2) which has a direct distribution, i.e., no effect compartment, a steady-state binding and a sigmoid transduction is considered. The model has the following model structure

$$\begin{aligned} \dot{x} &= -x \\ x(0) &= D \end{aligned} \tag{3.23}$$

with observation

$$y = \frac{E_m(R_{tot}x)^n}{(K_d + x)^n RC_{50}^n + (R_{tot}x)^n} \tag{3.24}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (E_m, K_d, RC_{50}, n, R_{tot}) \tag{3.25}$$

Table 3.2: Summary of the system equations of the investigated models.

N	Model equations	In/Out	ICs	Parameters
1	$E = k_e \frac{R_{tot}C_p}{K_d+C}$	C_p/E		R_{tot}, k_e, K_d
2	$E = \frac{E_m(R_{tot}C_p)^n}{(K_d+C_p)^n RC_{50}^n+(R_{tot}C_p)^n}$	C_p/E		$R_{tot}, E_m, RC_{50}, n, K_d$
3	$\dot{E} = k_{in}(1 + k_e \frac{R_{tot}C_p}{K_d+C_p}) - k_{out}E$	C_p/E	$E(0) = k_{out}/k_{in}$	$R_{tot}, k_{in}, k_{out}, k_e, K_d$
4	$\dot{E} = k_{in} - k_{out}(1 + k_e \frac{R_{tot}C_p}{K_d+C_p})E$	C_p/E	$E(0) = k_{out}/k_{in}$	$R_{tot}, k_{in}, k_{out}, k_e, K_d$
5	$\dot{RC} = k_{on}(R_{tot} - RC)C_p - k_{off}RC$ $E = k_eRC$	C_p/E	$RC(0) = 0$	$R_{tot}, k_{on}, k_{off}, k_e$
6	$\dot{RC} = k_{on}(R_{tot} - RC)C_p - k_{off}RC$ $E = \frac{E_m RC^n}{RC_{50}^n+RC^n}$	C_p/E	$RC(0) = 0$	$R_{tot}, k_{on}, k_{off}, E_m, RC_{50}, n$
7	$\dot{RC} = k_{on}(R_{tot} - RC)C_p - k_{off}RC$ $\dot{E} = k_{in}(1 + k_eRC) - k_{out}E$	C_p/E	$RC(0) = 0$ $E(0) = k_{out}/k_{in}$	$R_{tot}, k_{on}, k_{off}, k_{in}, k_{out}, k_e$
8	$\dot{RC} = k_{on}(R_{tot} - RC)C_p - k_{off}RC$ $\dot{E} = k_{in} - k_{out}(1 + k_eRC)E$	C_p/E	$RC(0) = 0$ $E(0) = k_{out}/k_{in}$	$R_{tot}, k_{on}, k_{off}, k_{in}, k_{out}, k_e$
9	$\dot{C_e} = k_{e0}(C_p - C_e)$ $E = k_e \frac{R_{tot}C_e}{K_d+C_e}$	C_p/E	$C_e(0) = 0$	$k_{e0}, R_{tot}, k_e, K_d$
10	$\dot{C_e} = k_{e0}(C_p - C_e)$ $E = \frac{E_m(R_{tot}C_e)^n}{(K_d+C_e)^n RC_{50}^n+(R_{tot}C_e)^n}$	C_p/E	$C_e(0) = 0$	$k_{e0}, R_{tot}, E_m, RC_{50}, n, K_d$
11	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{E} = k_{in}(1 + k_e \frac{R_{tot}C_e}{K_d+C_e}) - k_{out}E$	C_p/E	$C_e(0) = 0$ $E(0) = k_{out}/k_{in}$	$k_{e0}, R_{tot}, k_{in}, k_{out}, k_e, K_d$
12	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{E} = k_{in} - k_{out}(1 + k_e \frac{R_{tot}C_e}{K_d+C})E$	C_p/E	$C_e(0) = 0$ $E(0) = k_{out}/k_{in}$	$k_{e0}, R_{tot}, k_{in}, k_{out}, k_e, K_d$
13	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{RC} = k_{on}(R_{tot} - RC)C_e - k_{off}RC$ $E = k_eRC$	C_p/E	$C_e(0) = 0$ $RC(0) = 0$	$k_{e0}, R_{tot}, k_{on}, k_{off}, k_e$
14	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{RC} = k_{on}(R_{tot} - RC)C_e - k_{off}RC$ $E = \frac{E_m RC^n}{RC_{50}^n+RC^n}$	C_p/E	$C_e(0) = 0$ $RC(0) = 0$	$k_{e0}, R_{tot}, k_{on}, k_{off}, k_{in}, k_e$
15	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{RC} = k_{on}(R_{tot} - RC)C_e - k_{off}RC$ $\dot{E} = k_{in}(1 + k_eRC) - k_{out}E$	C_p/E	$C_e(0) = 0$ $RC(0) = 0$ $E(0) = k_{out}/k_{in}$	$k_{e0}, R_{tot}, k_{on}, k_{off}, k_{in}, k_{out}, k_e$
16	$\dot{C_e} = k_{e0}(C_p - C_e)$ $\dot{RC} = k_{on}(R_{tot} - RC)C_e - k_{off}RC$ $\dot{E} = k_{in} - k_{out}(1 + k_eRC)E$	C_p/E	$C_e(0) = 0$ $RC(0) = 0$ $E(0) = k_{out}/k_{in}$	$k_{e0}, R_{tot}, k_{on}, k_{off}, k_{in}, k_{out}, k_e$

and known dose D. Generating the input-output form of the model written in this form is not possible. This is because one of the model parameters n appears as an exponent. To handle this, the system can be augmented in such a way that the parameter n appears elsewhere in the model structure.

The augmentation of the original system was performed in the following way. Let a new state $A(t)$ be defined as

$$A(t) = \frac{(K_d + x(t))^n}{(R_{tot}x(t))^n} \tag{3.26}$$

which has the following time derivative

$$\dot{A}(t) = \frac{nK_dR_{tot}\dot{x}(t)\left(\frac{K_d+x(t)}{R_{tot}x(t)}\right)^{n+1}}{(K_d + x(t))^2}. \tag{3.27}$$

The initial condition of the new state $A(t)$ is

$$A(0) = \frac{(K_d + D)^n}{(R_{tot}D)^n}. \tag{3.28}$$

As can be seen, the problem of having a parameter as an exponent has now been removed from the output function y to the initial condition of $A(t)$. At this stage, this is handled by defining a dummy parameter a_0 as

$$a_0 = \frac{(K_d + D)^n}{(R_{tot}D)^n}. \tag{3.29}$$

Finally, the last augmentation of the original system is done by the following substitution

$$RC_{50}^* = RC_{50}^n. \tag{3.30}$$

The new augmented and reparameterised form of model (3.23) is then given by

$$\begin{aligned}\dot{x}(t) &= -x \\ \dot{A}(t) &= -\frac{nK_d}{K_d + x(t)}A(t) \\ x(0) &= D \\ A(0) &= a_0\end{aligned} \tag{3.31}$$

with observation

$$y = \frac{E_m}{A(t)RC_{50}^* + 1} \tag{3.32}$$

and with unknown parameter vector

$$\boldsymbol{\theta} = (a_0, n, K_d, E_m, RC_{50}^*). \tag{3.33}$$

The augmented model (3.31) is now in a form from which the input-output relation can be generated. The input-output relation was generated using Maple, (MapleSoft [2015]), see Appendix A for the relevant Maple code and details. The input-output relation is given by

$$-n\dot{y}yE_m + nE_myy\ddot{y} - nE_m\dot{y}^2 + n\dot{y}y^2 - n\ddot{y}y^2 + 2\,nyy\dot{y}^2 - E_m\dot{y}^2 = 0 \tag{3.34}$$

The initial conditions for the output function are

$$y(0) = \frac{Em}{a_0RC_{50}^* + 1} \tag{3.35}$$

$$\dot{y}(0) = -\frac{nK_da_0E_mRC_{50}^*}{(K_d + D)(a_0RC_{50}^* + 1)^2} \tag{3.36}$$

Again, by introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$ and collecting the coeffi-

cients in the input-output relation as

$$\sum_{k=1}^{l} c_k(\boldsymbol{\theta}, \bar{\boldsymbol{\theta}})\phi_k(y(t,\boldsymbol{\theta}), \dot{y}(t,\boldsymbol{\theta}), \ddot{y}(t,\boldsymbol{\theta}), \dots) = 0 \tag{3.37}$$

it is easy to see from the input-output relation that only one solution exists, namely

$$E_m = \bar{E}_m \tag{3.38}$$

and

$$n = \bar{n}. \tag{3.39}$$

Considering the initial conditions the following two relations can also be derived

$$\frac{E_m}{a_0 RC_{50}^* + 1} = \frac{\bar{E}_m}{\bar{a}_0 \overline{RC_{50}^*} + 1} \tag{3.40}$$

$$-\frac{nK_d a_0 E_m RC_{50}^*}{(K_d + D)(a_0 RC_{50}^* + 1)^2} = -\frac{\bar{n}\bar{K}_d \bar{a}_0 \bar{E}_m \overline{RC_{50}^*}}{(\bar{K}_d + D)(\bar{a}_0 \overline{RC_{50}^*} + 1)^2} \tag{3.41}$$

from which, using previous results from (3.38)–(3.39), the following solution can be found

$$K_d = \bar{K}_d \tag{3.42}$$

and

$$a_0 RC_{50}^* = \bar{a}_0 \overline{RC_{50}^*}. \tag{3.43}$$

Model (3.23) is therefore structurally unidentifiable. However, since the parameters K_d and n have been shown to be identifiable, the identifiability problem can be solved by fixing parameter R_{tot} which will lead to

$$a_0 = \bar{a}_0 \tag{3.44}$$

and thus

$$RC_{50}^{*} = \overline{RC_{50}^{*}}. \tag{3.45}$$

From (3.30) it is clear that if both RC_{50}^{*} and n are identifiable then so is RC_{50}. In summary, model (3.23) is structurally unidentifiable, but becomes structurally globally identifiable if either R_{tot} or RC_{50} is fixed.

In relation to this work, the importance of performing an analytical structural identifiability analysis was demonstrated by attempting to estimate parameters using a structurally unidentifiable model, Figure 3.2, using Monolix (Lixoft [2012]). An argument against performing structural identifiability analysis that is relatively often presented is the claim that if there are any issues with identifiability, such issues will appear when the used software estimates the uncertainty of the parameter estimates. In theory, an unidentifiable parameter has an infinitely large uncertainty, i.e., a flat likelihood with respect to the unidentifiable parameter. However, since computations of the estimates of the parameter uncertainties are done numerically they are subject to numerical noise and approximations. Because of this, estimated parameter uncertainties such as Relative Standard Error (RSE) should not be regarded as a substitute for a structural identifiability analysis. Figure 3.2 is an example of this where one set of initial estimates of the model parameters results in seamingly reasonable RSE-values, while with a different set on initial estimates the RSE-values indicate a potential structural identifiability problem.

3.2.2 Lung slice model

In this section the structural identifiability of four lung slice models will be considered. These models have been developed by Boger [2016] and the aim of the modelling efforts was to evaluate whether the lung absorption half-life of inhaled drugs can be predicted with rat lung slices. Traditionally, estimation of lung drug half-life is determined by measurement of total lung concentration at different timepoints after inhalation of a drug. This approach often requires a large number of

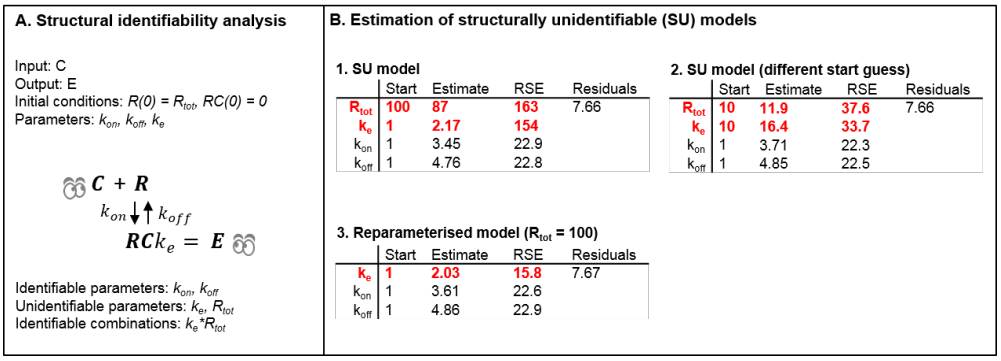


Figure 3.2: Parameter estimation results using a structurally unidentifiable model. In B1, the RSE-values indicates a potential identifiability issues. However, in B2 the reported RSE-values seems resonable. In B3 the R_{tot} parameter has been fixed resulting in a structurally globally identifiable model. Note also that the residuals in all three cases are more or less the same.

animals in each experiment, a problem which can be avoided if the proposed lung slice modelling approach proves to be successful as this alternative approach requires much fewer animals per study.

An important part in evaluating the feasibility of this alternative approach to evaluate the lung absorption half-life of inhaled drugs is to determine whether the models used are structurally identifiable. The structural identifiability analysis of the four lung slice models is presented below.

Model structures

Firstly, the model structures for all of the models considered will be presented, Figure 3.3. C_b is the buffer concentration of the drug, C_u is the unbound lung concentration, C_{u2} is the unbound lung concentration in the lysosomes, A_0 is the initial amount of drug in the lung slices, A_{b0} is the initial amount of drug in the buffer, V_b is the buffer volume, V_{lung} is given by

$$V_{lung} = W_{tot} V_{ulung} \tag{3.46}$$

where both W_{tot} (total weight of lung tissue) and V_{ulung} (apparent volume of distribution) can be measured experimentally (Bäckström et al. [2016]), V_{lung2} (apparent

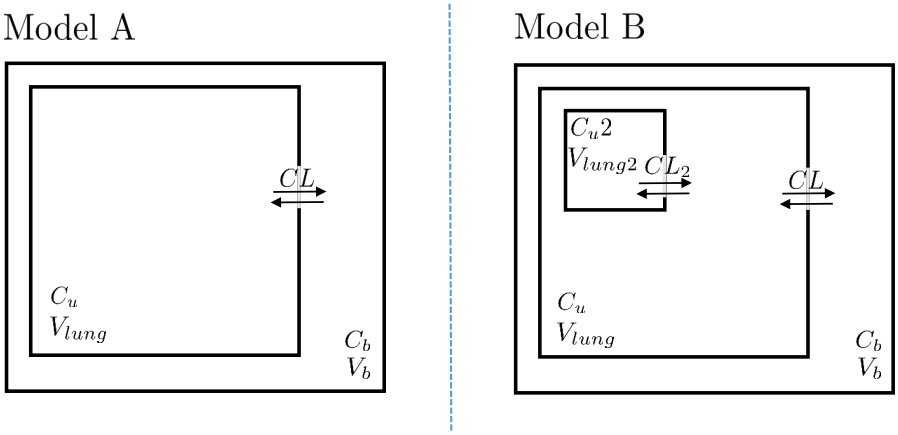


Figure 3.3: Model A has no lysosomal contribution while Model B has lysosomal contribution. There are two versions of both model A and model B: with and without mass balance.

volume of distribution of the lysosomes) is defined as

$$V_{lung2} = f_{lyso} W_{tot} V_{ulung} \alpha, \tag{3.47}$$

f_{lyso} is the volume fraction of lysosomes in the tissue which is assumed to be known, α assigns the relationship between V_{lung2}, i.e., how much greater V_{lung2} is for lysosomes, and can be computed from experimental measurements, f_0 is defined as

$$f_0 = \frac{\alpha f_{lyso}}{(\alpha f_{lyso} + (1 - f_{lyso}))}. \tag{3.48}$$

The first version of model A, i.e., without lysosomal contribution, has the following model structure

$$\begin{aligned} \dot{C}_u &= CL \frac{C_b - C_u}{V_{lung}} \\ \dot{C}_b &= CL \frac{C_u - C_b}{Vb} \\ C_u(0) &= \frac{A_0}{V_{lung}} \\ C_b(0) &= 0 \end{aligned} \tag{3.49}$$

with observations

$$\begin{aligned} y_1 &= (1 - V_0)C_u V_{ulung} + V_0 C_b \\ y_2 &= C_b \end{aligned} \tag{3.50}$$

and with the unknown parameter vector

$$\boldsymbol{\theta}_1 = (CL, A_0). \tag{3.51}$$

The second version of model A has the following model structure

$$\begin{aligned} \dot{C}_u &= CL\frac{C_b - C_u}{V_{lung}} \\ \dot{C}_b &= CL\frac{C_u - C_b}{Vb} \\ C_u(0) &= \frac{A_0}{V_{lung}} \\ C_b(0) &= \frac{A_{b0}}{V_b} \end{aligned} \tag{3.52}$$

with observations

$$\begin{aligned} y_1 &= (1 - V_0)C_u V_{ulung} + V_0 C_b \\ y_2 &= C_b \end{aligned} \tag{3.53}$$

and with the unknown parameter vector

$$\boldsymbol{\theta}_2 = (CL, A_0, A_{b0}). \tag{3.54}$$

The first version of model B, i.e., with lysosomal contribution, has the following model structure

$$\begin{aligned}
\dot{C}_u &= \frac{CLC_b - CLC_u + CL_2C_{u2} - CL_2C_u}{V_{lung}} \\
\dot{C}_b &= CL\frac{(C_u - C_b)}{V_b} \\
\dot{C}_{u2} &= CL_2\frac{(C_u - C_{u2})}{V_{lung2}} \\
C_u(0) &= \frac{(1-f_0)A_0}{V_{lung}} \\
C_b(0) &= 0 \\
C_{u2}(0) &= \frac{f_0A_0}{V_{lung2}}
\end{aligned} \tag{3.55}$$

with observations

$$\begin{aligned}
y_1 &= (1-V_0)\left(f_{lyso}C_{u2}V_{ulung}\alpha + (1-f_{lyso})C_uV_{ulung}\right) + V_0C_b \\
y_2 &= C_b
\end{aligned} \tag{3.56}$$

and the unknown parameter vector

$$\boldsymbol{\theta}_3 = (CL, CL_2, A_0) \tag{3.57}$$

The second version of model B has the following model structure

$$\begin{aligned}
\dot{C}_u &= \frac{CLC_b - CLC_u + CL_2C_{u2} - CL_2C_u}{V_{lung}} \\
\dot{C}_b &= CL\frac{(C_u - C_b)}{V_b} \\
\dot{C}_{u2} &= CL_2\frac{(C_u - C_{u2})}{V_{lung2}} \\
C_u(0) &= \frac{(1-f_0)A_0}{V_{lung}} \\
C_b(0) &= \frac{A_{b0}}{V_b} \\
C_{u2}(0) &= \frac{f_0A_0}{V_{lung2}}
\end{aligned} \tag{3.58}$$

with observations

$$\begin{aligned} y_1 &= (1 - V_0)\left(f_{lyso} C_{u2} V_{ulung} \alpha + (1 - f_{lyso}) C_u V_{ulung}\right) + V_0 C_b \\ y_2 &= C_b \end{aligned} \tag{3.59}$$

and with the unknown parameter vector

$$\boldsymbol{\theta}_4 = (CL, CL_2, A_0, A_{b0}). \tag{3.60}$$

Structural identifiability analysis

All four lung slice models were analysed using the Taylor series expansion approach, Pohjanpalo [1978], as outlined in Section 2.4.3. Although other structural identifiability analysis approaches also would have worked fine to analyse these four lung models, the Taylor series expansion approach was here chosen in order to show how it works in practice.

The first coefficient in the Taylor series expansion of the first output function y_1 of model (3.49) is

$$y_1(0) = (1 - V_0)\frac{A_0}{V_{lung}} \tag{3.61}$$

The first two coefficients in the Taylor series expansion of the second output function y_2 are

$$y_2(0) = 0 \tag{3.62}$$

$$\dot{y}_2(0) = \frac{CLA_0}{V_b V_{lung}}. \tag{3.63}$$

Introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}_1$ and equating the coefficients as

$$y_1(0, \boldsymbol{\theta}_1) = y_1(0, \bar{\boldsymbol{\theta}}_1) \tag{3.64}$$

$$\dot{y}_2(0, \boldsymbol{\theta}_1) = \dot{y}_2(0, \bar{\boldsymbol{\theta}}_1) \tag{3.65}$$

yields the following two equations

$$(1 - V_0)\frac{A_0}{V_{lung}} = (1 - V_0)\frac{\bar{A}_0}{V_{lung}} \tag{3.66}$$

$$\frac{CLA_0}{V_b V_{lung}} = \frac{\overline{CL}\bar{A}_0}{V_b V_{lung}} \tag{3.67}$$

which has only one solution, namely

$$A_0 = \bar{A}_0 \tag{3.68}$$

$$CL = \overline{CL} \tag{3.69}$$

and we therefore have that

$$\boldsymbol{\theta}_1 = \bar{\boldsymbol{\theta}}_1 \tag{3.70}$$

meaning that model (3.49) is structurally globally identifiable.

The first coefficient in the Taylor series expansion of the first output function y_1 of model (3.52) is given by

$$y_1(0) = (1 - V_0)\frac{A_0}{V_{lung}} + V_0\frac{A_{b0}}{V_b}. \tag{3.71}$$

The first two coefficients in the Taylor series expansion of the second output function y_2 of model (3.52) are given by

$$y_2(0) = \frac{A_{b0}}{V_{lung}} \tag{3.72}$$

$$\dot{y}_2(0) = CL\frac{\left(\frac{A_0}{V_{lung}} - \frac{A_b 0}{V_b}\right)}{V_b}. \tag{3.73}$$

Introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}_2$ and equating the coefficients as

$$y_1(0, \boldsymbol{\theta}_2) = y_1(0, \bar{\boldsymbol{\theta}}_2) \tag{3.74}$$

$$y_2(0, \boldsymbol{\theta}_2) = y_2(0, \bar{\boldsymbol{\theta}}_2) \tag{3.75}$$

$$\dot{y}_2(0, \boldsymbol{\theta}_2) = \dot{y}_2(0, \bar{\boldsymbol{\theta}}_2) \tag{3.76}$$

yields the following three equations

$$(1 - V_0)\frac{A_0}{V_{lung}} + V_0\frac{A_{b0}}{V_b} = (1 - V_0)\frac{\bar{A}_0}{V_{lung}} + V_0\frac{\bar{A}_{b0}}{V_b} \tag{3.77}$$

$$\frac{A_{b0}}{V_{lung}} = \frac{\bar{A}_{b0}}{V_{lung}} \tag{3.78}$$

$$CL\frac{\left(\frac{A_0}{V_{lung}} - \frac{A_b0}{V_b}\right)}{V_b} = \overline{CL}\frac{\left(\frac{\bar{A}_0}{V_{lung}} - \frac{\bar{A}_b0}{V_b}\right)}{V_b} \tag{3.79}$$

which have only one solution, namely

$$A_0 = \bar{A}_0 \tag{3.80}$$

$$A_{b0} = \bar{A}_{b0} \tag{3.81}$$

$$CL = \overline{CL} \tag{3.82}$$

and we therefore have that

$$\boldsymbol{\theta}_2 = \bar{\boldsymbol{\theta}}_2 \tag{3.83}$$

meaning that the model (3.52) is structurally globally identifiable.

The first coefficient in the Taylor series expansion of the first output function y_1 of model (3.58) is given by

$$y_1(0) = (1 - V_0)\left(\frac{A_0(1 - f_0)\,(1 - f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha A_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right). \tag{3.84}$$

The first three coefficients in the Taylor series expansion of the second output func-

tion y_2 are given by

$$y_2(0) = 0 \tag{3.85}$$

$$\dot{y}_2(0) = \frac{A_0 CL(1-f_0)}{V_b V_{lung}} \tag{3.86}$$

$$\ddot{y}_2(0) = \frac{CL\left(\frac{-\frac{A_0 CL(1-f_0)}{V_{lung}} - \frac{A_0 CL_2(1-f_0)}{V_{lung}} + \frac{A_0 CL_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{A_0 CL(1-f_0)}{V_b V_{lung}}\right)}{V_b}. \tag{3.87}$$

Introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}_3$ and equating the coefficients as

$$y_1(0, \boldsymbol{\theta}_3) = y_1(0, \bar{\boldsymbol{\theta}}_3) \tag{3.88}$$

$$\dot{y}_2(0, \boldsymbol{\theta}_3) = \dot{y}_2(0, \bar{\boldsymbol{\theta}}_3) \tag{3.89}$$

$$\ddot{y}_2(0, \boldsymbol{\theta}_3) = \ddot{y}_2(0, \bar{\boldsymbol{\theta}}_3) \tag{3.90}$$

yields the following three equations

$$(1-V_0)\left(\frac{A_0(1-f_0)\,(1-f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha A_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right) = (1-V_0)\left(\frac{\bar{A}_0(1-f_0)\,(1-f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha \bar{A}_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right) \tag{3.91}$$

$$\frac{A_0 CL(1-f_0)}{V_b V_{lung}} = \frac{\bar{A}_0 \overline{CL}(1-f_0)}{V_b V_{lung}} \tag{3.92}$$

$$\frac{CL\left(\frac{-\frac{A_0 CL(1-f_0)}{V_{lung}} - \frac{A_0 CL_2(1-f_0)}{V_{lung}} + \frac{A_0 CL_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{A_0 CL(1-f_0)}{V_b V_{lung}}\right)}{V_b} = \tag{3.93}$$

$$\frac{\overline{CL}\left(\frac{-\frac{\bar{A}_0 \overline{CL}(1-f_0)}{V_{lung}} - \frac{\bar{A}_0 \overline{CL}_2(1-f_0)}{V_{lung}} + \frac{\bar{A}_0 \overline{CL}_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{\bar{A}_0 \overline{CL}(1-f_0)}{V_b V_{lung}}\right)}{V_b} \tag{3.94}$$

which has only one solution, namely

$$CL = \overline{CL} \tag{3.95}$$

$$CL_2 = \overline{CL}_2 \tag{3.96}$$

$$A_0 = \bar{A}_0 \tag{3.97}$$

and we therefore have that

$$\boldsymbol{\theta}_3 = \bar{\boldsymbol{\theta}}_3 \tag{3.98}$$

meaning that model (3.55) is structurally globally identifiable.

The first coefficient in the Taylor series expansion of the first output function y_1 of model (3.58) is

$$y_1(0) = \frac{V_0 A_{b0}}{V_b} + (1 - V_0)\left(\frac{A_0(1-f_0)\,(1-f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha A_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right). \tag{3.99}$$

The first three coefficients in the Taylor series expansion of the second output function y_2 are given by

$$y_2(0) = \frac{A_{b0}}{V_b} \tag{3.100}$$

$$\dot{y}_2(0) = \frac{CL\left(\frac{A_0(1-f_0)}{V_{lung}} - \frac{A_{b0}}{V_b}\right)}{V_b} \tag{3.101}$$

$$\ddot{y}_2 = \frac{CL\left(\frac{\frac{CLA_{b0}}{V_b} - \frac{A_0 CL(1-f_0)}{V_{lung}} - \frac{A_0 CL_2(1-f_0)}{V_{lung}} + \frac{A_0 CL_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{CL\left(\frac{A_0(1-f_0)}{V_{lung}} - \frac{A_{b0}}{V_b}\right)}{V_b}\right)}{V_b}. \tag{3.102}$$

Introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}_4$ and equating the coefficients as

$$y_1(0, \boldsymbol{\theta}_4) = y_1(0, \bar{\boldsymbol{\theta}}_4) \tag{3.103}$$

$$y_2(0, \boldsymbol{\theta}_4) = y_2(0, \bar{\boldsymbol{\theta}}_4) \tag{3.104}$$

$$\dot{y}_2(0, \boldsymbol{\theta}_4) = \dot{y}_2(0, \bar{\boldsymbol{\theta}}_4) \tag{3.105}$$

$$\ddot{y}_2(0, \boldsymbol{\theta}_4) = \ddot{y}_2(0, \bar{\boldsymbol{\theta}}_4) \tag{3.106}$$

yields the following four equations

$$\frac{V_0 A_{b0}}{V_b} + (1 - V_0)\left(\frac{A_0(1-f_0)\,(1-f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha A_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right) =$$
$$\frac{V_0 \bar{A}_{b0}}{V_b} + (1 - V_0)\left(\frac{\bar{A}_0(1-f_0)\,(1-f_{lyso})\,V_{ulung}}{V_{lung}} + \frac{\alpha \bar{A}_0 f_0 f_{lyso} V_{ulung}}{V_{lung2}}\right) \tag{3.107}$$

$$\frac{A_{b0}}{V_b} = \frac{\bar{A}_{b0}}{V_b} \tag{3.108}$$

$$\frac{CL\left(\frac{A_0(1-f_0)}{V_{lung}} - \frac{A_{b0}}{V_b}\right)}{V_b} = \frac{\overline{CL}\left(\frac{\bar{A}_0(1-f_0)}{V_{lung}} - \frac{\bar{A}_{b0}}{V_b}\right)}{V_b} \tag{3.109}$$

$$\frac{CL\left(\frac{\frac{CLA_{b0}}{V_b} - \frac{A_0 CL(1-f_0)}{V_{lung}} - \frac{A_0 CL_2(1-f_0)}{V_{lung}} + \frac{A_0 CL_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{CL\left(\frac{A_0(1-f_0)}{V_{lung}} - \frac{A_{b0}}{V_b}\right)}{V_b}\right)}{V_b} =$$
$$\frac{\overline{CL}\left(\frac{\frac{\overline{CL}\bar{A}_{b0}}{V_b} - \frac{\bar{A}_0\overline{CL}(1-f_0)}{V_{lung}} - \frac{\bar{A}_0\overline{CL}_2(1-f_0)}{V_{lung}} + \frac{\bar{A}_0\overline{CL}_2 f_0}{V_{lung2}}}{V_{lung}} - \frac{\overline{CL}\left(\frac{\bar{A}_0(1-f_0)}{V_{lung}} - \frac{\bar{A}_{b0}}{V_b}\right)}{V_b}\right)}{V_b} \tag{3.110}$$

which have only one solution, namely

$$A_0 = \bar{A}_0 \tag{3.111}$$

$$A_{b0} = \bar{A}_{b0} \tag{3.112}$$

$$CL = \overline{CL} \tag{3.113}$$

$$CL_2 = \overline{CL}_2 \tag{3.114}$$

and we therefore have that

$$\boldsymbol{\theta}_4 = \bar{\boldsymbol{\theta}}_4 \tag{3.115}$$

meaning that the model (3.58) is structurally globally identifiable.

In summary, all four lung models have been shown to be structurally globally identifiable.

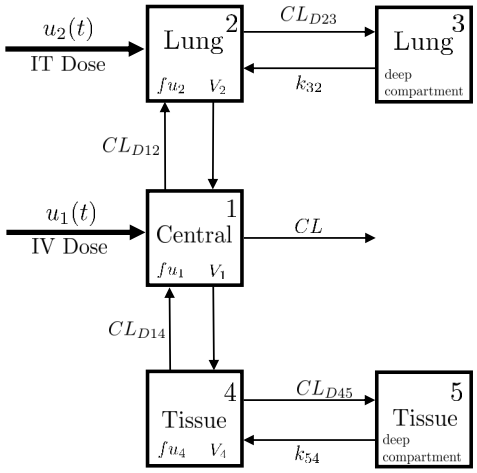


Figure 3.4: The five-compartment lung model.

3.2.3 Five-compartment lung PK-model

In this section a structural identifiability analysis on a five-compartment model is performed. This particular model, presented in Hendrickx et al. [2016] together with the structural identifiability analysis, aims to describe the PK-profile of a drug in the five following compartments; central A_1, lung A_2, lung deep A_3, tissue A_4 and tissue deep A_5, Figure 3.4. The purpose of this model was to use the PK-profile in rats following local delivery in the lung for prediction of lung and plasma PK-profiles in humans.

The model with an intravenous (IV) dosing has the following model structure

$$
\begin{aligned}
\dot{A}_1(t) &= -\frac{CLA_1(t)}{V_1} - \frac{CL_{D12}fu_1A_1(t)}{V_1} - \frac{CL_{D14}fu_1A_1(t)}{V_1} + \\
&\quad \frac{CL_{D12}fu_2A_2(t)}{V_2} + \frac{CL_{D14}fu_4A_4(t)}{V_4} + u_1(t) \\
\dot{A}_2(t) &= k_{32}A_3(t) + \frac{CL_{D12}fu_1A_1(t)}{V_1} - \frac{fu_2CL_{D12}A_2(t)}{V_2} - \frac{fu_2CL_{D23}A_2}{V_2} \\
\dot{A}_3(t) &= \frac{CL_{D23}fu_2A_2(t)}{V_2} - k_{32}A_3(t) \\
\dot{A}_4(t) &= \frac{CL_{D14}fu_1A_1(t)}{V_1} - \frac{CL_{D14}fu_4A_4(t)}{V_4} - \frac{CL_{D45}fu_4A_4(t)}{V_4} + k_{32}A_5(t) \\
\dot{A}_5(t) &= \frac{CL_{D45}fu_4A_4(t)}{V_4} - k_{32}A_5(t) \\
A_i(0) &= 0 \qquad i = 1, .., 5
\end{aligned}
\tag{3.116}
$$

with observations

$$
\begin{aligned}
y_1(t) &= \frac{A_2(t) + A_3(t)}{V_2} \\
y_2(t) &= \frac{A_1(t)}{V_1}
\end{aligned}
\tag{3.117}
$$

while the model with an intratracheal (IT) dosing has the following model structure

$$
\begin{aligned}
\dot{A}_1(t) &= -\frac{CLA_1(t)}{V_1} - \frac{CL_{D12}fu_1A_1(t)}{V_1} - \frac{CL_{D14}fu_1A_1(t)}{V_1} + \\
&\quad \frac{CL_{D12}fu_2A_2(t)}{V_2} + \frac{CL_{D14}fu_4A_4(t)}{V_4} \\
\dot{A}_2(t) &= k_{32}A_3(t) + \frac{CL_{D12}fu_1A_1(t)}{V_1} - \frac{fu_2CL_{D12}A_2(t)}{V_2} - \frac{fu_2CL_{D23}A_2}{V_2} + u_2(t) \\
\dot{A}_3(t) &= \frac{CL_{D23}fu_2A_2(t)}{V_2} - k_{32}A_3(t) \\
\dot{A}_4(t) &= \frac{CL_{D14}fu_1A_1(t)}{V_1} - \frac{CL_{D14}fu_4A_4(t)}{V_4} - \frac{CL_{D45}fu_4A_4(t)}{V_4} + k_{32}A_5(t) \\
\dot{A}_5(t) &= \frac{CL_{D45}fu_4A_4(t)}{V_4} - k_{32}A_5(t) \\
A_i(0) &= 0 \qquad i = 1, \ldots, 5
\end{aligned}
\tag{3.118}
$$

with observations

$$
\begin{aligned}
y_1(t) &= \frac{A_2(t) + A_3(t)}{V_2} \\
y_2(t) &= \frac{A_1(t)}{V_1}.
\end{aligned}
\tag{3.119}
$$

The unknown parameter vector for both models is given by

$$\boldsymbol{\theta} = (V_1, V_2, V_4, fu_4, CL, fu_1, fu_2, CL_{D12}, CL_{D14}, CL_{D45}, k_{32}, CL_{D23}). \quad (3.120)$$

Before applying any structural identifiability analysis techniques, it can be concluded directly from a visual inspection of the model structure that the two parameters fu_4 and V_4 can not be uniquely determined and the model is therefore structurally unidentifiable. This is because the two unidentifiable parameters always appear together as a fraction $\frac{fu_4}{V_4}$. Therefore, the following reparametrisation

$$\beta = \frac{fu_4}{V_4} \quad (3.121)$$

will be used in the subsequent structural identifiability analysis.

The Taylor series expansion approach and the input-output form approach were both applied to the models (3.116) and (3.118) in an effort to analyse them from a structural perspective. However, the symbolic computations proved to be infeasible and because of this no conclusion could be drawn using this approach.

Instead, the EAR approach (Karlsson et al. [2012]) was used. This was performed in Mathematica (Wolfram Research Inc. [2016]) and the Mathematica code and the computations can be found in Appendix B. The analysis showed that the five-compartment model with either IV dosing (3.116) or IT dosing (3.118) are structurally unidentifiable. The unidentifiable model parameter vector was found to be

$$(CL_{D12}, CL_{D14}, CL_{D23}, CL_{D45}, fu_1, fu_2, \beta). \quad (3.122)$$

However, the analysis with the EAR approach also showed that the two models are structurally unidentifiable with one degree of freedom, i.e., if one of the unidentifiable parameters in (3.122) is known then the model becomes at least structurally locally identifiable. In this particular case, the parameter fu_1, i.e., the free fraction of drug in the plasma, can be measured from seperate experiments and can therefore be

considered to be known in the structural identifiability analysis.

It can therefore be concluded that the five-compartment lung model with either IV dosing (3.116) or IT dosing (3.118) is at least structurally locally identifiable with the reparametrisation (3.121) and under the assumption of the parameter fu_1 being known.

3.2.4 Input estimation

In Trägårdh et al. [2017], methods are presented that can be used to estimate the time-profile of an input function to a nonlinear system given a known model structure, known model parameters and a known output function and these methods are exemplified. The structural identifiability analysis that was included in this work is presented below.

The structural identifiability of two PK-models presented in Gao and Jusko [2012] and Li et al. [2015] has been considered. The structural identifiability analysis in this paper has been performed using two approaches: The EAR approach (Karlsson et al. [2012]) and the Taylor series expansion approach (Pohjanpalo [1978]). The EAR approach was used to analyse the model structure itself and the Taylor series expansion approach was used to analyse the uniquenes of the input function to the system.

Gao and Jusko model

The structural identifiability analysis was done only of the PK-model in Gao and Jusko [2012]. The structure of the PK-model with IV administration is given by

$$\begin{aligned}
\dot{C}(t) &= \frac{u(t)}{V_c} - (k_{el} + k_{pt})C(t) + k_{tp}\frac{A_T(t)}{V_c} \\
&\quad - k_{on}(R_{tot} - RC(t))C(t) + k_{off}RC(t) \\
\dot{A}_T(t) &= k_{pt}C(t)V_C - k_{tp}A_T(t) \\
\dot{RC}(t) &= k_{on}(R_{tot} - RC(t))C(t) - (k_{off} + k_{int})RC(t) \\
C(0) &= 0 \\
A_T(0) &= 0 \\
RC(0) &= 0
\end{aligned} \tag{3.123}$$

with observation

$$y(t) = C(t) \tag{3.124}$$

where $C(t)$ is the drug concentration in the central compartment, $A_T(t)$ is the drug amount in a peripheral compartment, $RC(t)$ is the concentration of the drug-receptor complex, and $u(t)$ is the unknown input. The unknown parameter vector is

$$\boldsymbol{\theta}_1 = (V_c, k_{el}, k_{pt}, k_{tp}, k_{on}, k_{off}, k_{int}, R_{tot}). \tag{3.125}$$

The structure of the PK-model with subcutaneous (SC) administration is given by

$$\begin{aligned}
\dot{x}_{sc} &= -k_a x_{sc} \\
\dot{C}(t) &= \frac{k_a x_{sc}}{V_c} - (k_{el} + k_{pt})C(t) + k_{tp}\frac{A_T(t)}{V_c} \\
&\quad - k_{on}(R_{tot} - RC(t))C(t) + k_{off}RC(t) \\
\dot{A}_T(t) &= k_{pt}C(t)V_C - k_{tp}A_T(t) \\
\dot{RC}(t) &= k_{on}(R_{tot} - RC(t))C(t) - (k_{off} + k_{int})RC(t) \\
x_{sc}(0) &= FD \\
C(0) &= 0 \\
A_T(0) &= 0 \\
RC(0) &= 0
\end{aligned} \tag{3.126}$$

with observation

$$y(t) = C(t) \tag{3.127}$$

and with the unknown parameter vector

$$\boldsymbol{\theta}_2 = (V_c, k_{el}, k_{pt}, k_{tp}, k_{on}, k_{off}, k_{int}, R_{tot}, k_a, F) \tag{3.128}$$

and a known dose D.

Structural identifiability results

Three different dose administrations were considered: intravenous (IV) infusion, IV bolus dose and subcutaneous (SC) administration. The details of the analysis using the EAR approach can be found in Appendix D. The following are the results from the structural identifiability analysis using the EAR approach:

- The PK-model (3.123) with IV infusion is at least structurally locally identifiable.
- The PK-model (3.123) with bolus IV dose is at least structurally locally identifiable.

- The PK-model (3.126) with SC administration is structurally unidentifiable with one degree of freedom. The structurally unidentifiable parameters are F and V_c while the remaining model parameters can still be determined.

Since all of the model parameters, except for F and k_a, are shared between the IV case and the SC case two different methods can be applied to make the structurally unidentifiable SC model (3.126) structurally identifiable:

1. Fix the parameter V_c to the estimated value from the IV case.

2. Consider both models together in a joint inference problem.

In the paper Gao and Jusko [2012] the bioavailability parameter F was fixed to a value which solves the structural identifiability issue. However, this was not done as a direct result of a structural identifiability analysis.

Identifiability of the input function

Before applying the methods developed by Trägårdh et al. [2017] with the purpose to estimate the time-profile of the input function to a nonlinear system it must first be determined whether the input function is identifiable, i.e., whether there exists a unique time-profile for the input to a model for every given set of model parameters, model structure and output function.

To analyse whether the input function in the PK-model in Gao and Jusko [2012] is identifiable or not the Taylor series expansion approach, (Pohjanpalo [1978]), was used. By computing the Taylor series expansion around $t = 0$ of the output function

$$y(t) = C(t) \tag{3.129}$$

using Mathematica (Wolfram Research Inc. [2016]) the following coefficients are

obtained

$$y(0) = 0 \tag{3.130}$$

$$\dot{y}(0) = u(0) \tag{3.131}$$

$$\ddot{y}(0) = u(0)\left(-\left(k_{\text{el}} + k_{\text{pt}}\right)\right) - u(0)k_{\text{on}}R_{\text{tot}} + \dot{u}(0) \tag{3.132}$$

$$\begin{aligned} y^{(3)}(0) = &-\left(k_{\text{el}} + k_{\text{pt}}\right)\left(u(0)\left(-\left(k_{\text{el}} + k_{\text{pt}}\right)\right) - u(0)k_{\text{on}}R_{\text{tot}} + \dot{u}(0)\right) \\ &- k_{\text{on}}R_{\text{tot}}\left(u(0)\left(-\left(k_{\text{el}} + k_{\text{pt}}\right)\right) - u(0)k_{\text{on}}R_{\text{tot}} + \dot{u}(0)\right) + \\ &u(0)k_{\text{off}}k_{\text{on}}R_{\text{tot}} + u(0)k_{\text{pt}}k_{\text{tp}} + \ddot{u}(0) \end{aligned} \tag{3.133}$$

$$\begin{aligned} y^{4}(0) = &\, k_{\text{off}}k_{\text{on}}R_{\text{tot}}\left(-u(0)\left(k_{\text{el}} + k_{\text{pt}}\right) + u(0)\left(k_{\text{int}} - k_{\text{off}}\right) - u(0)k_{\text{on}}R_{\text{tot}} + \dot{u}(0)\right) \\ &- \left(k_{\text{el}} + k_{\text{pt}}\right)\left(\left(k_{\text{el}} + k_{\text{pt}}\right)\left(u(0)k_{\text{el}} + u(0)k_{\text{on}}R_{\text{tot}} + u(0)k_{\text{pt}} - \dot{u}(0)\right) + \right. \\ &k_{\text{on}}R_{\text{tot}}\left(u(0)k_{\text{el}} + u(0)k_{\text{on}}R_{\text{tot}} + u(0)k_{\text{pt}} - \dot{u}(0)\right) + u(0)k_{\text{off}}k_{\text{on}}R_{\text{tot}} + \\ &u(0)k_{\text{pt}}k_{\text{tp}} + \ddot{u}(0)\Big) - k_{\text{on}}R_{\text{tot}}\left(\left(k_{\text{el}} + k_{\text{pt}}\right)\left(u(0)k_{\text{el}} + u(0)k_{\text{on}}R_{\text{tot}}\right.\right. \\ &\left.\left. + u(0)k_{\text{pt}} - \dot{u}(0)\right) + k_{\text{on}}R_{\text{tot}}\left(u(0)k_{\text{el}} + u(0)k_{\text{on}}R_{\text{tot}}\right.\right. \\ &\left.\left. + u(0)k_{\text{pt}} - \dot{u}(0)\right) + u(0)k_{\text{off}}k_{\text{on}}R_{\text{tot}} + u(0)k_{\text{pt}}k_{\text{tp}} + \ddot{u}(0)\right) - \\ &k_{\text{pt}}k_{\text{tp}}\left(u(0)k_{\text{el}} + u(0)k_{\text{on}}R_{\text{tot}} + u(0)k_{\text{pt}} + u(0)k_{\text{tp}} - \dot{u}(0)\right) + \\ &3u(0)^{2}k_{\text{on}}^{2}R_{\text{tot}} + u^{(3)}(0) \end{aligned} \tag{3.134}$$

$$\vdots$$

The unknown terms in the equations above are

$$\left(u(0), \dot{u}(0), \ddot{u}(0), u^{(3)}(0), \dots\right) \tag{3.135}$$

since the model parameters, the output function $y(0)$ and its higher order derivatives are assumed to be known in this analysis. Because of the structure of this particular model the $u^{(n-1)}(0)$ term will always enter linearly in the expression (see last term respectively) for each $y^{(n)}(0)$ where $n = 1, 2, \dots$ In other words, from $\dot{y}(0)$ we can determine $u(0)$. In $\ddot{y}(0)$ all terms are known, including now also $u(0)$, and because of this we can determine $\dot{u}(0)$, and so on. We therefore have a triangular structure

of the higher order derivatives and from which it can be concluded that

$$(u(0), \dot{u}(0), \ddot{u}(0), u^{(3)}(0), \dots) \tag{3.136}$$

can be determined from the output function $y(t)$.

Next the Taylor series expansion of the input signal $u(t)$ around $t = 0$ is considered. The input signal $u(t)$ has the following Taylor series expansion

$$u(t) = u(0) + u^{(1)}(0)\frac{t}{1!} + u^{(2)}(0)\frac{t^2}{2!} + \cdots + u^{(k)}(0)\frac{t^k}{k!} + \dots \tag{3.137}$$

Since it has been shown that all of the coefficients in the Taylor series expansion of $u(t)$ can be determined it can be concluded that the input function $u(t)$ is identifiable since the coefficients in a Taylor series expansions of a function uniquely determines that function for this particular model structure.

Li et al.

A second model that was considered in Trägårdh et al. [2017] was a similar PK-model presented in Li et al. [2015]. The structural identifiability of this model was

also analysed. The model has the following structure

$$
\begin{aligned}
\dot{a}_1(t) &= -k_{tr}a_1(t) \\
\dot{a}_2(t) &= -k_{tr}(a_1(t) - a_2(t)) \\
\dot{a}_3(t) &= -k_{tr}(a_2(t) - a_3(t)) \\
\dot{a}_4(t) &= -k_{tr}(a_3(t) - a_4(t)) \\
\dot{a}_5(t) &= -k_{tr}(a_4(t) - a_5(t)) \\
\dot{a}_6(t) &= k_a a_5(t) - \frac{Q}{V_1}a_6(t) - \frac{CL}{V_1}a_6(t) + \frac{Q}{V_2}a_7(t) - k_{on}a_8(t)a_6(t) \\
&\quad + k_{off}a_9(t)V_1 \\
\dot{a}_7(t) &= \frac{Q}{V_1}a_6(t) - \frac{Q}{V_2}a_7(t) \\
\dot{a}_8(t) &= k_{syn} - k_{deg}a_8(t) - k_{on}a_8(t)\frac{a_6(t)}{V_1} + k_{off}a_9(t) \\
\dot{a}_9(t) &= k_{on}a_8(t)\frac{a_6(t)}{V_1} - k_{off}a_9(t) - k_{int}a_9(t) \\
a_1(0) &= (1 - F_1 - F_2 - F_3)FD \\
a_2(0) &= F_1 FD \\
a_3(0) &= F_2 FD \\
a_4(0) &= F_3 FD \\
a_5(0) &= 0 \\
a_6(0) &= 0 \\
a_7(0) &= 0 \\
a_8(0) &= \frac{k_{syn}}{k_{deg}} \\
a_9(0) &= 0
\end{aligned}
$$

with observation

$$y(t) = \frac{a_6(t)}{V_1} \tag{3.138}$$

and with the unknown parameter vector

$$\boldsymbol{\theta} = (F, F_1, F_2, F_3, k_{tr}, k_a, k_{on}, k_{off}, k_{syn}, k_{deg}, k_{int}, Q, CL, V_1, V_2) \tag{3.139}$$

and a known dose D.

The structural identifiability analysis using the EAR approach, the details of which can be seen in Appendix D, showed that the PK-model in Li et al. [2015] was structurally unidentifiable with one degree of freedom. From the EAR analysis it can be concluded that if any one of the following parameters

$$(CL, F, F_1, F_2, F_3, Q, V_1, V_2) \tag{3.140}$$

is assumed to be known, or can be measured separately experimentally, then the model (3.138) becomes at least structurally locally identifiable.

3.3 Summary

Conclusive results for all of the models analysed were obtained. The 16 analysed PD-models were all shown to be structurally unidentifiable, but by fixing either one of the two parameters R_{tot} or k_e all models become structurally globally identifiable. Given that such models are routinely used in practice, it is quite remarkable that no formal structural identifiability analysis of these models have been published previously. These results ensure the theoretical soundness of the models from a structural identifiability perspective.

All versions of the lung slice model were shown to be structurally globally identifiable. The models are quite complex with several parameters. However, although no formal analysis was done, the fact that most of the model parameters could be derived directly using experimental measurements or literature data, thus leaving only 2-4 unknown parameters, helped with the structural identifiability a great deal.

The five-compartment lung PK-model was shown to be at least structurally

locally identifiable after a reparametrisation and fixing a particular parameter. Both the Taylor series expansion and the input-output approaches were applied, but the subsequent symbolic computations proved to be too computationally demanding. Because of this, the EAR approach was applied instead. The reason why the EAR approach was applied after the Taylor series expansion approach and the input-output form approach has to do with the arguably main downside of the EAR approach. While the Taylor series expansion approach and the input-output form approach can be used to show whether a model is either structurally globally/locally identifiable, the EAR approach can only be used to show whether a model is either at least structurally locally identifiable or structurally unidentifiable. Before analysing the model, a visual inspection of the model equations can be used to conclude that the original model was structurally unidentifiable since a ratio of two parameters appeared only together. It was also shown using the EAR approach that even after reparametrising the fraction, the model was still structurally unidentifiable. A subset of the model parameters was identified, of which, if any one of the parameters of that subset was fixed, then the model becomes at least structurally locally identifiable. In this way, the five-compartment lung PK-model is an excellent example to illustrate that some structural identifiability issues can be found with a simple visual inspection while other identifiability issues can only be found using more sophisticated methods.

In terms of the input estimation models analysed, while the PK-model (3.123) in Gao and Jusko [2012] with either IV infusion or IV bolus dosing of the drug was found to be at least structurally locally identifiable, the PK-model (3.126) with SC administration of the drug was structurally unidentifiable. However, it was found that the structural identifiability problem for the SC administration model can be avoided if *i)* the estimates of the model parameters from the IV administration model are fixed when estimating the additional parameters in the SC administration model or *ii)* the parameters in the IV and the SC model are estimated simultaneously. The latter would be the preferable choice as this approach will most likely decrease the uncertainty of the shared parameters in terms of practical identifiability since more

information will be used.

It was also shown using the Taylor series expansion approach that the time-profile of the input function can (theoretically) be determined uniquely if the model parameters and the time-profile of the output function are known. This is an important result as this shows that the input estimation methods presented in Trägårdh et al. [2017] are therefore applicable to the PK-model considered.

Chapter 4

Structural Identifiability and Indistinguishability in Mixed-Effects Models

4.1 Introduction

The definitions of structural identifiability and indistinguishability as given in Section 2.4.2 and Section 2.5.2 are only applicable to models written in a non-mixed-effects state-space form (2.4). Furthermore, existing structural identifiability and indistinguishability analysis techniques are only applicable to such systems. The reason why is because mixed-effects models include a statistical submodel, resulting in a distribution of trajectories, rather than a single trajectory, of the output function(s).

In parallel to the development of methods for structural identifiability analysis of dynamic systems the concept of parameter identifiability has at the same time been of interest in a more purely statistical context, (Paulino and de Braganca Pereira [1994]; Picci [1977]; Martin and Quintana [2002]; Allman et al. [2009]; Koopmans and Reiersøl. [1950]; Goodman [1974]). In this thesis, structural identifiability of mixed-effects models has in some sense been treated as a combination of

the two, as it is both a structural and a statistical problem. Since examples of structural identifiability analysis of dynamical systems have been introduced in Chapter 3, a simple conceptual example of the identifiability problem of a statistical model will now be given.

Consider the simple statistical model

$$Z = X_1 + X_2 \tag{4.1}$$

where Z is observed and X_1 and X_2 are random variables with normal distributions given by

$$X_1 \sim N(\mu_1, \sqrt{\omega_1}) \tag{4.2}$$

$$X_2 \sim N(\mu_2, \sqrt{\omega_2}). \tag{4.3}$$

where μ_1 and μ_2 are the expected values and ω_1 and ω_2 are the variances. Since both X_1 and X_2 are normally distributed it follows that Z is a random variable with a normal distribution with the following parametrisation

$$Z \sim N(\mu_1 + \mu_2, \sqrt{\omega_1 + \omega_2}). \tag{4.4}$$

However, if only Z can be observed none of the statistical parameters

$$\{\mu_1, \mu_2, \omega_1, \omega_2\} \tag{4.5}$$

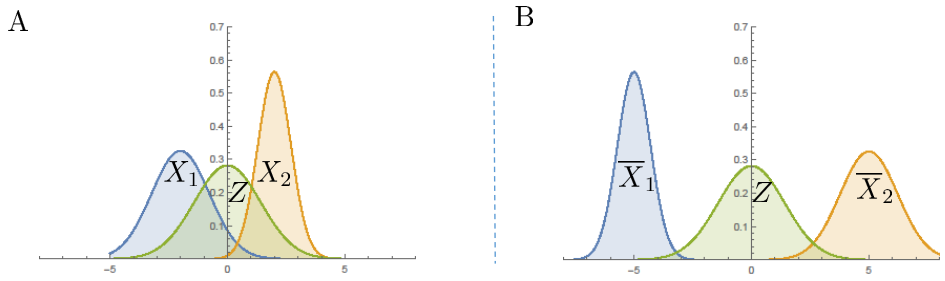
can be uniquely determined and the statistical model (4.1) is therefore unidentifiable. Instead, only the sum

$$\mu_Z = \mu_1 + \mu_2 \tag{4.6}$$

and the square root of the sum of the variance parameters

$$\omega_Z = \sqrt{\omega_1 + \omega_2} \tag{4.7}$$

Figure 4.1: An unidentifiable statistical model. The distributions of X_1 and X_2 in A are different than the distribution of $\bar{X}_1$ $\bar{X}_2$ in B since the statistical parameters have different numerical values. The distribution of the sum Z of the random variables $X_1 + X_2$ and $\bar{X}_1 + \bar{X}_2$ respectively however is identical in A and B. Therefore, the distribution of Z does not uniquely determine the underlying statistical parameters $\{\mu_1, \mu_1, \omega_1, \omega_2\}$.



can be determined from Z. In Figure 4.1, the distributions of Z, X_1 and X_2 are shown with two different sets of numerical values for the statistical parameters. The numerical values used in Figure 4.1A are

$$\mu_1 = -2 \tag{4.8}$$

$$\mu_2 = 2 \tag{4.9}$$

$$\omega_1 = \sqrt{1.5} \tag{4.10}$$

$$\omega_2 = \sqrt{0.5} \tag{4.11}$$

and in Figure 4.1B

$$\bar{\mu}_1 = -5 \tag{4.12}$$

$$\bar{\mu}_2 = 5 \tag{4.13}$$

$$\bar{\omega}_1 = \sqrt{0.5} \tag{4.14}$$

$$\bar{\omega}_2 = \sqrt{1.5}. \tag{4.15}$$

Clearly, the numerical values in the two cases are different. But still, as can be seen, while the distributions of X_1 and X_2 in Figure 4.1A are not the same as in Figure 4.1B, the distribution of Z is the same for the two cases.

In this chapter the concepts of structural identifiability and structural indistinguishability are extended to include mixed-effects models also written in the form (2.5). The new mathematical definitions of structural identifiability and structural indistinguishability for mixed-effects models developed and introduced in this thesis are given in Section 4.2.1 and 4.2.2 respectively. These definitions can be regarded as generalisations of the previous definitions for fixed-effects models as they collapse to the previous definitions when the variances of all model parameters are set to zero, resulting in identical individual trajectories of the output function.

With respect to these new definitions, several methods have also been developed within this thesis that are applicable to study the structural identifiability and indistinguishability of mixed-effects models. Methods for structural identifiability applicable to mixed-effects models are presented in Sections 4.3.1–4.3.6. Methods for structural indistinguishability applicable to mixed-effects models are presented in Sections 4.4.1–4.4.3.

In some sense, the reason why structural identifiability is often mentioned as a prerequisite for successful parameter estimation is because one considers whether it is possible to determine the model parameters uniquely or otherwise with respect to ideal experimental conditions. If it is not possible to determine the model parameters under ideal conditions, doing so under less than ideal conditions will obviously never be possible, hence the use of the term prerequisite. For a non-mixed-effects model, ideal experimental conditions means noise-free and time-continuous measurements, i.e., infinite number of measurements. For a mixed-effects model, ideal experimental conditions means, in addition to noise-free and time-continuous measurements, also an infinite number of subjects which is assumed in the following sections.

4.2 Structural Identifiability and Indistinguishability in Mixed-Effects Models

4.2.1 Definition of Structural Identifiability: Mixed-Effects Models

As mixed-effects models yields individual trajectories the structural identifiability concept needs to be extended to *distributions of output functions*, i.e., different parameter values may result in different or identical distributions.

Definition 4. Let $p(\boldsymbol{y}, t)$ denote the distribution of the output signals $\boldsymbol{y}$ at time t. Similar to the non-mixed-effects state-space case, let the generic parameter vector and matrix $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ belong to a feasible parameter space $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} \subset \boldsymbol{\Theta}$, consider the following two sets of parameters $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ and $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$. If $p(\boldsymbol{y}_{\{\boldsymbol{\theta},\boldsymbol{\Omega}\}}, t) = p(\boldsymbol{y}_{\{\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}\}}, t)$ for all t implies that $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ in a neighbourhood $\boldsymbol{N} \subset \boldsymbol{\Theta}$ then the model is structurally locally identifiable, and if $\boldsymbol{N} = \boldsymbol{\Theta}$ the model is structurally globally identifiable. For an unidentifiable parameter, $\theta_i \in \boldsymbol{\theta}$, or $\omega_i \in \boldsymbol{\Omega}$, every neighbourhood $\boldsymbol{N}$ around θ_i, or ω_i, has a parameter vector/matrix $\bar{\boldsymbol{\theta}}$, or $\bar{\boldsymbol{\Omega}}$, where $\theta_i \neq \bar{\theta}_i$, or $\omega_i \neq \bar{\omega}_i$, that gives rise to the same distribution of identical input-output relations. Note that if $\boldsymbol{\Omega} = \mathbf{0}$ where $\mathbf{0}$ is the null-matrix, i.e., a matrix with all entries being zero, then the definition of structural identifiability in mixed-effects models collapses to the definition for the non-mixed-effects state-space case.

4.2.2 Definition of Structural Indistinguishability: Mixed-Effects Models

Definition 5. Consider two systems $\boldsymbol{\Sigma}(\boldsymbol{\theta}, \boldsymbol{\Omega})$ and $\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}})$ where

$$\boldsymbol{\Sigma}(\boldsymbol{\theta}, \boldsymbol{\Omega}) = \begin{cases} \dot{\boldsymbol{x}}_i(t) = \boldsymbol{f}(\boldsymbol{x}_i(t), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \\ \boldsymbol{y}_i(t) = \boldsymbol{h}(\boldsymbol{x}_i(t), \boldsymbol{u}_i(t), \boldsymbol{\phi}_i) \\ \boldsymbol{x}(0) = \boldsymbol{x}_0(\boldsymbol{\phi}_i) \\ \boldsymbol{\phi}_i = g(\boldsymbol{\theta}, \boldsymbol{\eta}, \boldsymbol{C}_i) \\ \boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \end{cases} \tag{4.16}$$

and

$$\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}) = \begin{cases} \dot{\bar{\boldsymbol{x}}}_i(t) = \bar{\boldsymbol{f}}(\bar{\boldsymbol{x}}_i(t),\boldsymbol{u}_i(t),\bar{\boldsymbol{\phi}}_i) \\ \bar{\boldsymbol{y}}_i(t) = \bar{\boldsymbol{h}}(\bar{\boldsymbol{x}}_i(t),\boldsymbol{u}_i(t),\bar{\boldsymbol{\phi}}_i) \\ \bar{\boldsymbol{x}}(0) = \bar{\boldsymbol{x}}_0(\bar{\boldsymbol{\phi}}_i) \\ \bar{\boldsymbol{\phi}}_i = \bar{g}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}},\bar{\boldsymbol{C}}_i) \\ \bar{\boldsymbol{\eta}} \sim N(\boldsymbol{0},\bar{\boldsymbol{\Omega}}). \end{cases} \tag{4.17}$$

where i denotes the i:th individual and where $\boldsymbol{x}_i(t) \in \mathbb{R}^n$ and $\bar{\boldsymbol{x}}_i(t) \in \mathbb{R}^n$ is the state, $\boldsymbol{u}_i(t) \in \mathbb{R}^r$ and $\bar{\boldsymbol{u}}_i(t) \in \mathbb{R}^r$ is the input, $\boldsymbol{y}_i(t) \in \mathbb{R}^m$ and $\bar{\boldsymbol{y}}_i(t) \in \mathbb{R}^m$ is the output, $\boldsymbol{\theta} \in \boldsymbol{\Theta} \subset \mathbb{R}^q$ and $\bar{\boldsymbol{\theta}} \in \bar{\boldsymbol{\Theta}} \subset \mathbb{R}^d$ where $\boldsymbol{\Theta} \subset \mathbb{R}^q$ and $\bar{\boldsymbol{\Theta}} \subset \mathbb{R}^d$ is the set of possible parameter values in a feasible parameter space.

Let $p(\boldsymbol{y},t)$ denote the distribution of the output signal at time t. The two systems $\boldsymbol{\Sigma}(\boldsymbol{\theta},\boldsymbol{\Omega})$ and $\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}})$ are said to be *output indistinguishable*, denoted by $\boldsymbol{\Sigma}(\boldsymbol{\theta},\boldsymbol{\Omega}) \sim \bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}})$ if $p(\boldsymbol{y}_{\{\boldsymbol{\theta},\boldsymbol{\Omega}\}},t) = p(\bar{\boldsymbol{y}}_{\{\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}\}},t)$ for all t. If for generic $\{\boldsymbol{\theta},\boldsymbol{\Omega}\}$ there exists a $\{\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}\}$ such that $\boldsymbol{\Sigma}(\boldsymbol{\theta},\boldsymbol{\Omega}) \sim \bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}})$ and if for generic $\{\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}\}$ there exists a $\{\boldsymbol{\theta},\boldsymbol{\Omega}\}$ such that $\bar{\boldsymbol{\Sigma}}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}) \sim \boldsymbol{\Sigma}(\boldsymbol{\theta},\boldsymbol{\Omega})$ then the models are called *structurally indistinguishable*.

4.3 Structural Identifiability analysis approaches

In this section five different methods applicable to the study of study structural identifiability of mixed-effects models will be presented that have been developed within this thesis. Two of the developed methods are called the *repeated measurement approach* and the *augmented system approach* and are published in Janzén et al. [2015]. The other three methods are extended approaches of methods applicable to non-mixed-effects models. These include the *Laplace transform approach*, published in Janzén et al. [2016a], and modified versions of the *Taylor series approach* and the *input-output approach* published in Janzén et al. [2016b].

4.3.1 Repeated measurement approach

If a model is structurally unidentifiable it means that a subset of the model parameters can take on an uncountable number of different values while the output function $\boldsymbol{y}$ from the model remains unchanged. However, it is more common that more than one parameter is unidentifiable in a structurally unidentifiable model. It is quite common for a structurally unidentifiable model to have structurally identifiable combinations of the given model parameters, e.g., sums, products or ratios. For instance, in the example with the simple one-compartment model (2.11) we could conclude from the input-output relation (2.15) that the two model parameters $\{\theta_2, \theta_3\}$ are unidentifiable, but the product $\theta_2\theta_3$ is identifiable.

If a non-mixed-effects model with structurally identifiable combinations of parameters is defined in a mixed-effects framework, i.e., random effects are introduced to the model parameters, it follows that those combinations would have structurally identifiable distributions. The repeated measurement approach is to determine under what conditions more can be said about the identifiability of the underlying statistical parameters if the non-mixed-effects state-space model is defined in a mixed-effects framework. To state the problem more formally, consider

$$\mathbf{Z} = \lambda(\mathbf{\Phi}) \tag{4.18}$$

where $\mathbf{\Phi}$ is a vector containing a subset of the model parameters $\boldsymbol{\phi}_i$ with postulated parameterised distributions and $\mathbf{\Phi} \in \mathbb{R}^p$, $\mathbf{Z} \in \mathbb{R}^q$, $q \leq p$ and $\lambda(\cdot)$ is in general a nonlinear function of the model parameters, e.g., products, sums or ratios of two or more parameters. The repeated measurement approach determines under what conditions the original parameterised distributions of $\mathbf{\Phi}$ are determined by a lower or equal dimensional distribution of $\mathbf{Z}$. By considering the structural identifiability of a mixed-effects model in this way there exist several previously established statistical theorems that can be used to answer this question. These theorems are restated below.

Theorem 1. Radhakrishna [1971] Suppose P_1, P_2 and P_3 are three independent

real-valued random variables. Consider the two linear forms:

$$Z_1 = a_1P_1 + a_2P_2 + a_3P_3 \tag{4.19}$$

$$Z_2 = b_1P_1 + b_2P_2 + b_3P_3 \tag{4.20}$$

such that the ratios $a_i : b_i \neq a_j : b_j$ for $i \neq j$. If the characteristic function of (Z_1, Z_2) does not vanish, then the joint distribution of (Z_1, Z_2) determines the distribution of P_1, P_2 and P_3 up to a change of location.

Theorem 2. Radhakrishna [1971] In an extension of Theorem 1, consider p linear functions Z_i, $1 \leq i \leq p$, of n independent variables P_i. The smallest number p of linear functions Z_i, $1 \leq i \leq p$ such that the joint distribution specifies the distribution of each random variable P_i, $1 \leq i \leq n$, can be calculated from the following relation

$$\frac{p(p-1)}{2} < n \leq \frac{p(p+1)}{2} \tag{4.21}$$

Theorem 3. Rao [1992] Suppose P_1, P_2 and P_3 are three independent positive random variables. Let

$$Z_1 = \frac{P_1}{P_3} \tag{4.22}$$

$$Z_2 = \frac{P_2}{P_3} \tag{4.23}$$

If the characteristic function of (log Z_1, log Z_2) does not vanish then the distribution of (Z_1,Z_2) determines the distributions of P_1, P_2 and P_3 up to a change of scale.

Theorem 4. Szekely and Rao [2000] Let P_1, P_2,..., P_n be independent random variables. Given the moments $E[P_j^s]$ where s = 1,2,..., m and j = 1,2,..., n the joint distribution function of the linear forms

$$Z_i = \sum_{j=1}^{n} a_{ij}P_i, i = 1, 2, ...k \tag{4.24}$$

with an arbitrary nonvanishing joint characteristic function uniquely determines the

distributions of P_1, P_2,..., P_n if and only if

$$n \leq \binom{k+m}{m+1}. \tag{4.25}$$

Theorems 1–4 provide some general conditions on how the model parameters belonging to a distribution affect structural identifiability. The process in the repeated measurement approach is therefore

- Determine what combinations of parameters that can be uniquely determined, i.e, sums, products, ratios etc. In more simple cases, this can be done by visual inspection. Alternatively, derive the *exhaustive summary* (Walter [1982]; Chapman and Godfrey [1985]) and apply the subsequent extensions presented in Sections 4.3.3–4.3.6.

- Once the identifiable combinations of parameters have been identified the applicability of statistical theorems such as Theorem 1–4 should be checked. If any of the theorems can be applied then the identifiability of the model follows directly from what that particular theorem states.

A practical example of a structural identifiability analysis using the repeated measurement approach is given in Section 5.2.1.

4.3.2 Augmented system approach

One approach when studying a system is to transform the system to an alternative representation from which the subsequent analysis is simplified compared to the original system. A well-known example of this is to take Laplace transforms of a linear differential equation and transform from the time domain to the s-domain, which often simplifies solving the original differential equation.

The idea behind the augmented system approach is to represent the model in a different form in order to simplify/enable the structural identifiability analysis of the mixed-effects system.

The augmented system approach augments the original mixed-effects system (2.5) in such a way that the model parameters in the original mixed-effects system are differential equations with zero time-derivative and with initial conditions as random variables in the augmented system. The augmented system is written in a random differential equation (RDE) form (Soong [1973]) where the randomness enters only through the random variables as the initial conditions. Having the mixed-effects model rewritten in a RDE form allows for utilisation of existing theory for RDE systems, see Theorem 5, to study structural identifiability in the original mixed-effects system.

Theorem 5. Soong [1973] Consider the random system described by

$$\dot{\boldsymbol{x}}(t) = \boldsymbol{f}(\boldsymbol{x}(t), t) \tag{4.26}$$

$$\boldsymbol{x}(t_0) = \boldsymbol{x}_0 \tag{4.27}$$

where the initial condition vector $\boldsymbol{x}_0$ has elements that are random variables and $p_0(\boldsymbol{x}_0)$ is the joint density function of the initial condition $\boldsymbol{x}_0$. The general solution takes the form

$$\boldsymbol{x}(t) = \boldsymbol{q}(\boldsymbol{x_0}, t) \tag{4.28}$$

If $\boldsymbol{q(\cdot)}$ is continuous in $\boldsymbol{x_0}$, has continuous partial derivatives with respect to $\boldsymbol{x_0}$ and defines a one-to-one mapping, then the inverse transform can be written as

$$\boldsymbol{x}_0 = \boldsymbol{q}^{-1}(\boldsymbol{x}, t) \tag{4.29}$$

The joint density function $p(\boldsymbol{x}, t)$ of $\boldsymbol{x}(t)$ is then given by

$$p(\boldsymbol{x}, t) = p_0[\boldsymbol{x}_0 = \boldsymbol{q}^{-1}(\boldsymbol{x}, t)] \mid J \mid \tag{4.30}$$

where $J = \left| \frac{\partial \boldsymbol{x}_0}{\partial \boldsymbol{x}} \right|$.

The joint density function $p(\boldsymbol{x}, t)$ in (4.30) in Theorem 5 describes the distribution of all states $\boldsymbol{x}(t)$ at all times t. If each state is observed the structural

identifiability problem is then to determine whether $p(\boldsymbol{x}, t)$ is uniquely determined by (4.30) in Theorem 5.

However, in the analysis of the augmented system all models states will never be observed. If that was the case, then the model parameters would be observed directly since they are defined as states in the augmented system, thus making a structural identifiability analysis unnecessary to begin with. It is therefore necessary to describe the distribution of individual states. In Soong [1973] it is stated that the distribution of a single state from a RDE system can be described by

$$\mathbb{E}[x_i^m(t)] = \int_{-\infty}^{\infty} q_i^m(\boldsymbol{x}_0, t) p_0(\boldsymbol{x}_0) d\boldsymbol{x}_0 \tag{4.31}$$

where m denotes the order of a statistical moment. If the output function is a direct observation of some state $x_1(t)$ as

$$y(t) = x_1(t) \tag{4.32}$$

then the distribution of the output function can be described as

$$\mathbb{E}[y^m(t)] = E[x_1^m(t)] = \int_{-\infty}^{\infty} q_1^m(\boldsymbol{x}_0, t) p_0(\boldsymbol{x}_0) d\boldsymbol{x}_0 \tag{4.33}$$

where m denotes the order of a statistical moment. To see whether the distribution of the output function y is uniquely determined by $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ an alternative parameter vector and covariance matrix $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ can be introduced. By equating

$$\mathbb{E}[y^m(t, \boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[y^m(t, \bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{4.34}$$

where $m = 1, 2, \ldots$, and solving for $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ the structural identifiability of the original mixed-effects model can be determined. An example of a structural identifiability analysis using the augmented system approach is provided in Section 5.2.2.

4.3.3 Functions of random variables

The three remaining methods applicable to study the structural identifiability of mixed-effects models developed within this thesis are related to functions of random variables $Z_k(\boldsymbol{\theta}, \boldsymbol{\eta})$. In this section it will be shown how the uniqueness of the parameters involved in such functions can be studied with respect to the distribution of $Z_k(\boldsymbol{\theta}, \boldsymbol{\eta})$. These functions are then shown in this thesis to be related to structural identifiability and structural indistinguishability of mixed-effects models via the exhaustive summary (Walter [1982]).

Let

$$\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta}) = \begin{pmatrix} Z_1(\boldsymbol{\theta}, \boldsymbol{\eta}) \\ Z_2(\boldsymbol{\theta}, \boldsymbol{\eta}) \\ \vdots \end{pmatrix} \tag{4.35}$$

be a vector of functions of random variables. In the analysis, full knowledge of all of the statistical moments and covariances of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$ is assumed. This follows from the assumption of having ideal experimental conditions, i.e., noise-free, continuous-time measurements from an infinite number of subjects. The question is whether the statistical moments and covariance matrix of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$ uniquely determine $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ or otherwise.

By computing different orders m of the statistical moments and covariances of $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$, introducing alternative parameters $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ and equating the statistical moments and covariances as

$$\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[\boldsymbol{Z}^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{4.36}$$

$$\mathrm{Cov}(\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})) = \mathrm{Cov}(\boldsymbol{Z}(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})) \tag{4.37}$$

and solving for $\boldsymbol{\theta}$ and $\boldsymbol{\Omega}$ the uniqueness or otherwise of the model parameters can be determined. Note that by $\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta}, \boldsymbol{\eta})]$ means the m:th statistical moment element-wise in $\boldsymbol{Z}(\boldsymbol{\theta}, \boldsymbol{\eta})$ in this context.

As an example of how the uniqueness of the model parameters can be studied, consider the case of two functions of random variables, Z_1 and Z_2. The two functions

in this particular example are both lognormally distributed functions of random variables with an associated full covariance matrix $\boldsymbol{\Omega}$

$$\boldsymbol{Z} = \begin{pmatrix} Z_1 \\ Z_2 \end{pmatrix} = \begin{pmatrix} \theta_1 e^{\eta_1} \\ \theta_2 e^{\eta_2} \end{pmatrix} \tag{4.38}$$

$$\tag{4.39}$$

with the random effects vector

$$\boldsymbol{\eta} = (\eta_1, \eta_2) \tag{4.40}$$

which is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{4.41}$$

with the covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_1 & \omega_{12} \\ \omega_{12} & \omega_2 \end{pmatrix} \tag{4.42}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (\theta_1, \theta_2, \omega_1, \omega_2, \omega_{12}). \tag{4.43}$$

The first statistical moments of $\boldsymbol{Z}$ are

$$\mathbb{E}[\boldsymbol{Z}] = \begin{pmatrix} \theta_1 e^{\frac{\omega_1}{2}} \\ \theta_2 e^{\frac{\omega_2}{2}} \end{pmatrix}. \tag{4.44}$$

The covariance matrix for $\boldsymbol{Z}$ is given by

$$\begin{aligned}\mathrm{Cov}(\boldsymbol{Z}) &= \mathbb{E}[\boldsymbol{Z}\boldsymbol{Z}^T] - \mathbb{E}[\boldsymbol{Z}]\mathbb{E}[\boldsymbol{Z}]^T = \\ &= \begin{pmatrix} \mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 & \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] \\ \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] & \mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 \end{pmatrix}\end{aligned} \tag{4.45}$$

where the diagonal elements, i.e., the variances of Z_1 and Z_2, are given by

$$\mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 = \theta_1^2 e^{2\omega_1} - \theta_1^2 e^{\omega_1} \tag{4.46}$$

$$\mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 = \theta_2^2 e^{2\omega_2} - \theta_2^2 e^{\omega_2} \tag{4.47}$$

and the off-diagonal elements, i.e., the covariances between Z_1 and Z_2, are given by

$$\mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] = \theta_1\theta_2 e^{\frac{1}{2}(2\omega_{12}+\omega_1+\omega_2)} - \theta_1\theta_2 e^{\frac{\omega_1}{2}+\frac{\omega_2}{2}}. \tag{4.48}$$

By introducing an alternative parameter vector and matrix $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ and equating the statistical moments and covariance as in (4.36)–(4.37) from (4.44)–(4.48) the following equation system is obtained:

$$\theta_1 e^{\frac{\omega_1}{2}} = \bar{\theta}_1 e^{\frac{\bar{\omega}_1}{2}} \tag{4.49}$$

$$\theta_1^2 e^{2\omega_1} - \theta_1^2 e^{\omega_1} = \bar{\theta}_1^2 e^{2\bar{\omega}_1} - \bar{\theta}_1^2 e^{\bar{\omega}_1} \tag{4.50}$$

$$\theta_2 e^{\frac{\omega_2}{2}} = \bar{\theta}_2 e^{\frac{\bar{\omega}_2}{2}} \tag{4.51}$$

$$\theta_2^2 e^{2\omega_2} - \theta_2^2 e^{\omega_2} = \bar{\theta}_2^2 e^{2\bar{\omega}_2} - \bar{\theta}_2^2 e^{\bar{\omega}_2} \tag{4.52}$$

$$\theta_1\theta_2 e^{\frac{1}{2}(2\omega_{12}+\omega_1+\omega_2)} - \theta_1\theta_2 e^{\frac{\omega_1}{2}+\frac{\omega_2}{2}} = \bar{\theta}_1\bar{\theta}_2 e^{\frac{1}{2}(2\bar{\omega}_{12}+\bar{\omega}_1+\bar{\omega}_2)} - \bar{\theta}_1\bar{\theta}_2 e^{\frac{\bar{\omega}_1}{2}+\frac{\bar{\omega}_2}{2}} \tag{4.53}$$

which has only one solution, namely

$$\theta_1 = \bar{\theta}_1 \tag{4.54}$$

$$\theta_2 = \bar{\theta}_2 \tag{4.55}$$

$$\omega_1 = \bar{\omega}_1 \tag{4.56}$$

$$\omega_2 = \bar{\omega}_2 \tag{4.57}$$

$$\omega_{12} = \bar{\omega}_{12}. \tag{4.58}$$

This means that

$$\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\} \tag{4.59}$$

and the distribution of $\boldsymbol{Z}$ uniquely determines the parameters $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$.

To study the structural identifiability of a mixed-effects system using functions of random variables, the corresponding *exhaustive summary* $\boldsymbol{\sigma}(\boldsymbol{\theta})$ (Walter [1982]) for the mixed-effects system must be found. The exhaustive summary is a vector which contains all information about the model parameters that can be extracted from the knowledge of the input and output signal (Walter and Lecourtier [1982]). The functions of random variables can be generated from the exhaustive summary for the corresponding non-mixed-effects system, i.e., where

$$\boldsymbol{\Omega} = \mathbf{0}. \tag{4.60}$$

Once the functions of the random variables for the mixed-effects system have been found the structural identifiability of the mixed-effects system can be considered.

In Sections 4.3.4–4.3.6 it will be shown how the functions of random variables for the mixed-effects system can be found using established techniques for structural identifiability analysis of non-mixed-effects systems.

4.3.4 The Laplace transform approach

The Laplace transform approach to study structural identifiability of non-mixed-effects systems was covered in Section 2.4.3. In this section, functions of random variables are combined with the exhaustive summary generated from the moment invariants from the transfer function coefficient of the corresponding non-mixed-effects model to study the structural identifiability of the corresponding mixed-effects model.

To reiterate, the Laplace transformation approach is only applicable to linear models of the form

$$\begin{aligned} \dot{\boldsymbol{x}}(t,\boldsymbol{\theta}) &= \boldsymbol{A}(\boldsymbol{\theta})\boldsymbol{x}(t) + \boldsymbol{B}(\boldsymbol{\theta})\boldsymbol{u}(t) \\ \boldsymbol{x}(0,\boldsymbol{\theta}) &= 0 \\ \boldsymbol{y}(t,\boldsymbol{\theta}) &= \boldsymbol{C}(\boldsymbol{\theta})\boldsymbol{x}(t) \end{aligned} \tag{4.61}$$

where $\boldsymbol{A}(\boldsymbol{\theta})$, $\boldsymbol{B}(\boldsymbol{\theta})$ and $\boldsymbol{C}(\boldsymbol{\theta})$ are the system matrices. By calculating the Laplace transform of the system the input-output relation can be described by

$$\boldsymbol{Y}(s) = \boldsymbol{G}(s)\boldsymbol{U}(s) \tag{4.62}$$

where

$$\boldsymbol{G}(s) = \boldsymbol{C}(\boldsymbol{\theta})(s\boldsymbol{I} - \boldsymbol{A}(\boldsymbol{\theta}))^{-1}\boldsymbol{B}(\boldsymbol{\theta}) \tag{4.63}$$

is the transfer function matrix of the system. For simplicity, but without loss of generality, assume that the system only has one output function. The elements of the transfer function matrix can be written in the following form

$$G(s) = \frac{b_1(\boldsymbol{\theta})s^{n-1} + \cdots + b_{n-1}(\boldsymbol{\theta})s + b_n(\boldsymbol{\theta})}{s^n + a_1(\boldsymbol{\theta})s^{n-1} + \cdots + a_{n-1}(\boldsymbol{\theta})s + a_n(\boldsymbol{\theta})}. \tag{4.64}$$

The coefficients of the powers of s in the numerator and denominator are called the system's moment invariants and are unique with respect to the input-output

relationship. Let

$$\boldsymbol{\sigma}(\boldsymbol{\theta}) = \begin{pmatrix} a_1(\boldsymbol{\theta}) \\ \vdots \\ a_n(\boldsymbol{\theta}) \\ \vdots \\ b_1(\boldsymbol{\theta}) \\ \vdots \\ b_n(\boldsymbol{\theta}) \end{pmatrix}. \tag{4.65}$$

be the exhaustive summary and $\sigma_k(\boldsymbol{\theta})$ denote the k:th element in $\boldsymbol{\sigma}(\boldsymbol{\theta})$. By equating and solving

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \ldots \tag{4.66}$$

the structural identifiability of the non-mixed-effects system can be studied. Under the assumption of an infinite number of subjects, the moment invariants become distributed over the population with respect to the statistical sub-model. Therefore, in a mixed-effects system, the functions of random variables are given by

$$Z_k(\boldsymbol{\theta}, \boldsymbol{\eta}) = \sigma_k(\boldsymbol{\theta}, \boldsymbol{\eta}) \qquad k = 1, 2, \ldots, N \tag{4.67}$$

Using (4.36)–(4.37) with (4.67) the structural identifiability of the mixed-effects model can be studied.

4.3.5 Taylor series expansion approach

Mathematical definition

The Taylor series expansion approach to study structural identifiability in non-mixed-effects models was outlined in Section 2.4.3. Again, the Taylor series expansion of the output function y_i from subject i is given by

$$y_i(t,\boldsymbol{\theta}) = y_i(0,\boldsymbol{\theta}) + y_i^{(1)}(0,\boldsymbol{\theta})\frac{t}{1!} + y_i^{(2)}(0,\boldsymbol{\theta})\frac{t^2}{2!} + \cdots + y_i^{(k)}(0,\boldsymbol{\theta})\frac{t^k}{k!} + \ldots \tag{4.68}$$

In a mixed-effects modelling framework under ideal conditions, i.e., an infinite number of subjects, there is an infinite number of trajectories $y_i(t,\boldsymbol{p})$ where $i = 1,\ldots,\infty$. From this it follows that there are an infinite number of each coefficient in this Taylor series expansion. This means that each coefficient has a distribution which is dependent on the statistical sub-model in the mixed-effects model. Since the trajectory from each subject is measured, we also know what the distribution of each coefficient is.

Since all coefficients in the Taylor series expansion are unique for a particular model output, the uniqueness of the model parameters can be determined from these coefficients. The exhaustive summary is therefore the coefficients in the Taylor series expansion and by equating these as

$$\sigma_1(\boldsymbol{\theta}) = y(0,\boldsymbol{\theta}) \tag{4.69}$$

$$\sigma_{k+1}(\boldsymbol{\theta}) = y^{(k)}(0,\boldsymbol{\theta}) \qquad k = 1,2,\ldots \tag{4.70}$$

$$\sigma_i(\boldsymbol{\theta}) = \sigma_i(\bar{\boldsymbol{\theta}}) \qquad i = 1,2,\ldots \tag{4.71}$$

and solving for $\boldsymbol{\theta}$ the structural identifiability of the state-space model can be determined.

In the mixed-effects case, again under the assumption of an infinite number of subjects, the coefficients $\sigma_i(\boldsymbol{\theta})$ become distributed over the population. This distribution depends on the underlying statistical sub-model. Therefore, the coefficients in the Taylor series expansion can, in the mixed-effects case, be regarded as functions of random variables and is given by

$$Z_i(\boldsymbol{\theta},\boldsymbol{\eta}) = \sigma_i(\boldsymbol{\theta},\boldsymbol{\eta}) \qquad i = 1,2,\ldots. \tag{4.72}$$

By combining (4.36)–(4.37) with the exhaustive summary from the Taylor series co-

efficients (4.72) the structural identifiability of mixed-effects models can be studied.

Upper bounds Taylor series expansion

As mentioned in Section 2.4.3, there exist upper bounds on how many terms of higher order derivatives of the output function(s) need to be computed in order to show that a model is structurally unidentifiable. The upper bounds for a mixed-effects models is related to the bounds for the non-mixed-effects model in the following way. When studying the structural identifiability of a mixed-effects model we are studying the distribution of the exhaustive summary $\boldsymbol{\sigma}(\boldsymbol{\theta})$ of the corresponding non-mixed-effects model. If the bounds are higher in the mixed-effects case we would instead study the distribution of $\boldsymbol{\sigma}^*(\boldsymbol{\theta})$, a vector containing redundant information on $\boldsymbol{\theta}$ at the individual level. The distributions of $\boldsymbol{\sigma}^*(\boldsymbol{\theta})$ would therefore contain redundant information about $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$. If the bounds instead are lower for the mixed-effects case, then we study the distribution of $\boldsymbol{\sigma}^{**}(\boldsymbol{\theta})$, a vector containing some, but not all information, on $\boldsymbol{\theta}$ that is required to deduce structural identifiability from the output at an individual level. In other words, neither $\boldsymbol{\sigma}^*(\boldsymbol{\theta})$ nor $\boldsymbol{\sigma}^{**}(\boldsymbol{\theta})$ are exhaustive summaries. Therefore, the upper bounds for the Taylor series expansion in the non-mixed-effects case are the same as for the mixed-effects case.

4.3.6 Input-output form approach

Mathematical definition

A third way of generating the exhaustive summary of a system and extending it to functions of random variables is to rewrite the model in an input-output form. Starting with a state-space system, this can be done by iteratively differentiating the output function $\boldsymbol{y}$ and substituting in place of all of the model states $\boldsymbol{x}$, (Bearup et al. [2013]). A system rewritten in an input-output form has the following differential algebraic polynomial form

$$\sum_{k=1}^{l} \sigma_k(\boldsymbol{\theta}) g_k(y, \dot{y}, \ddot{y}, \dots) = 0. \tag{4.73}$$

Together with the initial conditions of the system, (4.73) determines uniquely the solution of the model output, Bearup et al. [2013]. Therefore, by setting up the equation system

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \dots, l \tag{4.74}$$

$$y(0, \boldsymbol{\theta}) = y(0, \bar{\boldsymbol{\theta}}) \tag{4.75}$$

$$\vdots$$

$$y^{(k)}(0, \boldsymbol{\theta}) = y^{(k)}(0, \bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \dots, n-1 \tag{4.76}$$

and solving for $\boldsymbol{\theta}$ the structural identifiability of the system can be determined. Note that for this to be true the terms in

$$g_k(y, \dot{y}, \ddot{y}, \dots) \tag{4.77}$$

must all be linearly independent. Generating the input-output form often requires handling of relatively complex analytical expressions and in practice it is therefore often neseccary to use software with symbolic computation capabilities, e.g., Maple (MapleSoft [2015]) or Mathematica (Wolfram Research Inc. [2016]).

In a mixed-effects model, using the same reasoning as for the Laplace transform and the Taylor series approaches, the input-output form (4.73) becomes

$$\sum_{k=1}^{l} \sigma_k(\boldsymbol{\theta}, \boldsymbol{\eta}) g_k(y, \dot{y}, \ddot{y}, \dots) = 0. \tag{4.78}$$

and the full set of functions of random variables is therefore given by

$$Z_k(\boldsymbol{\theta}, \boldsymbol{\eta}) = \sigma_k(\boldsymbol{\theta}, \boldsymbol{\eta}) \qquad k = 1, 2, \dots, l \tag{4.79}$$

$$Z_{l+1}(\boldsymbol{\theta}, \boldsymbol{\eta}) = y(0, \boldsymbol{\theta}, \boldsymbol{\eta}) \tag{4.80}$$

$$\vdots$$

$$Z_{l+1+r}(\boldsymbol{\theta}, \boldsymbol{\eta}) = y^{(r)}(0, \boldsymbol{\theta}, \boldsymbol{\eta}) \qquad r = 1, 2, \dots, n-1. \tag{4.81}$$

Again, by using (4.36)–(4.37) with (4.79)–(4.81) the structural identifiability of mixed-effects models can be studied.

4.4 Structural Indistinguishability methods

As mentioned above, structural identifiability is a special case of structural indistinguishability. Therefore, it should perhaps be no surprise that techniques for structural identifiability analysis of non-mixed-effects models can be modified to study the structural indistinguishability of non-mixed-effects models. The same is true for some of the methods developed here for structural identifiability analysis of mixed-effects models, namely the methods which study the distribution of the exhaustive summary of a model: Laplace transform approach, Taylor series expansion approach and the input-output approach. These methods can be modified slightly to study structural indistinguishability of mixed-effects model as well. In general, for linear models all models can be found that have the same input-output relation. However, for nonlinear models only pair-wise comparisons are possible.

4.4.1 Laplace transform approach

To determine whether two mixed-effects models are indistinguishable, or to find all linear mixed-effects models with the same input-output relation, their corresponding non-mixed-effect model is first considered. In this section a pair-wise comparison will be taken into consideration. By Laplace transform the non-mixed-effects version of the two systems their moment invariants $\boldsymbol{\sigma}_A(\boldsymbol{\theta})$ and $\boldsymbol{\sigma}_B(\bar{\boldsymbol{\theta}})$ can be computed. In a mixed-effects setting, these moment invariants becomes functions of random variables as

$$\boldsymbol{Z}_A(\boldsymbol{\theta}, \boldsymbol{\eta}) = \boldsymbol{\sigma}_A(\boldsymbol{\theta}, \boldsymbol{\eta}) \tag{4.82}$$

$$\boldsymbol{Z}_B(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}}) = \boldsymbol{\sigma}_B(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}}). \tag{4.83}$$

To determine whether two models are structurally indistinguishable the functions of random variables derived from the moment invariants of the two models are equated

as

$$\mathbb{E}[\boldsymbol{Z}_A^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[\boldsymbol{Z}_B^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{4.84}$$

where $k = 1, \ldots, N$ with the m:th statistical moment. If a generic relation between $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ and $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ can be deduced from (4.84) then the two models are structurally indistinguishable. If such a relation cannot be deduced the two mixed-effects models are structurally distinguishable. Note that with using the Laplace transform approach only structural indistinguishability between linear models can be studied.

4.4.2 Taylor series expansion approach

For two non-mixed-effects models to be structurally indistinguishable all coefficients in the Taylor series expansion are equal. For two mixed-effects models to be structurally indistinguishable the distribution of all coefficients in the Taylor series expansion from the two models must be equal. Therefore, to see whether two mixed-effects models are structurally indistinguishable or not using the Taylor series expansion approach the following is done. First, coefficients in the Taylor series expansion of the two corresponding non-mixed-effects models are computed

$$\sigma_1(\boldsymbol{\theta}) = y(0, \boldsymbol{\theta}) \tag{4.85}$$

$$\sigma_{k+1}(\boldsymbol{\theta}) = y^{(k)}(0, \boldsymbol{\theta}) \qquad k = 1, 2, \ldots \tag{4.86}$$

$$\bar{\sigma}_1(\bar{\boldsymbol{\theta}}) = \bar{y}(0, \bar{\boldsymbol{\theta}}) \tag{4.87}$$

$$\bar{\sigma}_{k+1}(\bar{\boldsymbol{\theta}}) = \bar{y}^{(k)}(0, \bar{\boldsymbol{\theta}}) \qquad k = 1, 2, \ldots \tag{4.88}$$

From these coefficients the functions of random variables are derived. By equating these functions as

$$\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[\bar{\boldsymbol{Z}}^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{4.89}$$

where $k = 1, \ldots, N$ with the m:th statistical moment the structural indistinguishability of two mixed-effects models can be studied. Again, if there is a generic relation

between $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ and $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ the two mixed-effects models are structurally indistinguishable.

4.4.3 Input-Output form approach

Lastly, the input-output approach can also be modified to study structural indistinguishability of mixed-effects models. Again, the non-mixed-effects version of the two mixed-effects models are first considered. These models can be rewritten in an input-output form as

$$\sum_{k=1}^{l} \sigma_k(\boldsymbol{\theta}) g_k(y, \dot{y}, \ddot{y}, \dots) = 0 \tag{4.90}$$

$$\sum_{k=1}^{l} \bar{\sigma}_k(\bar{\boldsymbol{\theta}}) g_k(\bar{y}, \dot{\bar{y}}, \ddot{\bar{y}}, \dots) = 0. \tag{4.91}$$

From here the functions of random variables can be derived from the coefficients $\sigma_k(\boldsymbol{\theta})$ and $\bar{\sigma}_k(\bar{\boldsymbol{\theta}})$. By equating these functions as

$$\mathbb{E}[\boldsymbol{Z}^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[\bar{\boldsymbol{Z}}^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{4.92}$$

where $k = 1, \dots, N$ with the m:th statistical moment the structural indistinguishability of two mixed-effects models can be studied. If a generic relation between $\{\boldsymbol{\theta}, \boldsymbol{\Omega}\}$ and $\{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}$ can be established then the two mixed-effects models are structurally indistinguishable.

Upper bounds statistical moments

Similar as for the case with upper bounds for the Taylor series expansion, upper bounds of the order of statistical moment m are relevant in this context. This is because structural local identifiability or unidentifiability cannot be proven until the upper bound is reached. For function of random variables that have standard distributions, e.g., normal distribution or lognormal distribution, the upper bound is $m = 2$ since such distributions are fully characterized by their first two moments,

i.e., mean and variance. However, for non-standard distributions finding the upper bound is often a non-trivial problem.

4.5 Summary

Mathematical definitions of structural identifiability and structural indistinguishability have prior to this work only existed for non-mixed-effects models. In this chapter, these two concepts have been generalised to include mixed-effects models as well. Based on these two more general definitions, several analytical methods have been presented in this chapter that are applicable to mixed-effects models.

Five different methods to analyse the structural identifiability of mixed-effects models have been presented. Three of these methods are extensions of previously established techniques for non-mixed-effects models: the Laplace transform approach, the Taylor series approach and the input-output approach. It is worth emphasizing that the input-output form of a model can be generated in several ways as described in Section 2.4.3.

In the non-mixed-effects case, structural identifiability is a special case of structural indistinguishability. With respect to the two new definitions, this is also true in the mixed-effects case. The Laplace transform approach, the Taylor series approach and the Input-Output approach can therefore be modified to be applicable to study structural indistinguishability of mixed-effects models as well. Again, the input-output form of a model can be generated in several ways offering additional ways of studying the structural indistinguishability of mixed-effects models.

Of the presented methods in this thesis it is perhaps the Laplace transform approach, the Taylor series approach and the input-iutput form approach that are the most readily applicable. While the Laplace transform approach is only applicable to linear systems, the Taylor series approach and the input-output form approach are applicable to nonlinear systems. These methods are applicable to models with both either diagonal or non-zero off-diagonal covariance matrix $\boldsymbol{\Omega}$ and any arbitrary form of distribution of the random effects $\boldsymbol{\eta}$.

The concepts of structural identifiability and structural indistinguishability have been generalised. Two more general mathematical definitions have been presented which collapse to the old definition of identifiability and indistinguishability when all variance parameters of the random effects in a mixed-effects modelling framework are set to zero. Based on these new definitions, five novel approaches have been developed and are presented in this chapter.

The repeated measurement approach is perhaps the least applicable in its current form. It relies on certain combinations of random effects, i.e., random variables, to appear. If such combinations do appear, the idea is to apply previously existing theorems from statistics where the identifiability problem is also recognised. Such theorems are presented in this chapter and outlines what can be derived about the underlying statistical parameters.

The augmented system approach is more applicable than the repeated measurement approach however arguably the biggest downside of this approach is the requirement of finding the system solution. This is often not possible which limits the applicability.

In the next chapter examples of structural identifiability analysis of mixed-effects models will be provided to show how the methods presented in this chapter work in practice.

Chapter 5

Structural Identifiability analysis of Mixed-Effects Models: Case studies

5.1 Introduction

In this chapter the structural identifiability analysis methods applicable to mixed-effects models that are presented in Chapter 4 are applied to a set of mixed-effects models. The particular mixed-effects models that have been analysed have been chosen for two main reasons.

Firstly, the purpose of this chapter is to illustrate how the developed methods work in practice. Because of this, models have been chosen where the subsequent analytical analysis was not too complicated, i.e., relatively simple symbolic expressions.

Secondly, some of the models are routinely used in modelling efforts in pharmaceutical research and development projects and the results from the analysis of the models is therefore of direct interest to the pharmaceutical community.

All of the developed methods will be applied to at least one model to test their relative ease of application and to see if any implications arises out of the

application.

5.2 Structural identifiability analysis

5.2.1 Repeated measurement approach

The outline of the repeated measurement approach was provided in Section 4.3.1 and will be exemplified here using a simple tumour growth model as an example.

Simple tumour growth model

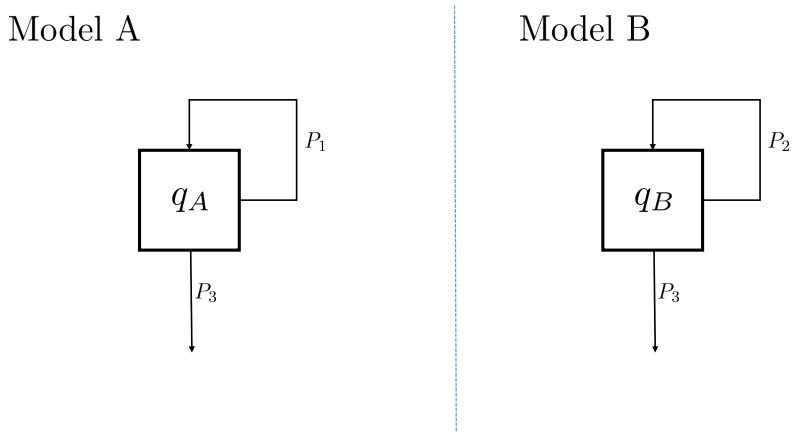


Figure 5.1: Two tumor growth models with one shared parameter P_3 representing the rate of natural cell death. The cell division rate P_1 and P_2 in the two models are different due to drug intervention.

This example involves two simple models with one shared parameter, Figure 5.1. The models represent tumour growth and have the following structures

$$\begin{aligned} \dot{q}_A(t) &= (P_1 - P_3)q_A(t) \\ q_A(0) &= q_{A0} \end{aligned} \tag{5.1}$$

with observation

$$y_1(t) = q_A(t) \tag{5.2}$$

and

$$\begin{aligned} \dot{q}_B(t) &= (P_2 - P_3)q_B(t) \\ q_B(0) &= q_{B0} \end{aligned} \tag{5.3}$$

with observation

$$y_2(t) = q_B(t) \tag{5.4}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (P_1, P_2, P_3) \tag{5.5}$$

where

$$P_i = e^{\eta_i} \tag{5.6}$$

and

$$\eta_i \in N(0, \sqrt{\omega}_i), \tag{5.7}$$

i.e., a lognormal distribution of all three parameters has been postulated as this is a common shape of distribution in biology in general (Grönholm and Annila [2007]) and it also ensures positivity. In the model it was, for simplicity, assumed that P1 = P2 = P3 = 1 but the identifiability result still holds for all positive numerical values of these parameters. The parameters P_1 and P_2 represent the growth rate of the tumour with no drug present and with drug present respectively, parameter P_3 is the rate of natural cell death, $q_A(t)$ tracks the tumour growth without the drug and $q_B(t)$ tracks the tumour growth with the drug present. This model can be used to study anti-proliferation effects of a drug. Note that the parameter P_3 is shared between the two models. Also note that as a consequence of fixing the expected values of the parameters P_1, P_2 and P_3 to 1 the tumour sizes $q_A(t)$ and $q_B(t)$ are on

average constant since the derivatives $\dot{q}_A(t)$ and $\dot{q}_B(t)$ are on average zero.

Already by visual inspection it is clear that the two functions of random variables

$$Z_1 = P_1 - P_3 \tag{5.8}$$

$$Z_2 = P_2 - P_3 \tag{5.9}$$

are identifiable, i.e., the statistical moments of Z_1 and Z_2 are uniquely determined by the distributions of the output functions y_1 and y_2. The question is whether the distribution of Z_1 and Z_2 determine the statistical parameters

$$\{\omega_1, \omega_2, \omega_3\} \tag{5.10}$$

of P_1, P_2 and P_3 uniquely. If the model parameters in the two models are estimated in two separate inference problems the models are structurally unidentifiable. However, if the model parameters in the two models are considered in a joint inference problem the equations are of such a form precisely stated in Theorem 1 in Chapter 4. The theorem states that if Z_1 and Z_2 have such forms then it follows directly that all statistical moments of $\{P_1, P_2, P_3\}$ up to the first moment are uniquely determined, i.e., the shape of the distribution of $\{P_1, P_2, P_3\}$ can be determined but not their mean (population parameter). In this particular simple illustrative example the first statistical moment, i.e., the mean, was assumed to be known as it was set to 1. Therefore, if the model parameters from the two models are estimated simultaneously, both models are structurally globally identifiable.

5.2.2 Augmented systems approach

The augmented systems approach was presented in Section 4.3.2 and will here be exemplified using a simple one-parameter model.

Simple one-parameter model

Consider the following linear model with only one state compartment and an unknown dose parameter Z_D

$$\begin{aligned} \dot{x}_1(t) &= -x_1(t) \\ x_1(0) &= Z_D \end{aligned} \tag{5.11}$$

with observation

$$y(t, \boldsymbol{\theta}) = x_1(t) \tag{5.12}$$

and with the probability distribution function

$$p_0(Z_D) = \frac{e^{-\frac{(Z_D-\mu_D)^2}{2\omega_D^2}}}{\sqrt{2\pi}\omega_D}. \tag{5.13}$$

Note that this particular model already has the parameter as an initial condition, namely Z_D. The dose parameter Z_D is considered to be a normally distributed random variable, e.g.,

$$Z_D \sim N(\mu_D, \omega_D) \tag{5.14}$$

with $p_0(Z_D)$ denoting its probability distribution with the unknown parameter vector

$$\boldsymbol{\theta} = (\mu_D, \omega_D). \tag{5.15}$$

The model (5.11) has the solution

$$y(t, \boldsymbol{\theta}) = Z_D e^{-t}. \tag{5.16}$$

The first and second statistical moments of the observation function $y(t, \boldsymbol{\theta})$ are, using eq. (4.33), given by

$$E[y(t,\boldsymbol{\theta})] = E[x_1(t)] = \int_{-\infty}^{\infty} Z_D e^{-t} \frac{e^{\left[-\frac{(Z_D-\mu_D)^2}{2\omega_D^2}\right]}}{\sqrt{2\pi}\omega_D} dZ_D = \\ = e^{-t}\mu_D \tag{5.17}$$

$$E[y^2(t,\boldsymbol{\theta})] = E[x_1^2(t)] = \int_{-\infty}^{\infty} (Z_D e^{-t})^2 \frac{e^{\left[-\frac{(Z_D-\mu_D)^2}{2\omega_D^2}\right]}}{\sqrt{2\pi}\omega_D} dZ_D = \\ = e^{-2t}\left(\mu_D^2 + \omega_D^2\right) \tag{5.18}$$

The last step in the identifiability analysis is to determine whether the distribution of the output function $y(t, \boldsymbol{\theta})$ uniquely determines the statistical parameters $\boldsymbol{\theta}$. By introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$ and equating the statistical moments of the output function as

$$E[y^n(t,\boldsymbol{\theta})] = E[y^n(t,\bar{\boldsymbol{\theta}})] \tag{5.19}$$

then solving for $\boldsymbol{\theta}$ the structural identifiability of the mixed-effects model (5.11) can be determined. This yields the following two equations

$$e^{-t}\mu_D = e^{-t}\bar{\mu}_D \tag{5.20}$$

$$e^{-2t}\left(\mu_D^2 + \omega_D^2\right) = e^{-2t}\left(\bar{\mu}_D^2 + \bar{\omega}_D^2\right) \tag{5.21}$$

for which there is only one solution, namely

$$\mu_D = \bar{\mu}_D \tag{5.22}$$

$$\omega_D = \bar{\omega}_D. \tag{5.23}$$

This means that

$$\boldsymbol{\theta} = \bar{\boldsymbol{\theta}} \tag{5.24}$$

and the mixed-effects model (5.11) is therefore structurally globally identifiable.

5.2.3 Laplace transform approach

The Laplace transform approach to study the structural identifiability of mixed-effects models was presented in Section 4.3.4. In this section, the Laplace transform approach will be applied to a set of compartmental models to exemplify how the method works in practice.

Two-compartment model

The model, the structure of which is illustrated in Figure 5.2, is given by the following system of equations

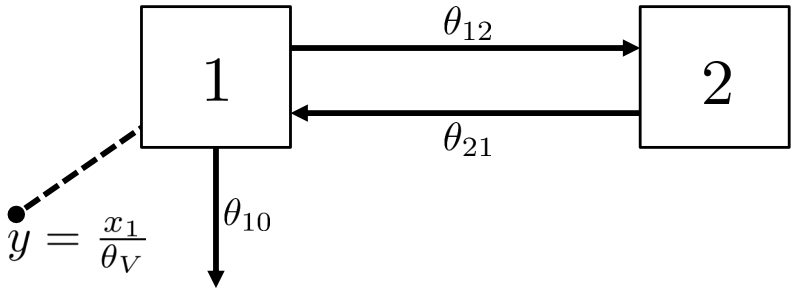


Figure 5.2: Two-compartment model with four unknown model parameters with a linear elimination from compartment 1.

$$\begin{aligned} \dot{x}_1 &= -(\theta_{12} + \theta_{10})x_1 + \theta_{21}x_2 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ x_1(0) &= D \\ x_2(0) &= 0 \end{aligned} \tag{5.25}$$

with observation

$$y = \frac{x_1}{\theta_V} \tag{5.26}$$

with the unknown parameter vector

$$\boldsymbol{\theta} = (\theta_{10}, \theta_V, \theta_{12}, \theta_{21}) \tag{5.27}$$

and a known dose parameter D as initial condition for x_1.

First the structural identifiability of the non-mixed-effects model will be considered. By taking Laplace transforms of the system the following transfer function can be generated

$$G(s) = \frac{\frac{s}{\theta_V} + \frac{\theta_{21}}{\theta_V}}{s^2 + (\theta_{12} + \theta_{10} + \theta_{21})s + \theta_{10}\theta_{21}} \tag{5.28}$$

from which the following moment invariants can be derived

$$b_1(\boldsymbol{\theta}) = \frac{1}{\theta_V}, \tag{5.29}$$

$$b_2(\boldsymbol{\theta}) = \frac{\theta_{21}}{\theta_V}, \tag{5.30}$$

$$a_1(\boldsymbol{\theta}) = \theta_{10}\theta_{21}, \tag{5.31}$$

$$a_2(\boldsymbol{\theta}) = \theta_{12} + \theta_{10} + \theta_{21}. \tag{5.32}$$

Introducing an alternative parameter $\bar{\boldsymbol{\theta}}$ and equating the corresponding moment invariants yields the following equation system

$$\frac{1}{\theta_V} = \frac{1}{\bar{\theta}_V}, \tag{5.33}$$

$$\frac{\theta_{21}}{\theta_V} = \frac{\bar{\theta}_{21}}{\bar{\theta}_V}, \tag{5.34}$$

$$\theta_{10}\theta_{21} = \bar{\theta}_{10}\bar{\theta}_{21}, \tag{5.35}$$

$$\theta_{12} + \theta_{10} + \theta_{21} = \bar{\theta}_{12} + \bar{\theta}_{10} + \bar{\theta}_{21}. \tag{5.36}$$

From (5.33) it is easy to see that

$$\theta_V = \bar{\theta}_V. \tag{5.37}$$

Using (5.37) in (5.34) yields a single solution, namely

$$\theta_{21} = \bar{\theta}_{21}. \tag{5.38}$$

Using (5.38) in (5.35) yields a single solution, namely

$$\theta_{10} = \bar{\theta}_{10}. \tag{5.39}$$

Combining (5.38)–(5.39) yields a single solution, namely

$$\theta_{10} = \bar{\theta}_{10}. \tag{5.40}$$

In summary, we have that

$$\boldsymbol{\theta} = \bar{\boldsymbol{\theta}} \tag{5.41}$$

and the non-mixed-effects two-compartment model (5.25) is therefore structurally globally identifiable.

Considering a mixed-effects version of the two-compartment model (5.25), random effects with lognormal distribution are added to all structural model parameters in this particular example in order to ensure positivity. The functions of random variables derived from the moment invariants are therefore as follows

$$Z_1 = \frac{1}{\theta_V e^{\eta_V}} \tag{5.42}$$

$$Z_2 = \frac{\theta_{21} e^{\eta_{21}}}{\theta_V e^{\eta_V}} \tag{5.43}$$

$$Z_3 = \theta_{10} e^{\eta_{10}} \theta_{21} e^{\eta_{21}} \tag{5.44}$$

$$Z_4 = \theta_{12} e^{\eta_{12}} + \theta_{10} e^{\eta_{10}} + \theta_{21} e^{\eta_{21}} \tag{5.45}$$

where the random effects vector

$$\boldsymbol{\eta} = (\eta_V, \eta_{10}, \eta_{12}, \eta_{21}) \tag{5.46}$$

is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{5.47}$$

with a diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_V & 0 & 0 & 0 \\ 0 & \omega_{10} & 0 & 0 \\ 0 & 0 & \omega_{12} & 0 \\ 0 & 0 & 0 & \omega_{21} \end{pmatrix} \tag{5.48}$$

where the elements in $\boldsymbol{\Omega}$ are unknown variance parameters. Even though the most simple covariance matrix $\boldsymbol{\Omega}$ has been used for this particular example, structural identifiability of mixed-effects models with off-diagonal elements in the covariance matrix can also be studied which will be shown in later examples.

Since the function of random variables of the mixed-effects model are known, it can be studied whether their distribution determine the underlying parameters uniquely or otherwise by equating the statistical moments, e.g.,

$$\mathbb{E}[Z_i^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_i^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.49}$$

where $i = 1, 2, 3, 4$ and $m = 1, 2$. Considering Z_1, the following equations are obtained for $m = 1$ and $m = 2$

$$\frac{e^{\frac{\omega_V}{2}}}{\theta_V} = \frac{e^{\frac{\bar{\omega}_V}{2}}}{\bar{\theta}_V} \tag{5.50}$$

$$\frac{e^{\omega_V}\left(e^{\omega_V} - 1\right)}{\theta_V^2} = \frac{e^{\bar{\omega}_V}\left(e^{\bar{\omega}_V} - 1\right)}{\bar{\theta}_V^2} \tag{5.51}$$

for which only one solution exists, namely

$$\theta_V = \bar{\theta}_V \tag{5.52}$$

$$\omega_V = \bar{\omega}_V. \tag{5.53}$$

Considering Z_2, and using the previous results (5.52)–(5.53), the following equations are obtained

$$\frac{\theta_{21} e^{\frac{\omega_{21}}{2}+\frac{\omega_V}{2}}}{\theta_V} = \frac{\bar{\theta}_{21} e^{\frac{\bar{\omega}_{21}}{2}+\frac{\omega_V}{2}}}{\theta_V} \tag{5.54}$$

$$\frac{\theta_{21}^2 e^{\omega_{21}+\omega_V}\left(e^{\omega_{21}+\omega_V}-1\right)}{\theta_V^2} = \frac{\bar{\theta}_{21}^2 e^{\bar{\omega}_{21}+\omega_V}\left(e^{\bar{\omega}_{21}+\omega_V}-1\right)}{\theta_V^2} \tag{5.55}$$

for which there is only one solution, namely

$$\theta_{21} = \bar{\theta}_{21} \tag{5.56}$$

$$\omega_{21} = \bar{\omega}_{21}. \tag{5.57}$$

Considering Z_3, and using the previous results (5.56)–(5.57), the following equations are obtained

$$\theta_{10}\theta_{21} e^{\frac{\omega_{10}}{2}+\frac{\omega_{21}}{2}} = \bar{\theta}_{10}\theta_{21} e^{\frac{\bar{\omega}_{10}}{2}+\frac{\omega_{21}}{2}} \tag{5.58}$$

$$\theta_{10}^2\theta_{21}^2 e^{\omega_{10}+\omega_{21}}\left(e^{\omega_{10}+\omega_{21}}-1\right) = \bar{\theta}_{10}^2\theta_{21}^2 e^{\bar{\omega}_{10}+\omega_{21}}\left(e^{\bar{\omega}_{10}+\omega_{21}}-1\right) \tag{5.59}$$

for which there is only one solution, namely

$$\theta_{10} = \bar{\theta}_{10} \tag{5.60}$$

$$\omega_{10} = \bar{\omega}_{10}. \tag{5.61}$$

Lastly, considering Z_4, and using the previous results (5.56)–(5.57) and (5.60)–

(5.61), the following equations are obtained

$$\theta_{12}e^{\frac{\omega_{12}}{2}} + \theta_{10}e^{\frac{\omega_{10}}{2}} + \theta_{21}e^{\frac{\omega_{21}}{2}} = \bar{\theta}_{12}e^{\frac{\bar{\omega}_{12}}{2}} + \theta_{10}e^{\frac{\omega_{10}}{2}} + \theta_{21}e^{\frac{\omega_{21}}{2}} \tag{5.62}$$

$$\theta_{12}^2 e^{\omega_{12}}\left(e^{\omega_{12}} - 1\right) + \theta_{10}^2 e^{\omega_{10}}\left(e^{\omega_{10}} - 1\right) + \theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right) = \tag{5.63}$$

$$\bar{\theta}_{12}^2 e^{\bar{\omega}_{12}}\left(e^{\bar{\omega}_{12}} - 1\right) + \theta_{10}^2 e^{\omega_{10}}\left(e^{\omega_{10}} - 1\right) + \theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right) \tag{5.64}$$

for which there is only one solution, namely

$$\theta_{12} = \bar{\theta}_{12} \tag{5.65}$$

$$\omega_{12} = \bar{\omega}_{12}. \tag{5.66}$$

It has therefore been shown that

$$\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\} \tag{5.67}$$

which means that the corresponding mixed-effects model is also structurally globally identifiable.

5.2.4 Taylor series expansion approach

Linear one-compartment model

To exemplify the Taylor series expansion approach to study the structural identifiability of mixed-effects models consider the following linear one-compartment model

$$\begin{aligned} \dot{x}_1 &= -\theta_{10}x_1 \\ x_1(0) &= D \end{aligned} \tag{5.68}$$

with observation

$$y = \theta_c x_1 \tag{5.69}$$

and unknown parameter vector

$$\boldsymbol{\theta} = (\theta_{10}, \theta_c) \tag{5.70}$$

and known initial condition, i.e., D. The first and second coefficients in the Taylor series expansion around $t = 0$ are given by

$$y(0) = \theta_c D \tag{5.71}$$

$$\dot{y}(0) = -\theta_{10}\theta_c D. \tag{5.72}$$

Introducing lognormally distributed random effects on the structural parameters θ_c and θ_{10} to ensure positivity, the following two functions of random variables are derived

$$Z_1 = \theta_c e^{\eta_c} D \tag{5.73}$$

$$Z_2 = \theta_{10} e^{\eta_{10}} \theta_c e^{\eta_c} D \tag{5.74}$$

The random effects vector

$$\boldsymbol{\eta} = (\eta_c, \eta_{10}) \tag{5.75}$$

is assumed to be normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{5.76}$$

with a full covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_{10} & \omega_{10c} \\ \omega_{10c} & \omega_c \end{pmatrix}. \tag{5.77}$$

The vector of all unknown parameters in the mixed-effects model is therefore

$$\boldsymbol{\gamma} = (\theta_{10}, \theta_c, \omega_{10}, \omega_c, \omega_{10c}). \tag{5.78}$$

By introducing two alternative parameter vectors $\bar{\boldsymbol{\theta}}$ and $\bar{\boldsymbol{\eta}}$ and equating

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.79}$$

for $m = 1, 2$ the following moment equations are obtained

$$D\theta_c e^{\frac{\omega_c}{2}} = D\bar{\theta}_c e^{\frac{\bar{\omega}_c}{2}} \tag{5.80}$$

$$D^2\theta_c^2 e^{\omega_c}\left(e^{\omega_c} - 1\right) = D^2\bar{\theta}_c^2 e^{\bar{\omega}_c}\left(e^{\bar{\omega}_c} - 1\right) \tag{5.81}$$

for which there is only one solution, namely

$$\theta_c = \bar{\theta}_c \tag{5.82}$$

$$\omega_c = \bar{\omega}_c. \tag{5.83}$$

Let

$$\boldsymbol{Z} = \begin{pmatrix} Z_1 \\ Z_2 \end{pmatrix} \tag{5.84}$$

and consider the covariance matrix of $\boldsymbol{Z}$ which is given by

$$\begin{aligned} &\mathrm{Cov}(\boldsymbol{Z}) = \mathbb{E}[\boldsymbol{Z}\boldsymbol{Z}^T] - \mathbb{E}[\boldsymbol{Z}]\mathbb{E}[\boldsymbol{Z}]^T = \\ &= \begin{pmatrix} \mathbb{E}[Z_1^2] - \mathbb{E}[Z_1]^2 & \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] \\ \mathbb{E}[Z_1 Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] & \mathbb{E}[Z_2^2] - \mathbb{E}[Z_2]^2 \end{pmatrix}. \end{aligned}$$

By equating

$$\mathbb{E}[Z_2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2(\boldsymbol{Z}(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}}))] \tag{5.85}$$

and

$$\mathrm{Cov}(\boldsymbol{Z}(\boldsymbol{\theta},\boldsymbol{\eta})) = \mathrm{Cov}(\boldsymbol{Z}(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})) \tag{5.86}$$

the following equations can be obtained,

$$\theta_{10}\theta_c e^{\frac{1}{2}(\omega_c+2\omega_{10c}+\omega_{10})} = \bar{\theta}_{10}\theta_c e^{\frac{1}{2}(\omega_c+2\bar{\omega}_{10c}+\bar{\omega}_{10})} \tag{5.87}$$

$$\frac{\theta_{10}^2\sqrt{\omega_{10}}\theta_c^2 e^{\omega_c+2\omega_{10c}+\omega_{10}}\left(e^{\omega_c+2\omega_{10c}+\omega_{10}}-1\right)\left(\omega_{10c}^2\sqrt{\omega_c-\frac{\omega_{10c}^2}{\omega_{10}}}-\omega_c\sqrt{\omega_{10}\left(\omega_{10}\omega_c-\omega_{10c}^2\right)}\right)}{\left(\omega_{10}\omega_c-\omega_{10c}^2\right)^{3/2}} =$$

$$\frac{\bar{\theta}_{10}^2\sqrt{\bar{\omega}_{10}}\theta_c^2 e^{\omega_c+2\bar{\omega}_{10c}+\bar{\omega}_{10}}\left(e^{\omega_c+2\bar{\omega}_{10c}+\bar{\omega}_{10}}-1\right)\left(\bar{\omega}_{10c}^2\sqrt{\omega_c-\frac{\bar{\omega}_{10c}^2}{\bar{\omega}_{10}}}-\omega_c\sqrt{\bar{\omega}_{10}\left(\bar{\omega}_{10}\omega_c-\bar{\omega}_{10c}^2\right)}\right)}{\left(\bar{\omega}_{10}\omega_c-\bar{\omega}_{10c}^2\right)^{3/2}} \tag{5.88}$$

$$\theta_{10}\theta_c^2 e^{\omega_c+\omega_{10c}+\frac{\omega_{10}}{2}}\left(e^{\omega_c+\omega_{10c}}-1\right) = \bar{\theta}_{10}\theta_c^2 e^{\omega_c+\bar{\omega}_{10c}+\frac{\bar{\omega}_{10}}{2}}\left(e^{\omega_c+\bar{\omega}_{10c}}-1\right). \tag{5.89}$$

By using a software package with support for symbolic computation such as Mathematica (Wolfram Research Inc. [2016]) or Maple (MapleSoft [2015]), it can be shown that the equations above have a unique solution, namely

$$\theta_{10} = \bar{\theta}_{10} \tag{5.90}$$

$$\omega_{10} = \bar{\omega}_{10} \tag{5.91}$$

$$\omega_{10c} = \bar{\omega}_{10c}. \tag{5.92}$$

All of the parameters in the model have therefore been shown to be uniquely identifiable, i.e.,

$$\{\boldsymbol{\theta},\boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\Omega}}\}, \tag{5.93}$$

and the mixed-effects model (5.68) is therefore structurally globally identifiable.

5.2.5 Input-output form approach

Linear one-compartment model

In this example a one-compartment model with linear elimination and unknown scaling parameters for both the input u and output y is considered. The model structure is given by

$$\begin{aligned} \dot{x}_1 &= -\theta_{10} x_1 + \theta_F u \\ x_1(0) &= 0 \end{aligned} \tag{5.94}$$

with observation

$$y = \theta_c x_1 \tag{5.95}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (\theta_c, \theta_{10}, \theta_F). \tag{5.96}$$

By calculating the time derivative of the output signal y and substituting it in place for the model state x_1, the model (5.94) can be rewritten in the following input-output form

$$\dot{y} + \theta_{10} y - \theta_c \theta_F u = 0. \tag{5.97}$$

The mixed-effects version of model (5.94) considered in this example has the following random effects vector

$$\boldsymbol{\eta} = (\eta_{10}, \eta_c, \eta_F) \tag{5.98}$$

which is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{5.99}$$

with a diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_{10} & 0 & 0 \\ 0 & \omega_c & 0 \\ 0 & 0 & \omega_F \end{pmatrix}. \tag{5.100}$$

The exhaustive summary $\boldsymbol{\sigma}$ of the model (5.94) consists of the coefficients in the input-output form (5.97), i.e.,

$$\boldsymbol{\sigma} = (\theta_{10}, \theta_c \theta_F) \tag{5.101}$$

from which the functions of random variables for the mixed-effects model when the random effects enter lognormally can be derived, namely

$$Z_1(\boldsymbol{\theta}, \boldsymbol{\eta}) = \theta_{10} e^{\eta_{10}} \tag{5.102}$$

$$Z_2(\boldsymbol{\theta}, \boldsymbol{\eta}) = \theta_c e^{\eta_c} \theta_F e^{\eta_F}. \tag{5.103}$$

Calculating and equating the first and second statistical moments of Z_1 as

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.104}$$

for $m = 1, 2$ yields

$$\theta_{10} e^{\frac{\omega_{10}}{2}} = \bar{\theta}_{10} e^{\frac{\bar{\omega}_{10}}{2}} \tag{5.105}$$

$$\theta_{10}^2 e^{\omega_{10}} \left(e^{\omega_{10}} - 1\right) = \bar{\theta}_{10}^2 e^{\bar{\omega}_{10}} \left(e^{\bar{\omega}_{10}} - 1\right) \tag{5.106}$$

which have only one solution, namely

$$\theta_{10} = \bar{\theta}_{10} \tag{5.107}$$

$$\omega_{10} = \bar{\omega}_{10}. \tag{5.108}$$

Calculating and equating the first and second statistical moments of Z_2 as

$$\mathbb{E}[Z_2^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.109}$$

for $m = 1, 2$ yields

$$\theta_c \theta_F e^{\frac{1}{2}(\omega_c + \omega_F)} = \bar{\theta}_c \bar{\theta}_F e^{\frac{1}{2}(\bar{\omega}_c + \bar{\omega}_F)} \tag{5.110}$$

$$\theta_c^2 \theta_F^2 e^{\omega_c + \omega_F} \left(e^{\omega_c + \omega_F} - 1\right) = \bar{\theta}_c^2 \bar{\theta}_F^2 e^{\bar{\omega}_c + \bar{\omega}_F} \left(e^{\bar{\omega}_c + \bar{\omega}_F} - 1\right). \tag{5.111}$$

With the two substitutions

$$\beta_\theta = \theta_c \theta_F \tag{5.112}$$

$$\beta_\omega = \omega_c + \omega_F \tag{5.113}$$

the equation system for the statistical moments of Z_2 can be rewritten as

$$\beta_\theta e^{\frac{1}{2}\beta_\omega} = \bar{\beta}_\theta e^{\frac{1}{2}\bar{\beta}_\omega} \tag{5.114}$$

$$\beta_\theta^2 e^{\beta_\omega} \left(e^{\beta_\omega} - 1\right) = \bar{\beta}_\theta^2 e^{\bar{\beta}_\omega} \left(e^{\bar{\beta}_\omega} - 1\right) \tag{5.115}$$

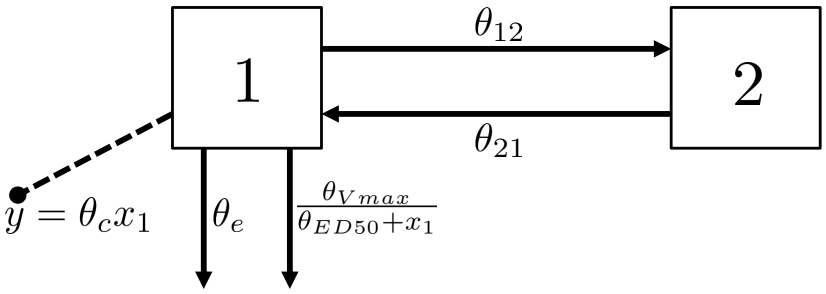
which has only one solution, namely

$$\beta_\theta = \bar{\beta}_\theta \tag{5.116}$$

$$\beta_\omega = \bar{\beta}_\omega. \tag{5.117}$$

The distribution of Z_2 is lognormal and therefore the first two statistical moments fully characterise Z_2. Because of this, it can be concluded that only the product $\theta_c \theta_F$ and the sum $\omega_c + \omega_F$ are globally identifiable, but not the individual contribution from those parameters. The mixed-effects model (5.94) is therefore structurally unidentifiable.

Figure 5.3: Nonlinear two-compartment model with a linear and a nonlinear elimination from compartment 1.



Nonlinear two-compartment model

To demonstrate that structural identifiability of more complex mixed-effects models than those analysed so far can be studied with the techniques developed within this thesis, a nonlinear two-compartment model will now be analysed, Figure 5.3. There are two routes of elimination from this model. These eliminations are both from compartment 1 and comprise a nonlinear and parallel linear elimination. The model has the following structure

$$\begin{aligned} \dot{x}_1 &= -(\frac{\theta_{Vmax}}{\theta_{ED50} + x_1} + \theta_e + \theta_{12})x_1 + \theta_{21}x_2 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ x_1(0) &= D \\ x_2(0) &= 0, \end{aligned} \tag{5.118}$$

with observation

$$y = \theta_c x_1 \tag{5.119}$$

and unknown parameter vector

$$\boldsymbol{\theta} = (\theta_{12}, \theta_{21}, \theta_e, \theta_c, \theta_{Vmax}, \theta_{ED50}) \tag{5.120}$$

and known initial conditions, i.e., D and zero. The model rewritten in an input-output form using Maple (MapleSoft [2015]) is given by

$$\begin{aligned}
&\ddot{y}y^2 + \theta_e\theta_{21}y^3 + 2\,y^2\theta_{21}\theta_c\theta_e\theta_{ED50} + y^2\theta_{21}\theta_c\theta_{Vmax} + y^2\dot{y}\theta_e + y^2\dot{y}\theta_{21} + y^2\dot{y}\theta_{12} + \\
&y{\theta_c}^2\theta_{21}{\theta_{ED50}}^2\theta_e + 2\,\ddot{y}\theta_c\theta_{ED50}y + 2\,y\theta_{12}\dot{y}\theta_{ED50}\theta_c + y{\theta_c}^2\theta_{21}\theta_{ED50}\theta_{Vmax} + \\
&2\,y\theta_{21}\dot{y}\theta_{ED50}\theta_c + 2\,y\theta_e\dot{y}\theta_{ED50}\theta_c + \ddot{y}{\theta_{ED50}}^2{\theta_c}^2 + {\theta_c}^2\theta_e{\theta_{ED50}}^2\dot{y} + \\
&{\theta_c}^2\theta_{12}{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{21}{\theta_{ED50}}^2\dot{y} + {\theta_c}^2\theta_{Vmax}\theta_{ED50}\dot{y} = 0
\end{aligned} \tag{5.121}$$

and the initial condition for the output function is

$$y(0) = \theta_c D \tag{5.122}$$

$$\dot{y}(0) = -\theta_c(\frac{\theta_{Vmax}}{\theta_{ED50} + D} + \theta_e + \theta_{12})D. \tag{5.123}$$

The coefficients in the input-output relation (5.121) and the initial conditions (5.122) are given by

$$\sigma_1(\boldsymbol{\theta}) = \theta_{ED50}^2\theta_c^2 \tag{5.124}$$

$$\sigma_2(\boldsymbol{\theta}) = \theta_{21}\theta_e \tag{5.125}$$

$$\sigma_3(\boldsymbol{\theta}) = 2\theta_{ED50}\theta_c \tag{5.126}$$

$$\sigma_4(\boldsymbol{\theta}) = \theta_c\theta_{21}\theta_{Vmax} + 2\theta_c\theta_{21}\theta_e\theta_{ED50} \tag{5.127}$$

$$\sigma_5(\boldsymbol{\theta}) = \theta_c^2\theta_{21}\theta_e\theta_{ED50}^2 + \theta_c^2\theta_{21}\theta_{Vmax}\theta_{ED50} \tag{5.128}$$

$$\sigma_6(\boldsymbol{\theta}) = \theta_{12} + \theta_e + \theta_{21} \tag{5.129}$$

$$\sigma_7(\boldsymbol{\theta}) = 2\theta_c\theta_{ED50}\theta_{21} + 2\theta_c\theta_{ED50}\theta_e + 2\theta_c\theta_{ED50}\theta_{12} \tag{5.130}$$

$$\sigma_8(\boldsymbol{\theta}) = \theta_c^2\theta_{ED50}^2\theta_{12} + \theta_c^2\theta_{ED50}^2\theta_e + \theta_c^2\theta_{ED50}\theta_{Vmax} + \theta_c^2\theta_{ED50}^2\theta_{21} \tag{5.131}$$

$$\sigma_9(\boldsymbol{\theta}) = \theta_c D \tag{5.132}$$

$$\sigma_{10}(\boldsymbol{\theta}) = -\theta_c(\frac{\theta_{Vmax}}{\theta_{ED50} + D} + \theta_e + \theta_{12})D. \tag{5.133}$$

Introducing the following random effects vector

$$\boldsymbol{\eta} = (\eta_{12}, \eta_{21}, \eta_e, \eta_c, \eta_{Vmax}, \eta_{ED50}) \tag{5.134}$$

which is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{5.135}$$

with a diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_{12} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \omega_{21} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \omega_e & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \omega_c & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \omega_{Vmax} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \omega_{ED50} \end{pmatrix} \tag{5.136}$$

in a lognormal fashion to all structural parameters $\boldsymbol{\theta}$ to ensure positivity yields the following functions of random variables

$$Z_1 = (\theta_{ED50} e^{\eta_{ED50}})^2 (\theta_c e^{\eta_c})^2 \tag{5.137}$$

$$Z_2 = \theta_{21} e^{\eta_{21}} \theta_{ke} e^{\eta_e} \tag{5.138}$$

$$Z_3 = 2\theta_{ED50} e^{\eta_{ED50}} \theta_c e^{\eta_c} \tag{5.139}$$

$$Z_4 = \theta_c e^{\eta_c} \theta_{21} e^{\eta_{21}} \theta_{Vmax} e^{\eta_{Vmax}} + 2\theta_c e^{\eta_c} \theta_{21} e^{\eta_{21}} \theta_e e^{\eta_e} \theta_{ED50} e^{\eta_{ED50}} \tag{5.140}$$

$$Z_5 = (\theta_c e^{\eta_c})^2 \theta_{21} e^{\eta_{21}} \theta_e e^{\eta_e} (\theta_{ED50} e^{\eta_{ED50}})^2 + (\theta_c e^{\eta_c})^2 \theta_{21} e^{\eta_{21}} \theta_{Vmax} \theta_{ED50} e^{\eta_{ED50}} \tag{5.141}$$

$$Z_6 = \theta_{12} e^{\eta_{12}} + \theta_e e^{\eta_e} + \theta_{21} e^{\eta_{21}} \tag{5.142}$$

$$Z_7 = 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_{21} e^{\eta_{21}} + 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_e e^{\eta_e} + 2\theta_c e^{\eta_c} \theta_{ED50} e^{\eta_{ED50}} \theta_{12} e^{\eta_{12}} \tag{5.143}$$

$$Z_8 = (\theta_c e^{\eta_c})^2 \theta_{ED50}^2 \theta_{12} e^{\eta_{12}} + (\theta_c e^{\eta_c})^2 (\theta_{ED50} e^{\eta_{ED50}})^2 \theta_e e^{\eta_e} + (\theta_c e^{\eta_c})^2 \theta_{ED50} e^{\eta_{ED50}} \theta_{Vmax} e^{\eta_{Vmax}} + (\theta_c e^{\eta_c})^2 (\theta_{ED50} e^{\eta_{ED50}})^2 \theta_{21} e^{\eta_{21}} \tag{5.144}$$

$$Z_9 = \theta_c e^{\eta_c} D \tag{5.145}$$

$$Z_{10} = -\theta_c e^{\eta_c} \left(\frac{\theta_{Vmax} e^{\eta_{Vmax}}}{\theta_{ED50} e^{\eta_{ED50}} + D} + \theta_e e^{\eta_e} + \theta_{12} e^{\eta_{12}} \right) D. \tag{5.146}$$

The first and second moments of Z_9 are given by

$$\mathbb{E}[Z_9(\boldsymbol{\theta}, \boldsymbol{\eta})] = D\theta_c e^{\frac{\omega_c}{2}} \tag{5.147}$$

$$\mathbb{E}[Z_9^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = D^2 \theta_c^2 e^{\omega_c} \left(e^{\omega_c} - 1 \right). \tag{5.148}$$

The equation system derived from the first and second moment in equation (5.147)–(5.148) is

$$D\theta_c e^{\frac{\omega_c}{2}} = D\bar{\theta}_c e^{\frac{\bar{\omega}_c}{2}} \tag{5.149}$$

$$D^2 \theta_c^2 e^{\omega_c} \left(e^{\omega_c} - 1 \right) = D^2 \bar{\theta}_c^2 e^{\bar{\omega}_c} \left(e^{\bar{\omega}_c} - 1 \right) \tag{5.150}$$

which have only one solution, namely

$$\theta_c = \bar{\theta}_c \tag{5.151}$$

$$\omega_c = \bar{\omega}_c. \tag{5.152}$$

The first and second moments of Z_3 are given by

$$\mathbb{E}[Z_3(\boldsymbol{\theta}, \boldsymbol{\eta})] = 2\theta_c \theta_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \omega_{\text{ED50}})} \tag{5.153}$$

$$\mathbb{E}[Z_3^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = 4\theta_c^2 \theta_{\text{ED50}}^2 e^{\omega_c + \omega_{\text{ED50}}} \left(e^{\omega_c + \omega_{\text{ED50}}} - 1\right). \tag{5.154}$$

Using (5.151)–(5.152) and solving the first and second moments of Z_3 yields

$$2\theta_c \theta_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \omega_{\text{ED50}})} = 2\theta_c \bar{\theta}_{\text{ED50}} e^{\frac{1}{2}(\omega_c + \bar{\omega}_{\text{ED50}})} \tag{5.155}$$

$$4\theta_c^2 \theta_{\text{ED50}}^2 e^{\omega_c + \omega_{\text{ED50}}} \left(e^{\omega_c + \omega_{\text{ED50}}} - 1\right) = 4\theta_c^2 \bar{\theta}_{\text{ED50}}^2 e^{\omega_c + \bar{\omega}_{\text{ED50}}} \left(e^{\omega_c + \bar{\omega}_{\text{ED50}}} - 1\right) \tag{5.156}$$

which gives only one solution, namely

$$\theta_{ED50} = \bar{\theta}_{ED50} \tag{5.157}$$

$$\omega_{ED50} = \bar{\omega}_{ED50}. \tag{5.158}$$

The first and second moments of Z_8 are given by

$$\begin{aligned}\mathbb{E}[Z_8(\boldsymbol{\theta},\boldsymbol{\eta})] &= \theta_c^2\theta_{\mathrm{ED50}}e^{2\omega_c+\frac{\omega_{\mathrm{ED50}}}{2}}(\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}(\theta_{12}e^{\frac{\omega_{12}}{2}}+\\ &\quad \theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}})+\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}) \qquad (5.159)\\ \mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})] &= \theta_c^4\theta_{\mathrm{ED50}}^2e^{4\omega_c+\omega_{\mathrm{ED50}}}(\theta_{\mathrm{ED50}}^2e^{3\omega_{\mathrm{ED50}}}(\theta_{12}^2e^{\omega_{12}}(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{12}}-1)\\ &\quad +2\theta_{12}e^{\frac{\omega_{12}}{2}}\left(e^{4(\omega_c+\omega_{\mathrm{ED50}})}-1\right)\left(\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right)+\\ &\quad \theta_{21}^2e^{\omega_{21}}\left(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{21}}-1\right)+\\ &\quad 2\theta_{21}\theta_{\mathrm{e}}\left(e^{4(\omega_c+\omega_{\mathrm{ED50}})}-1\right)e^{\frac{1}{2}(\omega_{21}+\omega_{\mathrm{e}})}+\\ &\quad \theta_{\mathrm{e}}^2e^{\omega_{\mathrm{e}}}\left(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}}-1\right))+\\ &\quad 2\theta_{\mathrm{ED50}}\theta_{\mathrm{Vmax}}\left(e^{4\omega_c+2\omega_{\mathrm{ED50}}}-1\right)e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{Vmax}})}\\ &\quad \left(\theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right)+\\ &\quad \theta_{\mathrm{Vmax}}^2e^{\omega_{\mathrm{Vmax}}}\left(e^{4\omega_c+\omega_{\mathrm{ED50}}+\omega_{\mathrm{Vmax}}}-1\right)). \qquad (5.160)\end{aligned}$$

The first and second moments of Z_6 are given by

$$\mathbb{E}[Z_6(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}} \qquad (5.161)$$

$$\mathbb{E}[Z_6^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{12}^2e^{\omega_{12}}\left(e^{\omega_{12}}-1\right)+\theta_{21}^2e^{\omega_{21}}\left(e^{\omega_{21}}-1\right)+\theta_{\mathrm{e}}^2e^{\omega_{\mathrm{e}}}\left(e^{\omega_{\mathrm{e}}}-1\right). \qquad (5.162)$$

Using the previous results (5.151)–(5.152) and (5.157)–(5.158) the following equation can be generated from (5.159)

$$\begin{aligned}&\theta_c^2\theta_{\mathrm{ED50}}e^{2\omega_c+\frac{\omega_{\mathrm{ED50}}}{2}}(\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}(\theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}})+\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}})=\\ &\bar{\theta}_c^2\bar{\theta}_{\mathrm{ED50}}e^{2\bar{\omega}_c+\frac{\bar{\omega}_{\mathrm{ED50}}}{2}}(\bar{\theta}_{\mathrm{ED50}}e^{\frac{3\bar{\omega}_{\mathrm{ED50}}}{2}}(\bar{\theta}_{12}e^{\frac{\bar{\omega}_{12}}{2}}+\bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}}+\bar{\theta}_{\mathrm{e}}e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}})+\bar{\theta}_{\mathrm{Vmax}}e^{\frac{\bar{\omega}_{\mathrm{Vmax}}}{2}})\end{aligned} \qquad (5.163)$$

which can be simplified to give

$$\left(\theta_{12}e^{\frac{\omega_{12}}{2}}+\theta_{21}e^{\frac{\omega_{21}}{2}}+\theta_{\mathrm{e}}e^{\frac{\omega_{\mathrm{e}}}{2}}\right)+\frac{\theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}}{\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}}=$$
$$\left(\bar{\theta}_{12}e^{\frac{\bar{\omega}_{12}}{2}}+\bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}}+\bar{\theta}_{\mathrm{e}}e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}}\right)+\frac{\bar{\theta}_{\mathrm{Vmax}}e^{\frac{\bar{\omega}_{\mathrm{Vmax}}}{2}}}{\theta_{\mathrm{ED50}}e^{\frac{3\omega_{\mathrm{ED50}}}{2}}}. \tag{5.164}$$

From the equation generated from (5.161) the relation

$$\mathbb{E}[Z_6(\boldsymbol{\theta},\boldsymbol{\eta})]=\mathbb{E}[Z_6(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.165}$$

can be used to simplify (5.164) further to give:

$$\theta_{Vmax}e^{\frac{\omega_{Vmax}}{2}}=\bar{\theta}_{Vmax}e^{\frac{\bar{\omega}_{Vmax}}{2}}. \tag{5.166}$$

Using (5.151)–(5.152), (5.157)–(5.158) and (5.161), the equation system arising from

$$\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})]=\mathbb{E}[Z_8^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.167}$$

has been omitted since the expression is very large, but which yields

$$\theta_{Vmax}e^{\frac{\omega_{Vmax}}{2}}=\bar{\theta}_{Vmax}e^{\frac{\bar{\omega}_{Vmax}}{2}} \tag{5.168}$$
$$\mathbb{E}[Z_8^2(\boldsymbol{\theta},\boldsymbol{\eta})]=\mathbb{E}[Z_8^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.169}$$

and has only one solution

$$\theta_{Vmax}=\bar{\theta}_{Vmax} \tag{5.170}$$
$$\omega_{Vmax}=\bar{\omega}_{Vmax}. \tag{5.171}$$

The first and second moments of Z_5 are given by

$$\mathbb{E}[Z_5(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_c^2\theta_{\mathrm{ED50}}\theta_{21}e^{\frac{1}{2}(4\omega_c+\omega_{\mathrm{ED50}}+\omega_{21})}(\theta_{\mathrm{ED50}}\theta_{\mathrm{e}}e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}})} + \theta_{\mathrm{Vmax}}e^{\frac{\omega_{\mathrm{Vmax}}}{2}}) \tag{5.172}$$

$$\begin{aligned}\mathbb{E}[Z_5^2(\boldsymbol{\theta},\boldsymbol{\eta})] =& \theta_c^4\theta_{\mathrm{ED50}}^2\theta_{21}^2e^{4\omega_c+\omega_{\mathrm{ED50}}+\omega_{21}} \qquad (5.173)\\ & (\theta_{\mathrm{ED50}}^2\theta_{\mathrm{e}}^2e^{3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}}\left(e^{4\omega_c+4\omega_{\mathrm{ED50}}+\omega_{21}+\omega_{\mathrm{e}}}-1\right)+\\ & 2\theta_{\mathrm{ED50}}\theta_{\mathrm{e}}\theta_{\mathrm{Vmax}}\left(e^{4\omega_c+2\omega_{\mathrm{ED50}}+\omega_{21}}-1\right)\\ & e^{\frac{1}{2}(3\omega_{\mathrm{ED50}}+\omega_{\mathrm{e}}+\omega_{\mathrm{Vmax}})}+\\ & \theta_{\mathrm{Vmax}}^2e^{\omega_{\mathrm{Vmax}}}\left(e^{4\omega_c+\omega_{\mathrm{ED50}}+\omega_{21}+\omega_{\mathrm{Vmax}}}-1\right)). \qquad (5.174)\end{aligned}$$

The first and second moments of Z_2 are given by

$$\mathbb{E}[Z_2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{21}\theta_{\mathrm{e}}e^{\frac{1}{2}(\omega_{21}+\omega_{\mathrm{e}})} \tag{5.175}$$

$$\mathbb{E}[Z_2^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \theta_{21}^2\theta_{\mathrm{e}}^2e^{\omega_{21}+\omega_{\mathrm{e}}}\left(e^{\omega_{21}+\omega_{\mathrm{e}}}-1\right). \tag{5.176}$$

Using (5.151)–(5.152), (5.157)–(5.158), (5.170)–(5.171) and (5.175) the equation system

$$\mathbb{E}[Z_5(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_5(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.177}$$

$$\mathbb{E}[Z_5^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_5^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.178}$$

has the unique solution

$$\theta_{21} = \bar{\theta}_{21} \tag{5.179}$$

$$\omega_{21} = \bar{\omega}_{21}. \tag{5.180}$$

Using (5.179)–(5.180) the following equation system

$$\mathbb{E}[Z_2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.181}$$

$$\mathbb{E}[Z_2^2(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2^2(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{5.182}$$

has the unique solution

$$\theta_e = \bar{\theta}_e \tag{5.183}$$

$$\omega_e = \bar{\omega}_e. \tag{5.184}$$

Finally, we make use of (5.179)–(5.180) and (5.183)–(5.184) and solve

$$\mathbb{E}[Z_6(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.185}$$

$$\mathbb{E}[Z_6^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_6^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{5.186}$$

which has the unique solution

$$\theta_{12} = \bar{\theta}_{12} \tag{5.187}$$

$$\omega_{12} = \bar{\omega}_{12}. \tag{5.188}$$

From (5.151)–(5.152),(5.157)–(5.158), (5.170)–(5.171), (5.179)–(5.180), (5.183)–(5.184) and (5.187)–(5.188) it can be concluded that

$$\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\} \tag{5.189}$$

which means that the mixed-effects model (5.118) is structurally globally identifiable.

5.3 Summary

The methods for structural identifiability analysis applicable to mixed-effects models developed within this thesis have all been applied to mixed-effects models to show how they work in practice. It has been shown that all developed methods can be used to conclude whether a mixed-effects model is structurally identifiable or otherwise.

It has been shown that the three approaches related to the exhaustive summary; the Laplace transform approach, the Taylor series expansion approach and the Input-Output approach, can be applied to mixed-effects models with any form

of distribution of the random effects $\boldsymbol{\eta}$ and any structure of the covariance matrix $\boldsymbol{\Omega}$.

In the repeated measurement approach the idea is to use already existing statistical theorems that are based on certain expressions of parameter to appear in order to determine whether or not the model is identifiable. Although not shown here, the elements in the vector containing the exhaustive summary can be used to find such parameter expressions as well.

It is not possible to say which approach that should be considered the best in general. In terms of ease of application the repeated measurement approach comes out on top. This is because no computations are needed at all in this approach since previously established statistical theorems are applied instead. However, the method does require the model equations to be in such a form as stated in the theorems which limits the applicability of the approach. The augmented system approach is more generally applicable compared to the repeated measurement approach since it does not rely on the model equations being in a particular form. The downside with the augmented system approach is that it is necessary to find the system solution which is not always possible. The Laplace transform approach is limited to structural identifiability analysis on linear models. The Taylor series expansion approach and the input-output approach are both applicable to nonlinear systems and are thus in this sense the most applicable methods presented in this thesis to study structural identifiability of mixed-effects models. Analytical structural identifiability analysis techniques for mixed-effects models prior to this thesis have not appeared in the literature, the analysed models in this chapter are the first mixed-effects models ever to be analysed in this way.

Chapter 6

Structural identifiability for mixed-effects models and its dependency on the statistical sub-model

6.1 Introduction

In a mixed-effects model there is a statistical sub-model. When defining the statistical sub-model for a particular model structure there are three different components that need to be specified. Firstly, it needs to be specified which structural parameters $\boldsymbol{\theta}$ should have an associated random effect $\boldsymbol{\eta}$. Secondly, the form of the distribution of the different random effects needs to be defined. Perhaps the most commonly used form of distribution for random effects when modelling pharmaceuticals is a lognormal distribution. This is both to ensure positivity and because many biological processes are in fact lognormally distributed, see Grönholm and Annila [2007]. Thirdly, the structure of the associated covariance matrix $\boldsymbol{\Omega}$ needs to be defined, i.e., which of the random effects have a relation via a covariance parameter.

Because of the additional statistical sub-model, and the multiple choices that

come with it, it is not obvious whether structural identifiability analysis results from the corresponding non-mixed-effects model are directly translatable to the mixed-effects model. In other words, it is not obvious whether a structurally unidentifiable model, or a structurally locally identifiable in the non-mixed-effects case, is always unidentifiable or locally identifiable similarily in the mixed-effects case.

In this chapter the developed structural identifiability analysis techniques presented in Chapter 4 will be used to study how structural identifiability results for non-mixed-effects models may translate to the mixed-effects case. In particular, the following three aspects will be studied

- Where the random effects enter into the structural model
- The form, i.e., the distribution, of the random effects
- The structure of the associated covariance matrix $\mathbf{\Omega}$.

The results and insights presented in this chapter have been collected together in the paper Janzén et al. [2016c].

6.2 Combining a structurally identifiable structural sub-model and a statistical submodel

Before studying more complex cases the most trivial case should first be discussed, namely when a structurally globally identifiable structural sub-model is combined with a globally identifiable statistical sub-model. Such a combination always results in a structurally globally identifiable mixed-effects model.

To realise this, consider first a structurally globally identifiable non-mixed-effects model, i.e., the structural sub-model only. In such a model, each structural parameter is by definition uniquely determined by the model output. If such a model is applied to an infinite number of subjects, where each subject is treated as a separate inference problem, a distribution for each structural parameter will be obtained. Since each structural parameter from every subject is uniquely determined it follows directly that the distribution of those parameters also is uniquely determined. If a

structurally globally identifiable statistical sub-model, i.e., a parametrised distribution, is used to describe the distribution of the structural parameters it means that the distribution determines the statistical parameters uniquely. Examples of such distributions that are commonly used in practice are normal and lognormal distributions. To exemplify this, consider a simple one-compartment non-mixed-effects model, i.e., a structural sub-model given as

$$\begin{aligned} \dot{x} &= -\theta_1 x \\ x(0) &= D \end{aligned} \tag{6.1}$$

with observation

$$y = \theta_2 x \tag{6.2}$$

and unknown parameter vector

$$\boldsymbol{\theta} = (\theta_1, \theta_2). \tag{6.3}$$

and a known dose parameter D. The first two coefficients in the Taylor series expansion of the output function y are given by

$$y(0, \boldsymbol{\theta}) = \theta_2 D \tag{6.4}$$

$$\dot{y}(0, \boldsymbol{\theta}) = -\theta_1 \theta_2 D. \tag{6.5}$$

Introducing an alternative parameter vector $\boldsymbol{\theta}$ and equating the first two coefficients as

$$y(0, \boldsymbol{\theta}) = y(0, \bar{\boldsymbol{\theta}}) \tag{6.6}$$

$$\dot{y}(0, \boldsymbol{\theta}) = \dot{y}(0, \bar{\boldsymbol{\theta}}) \tag{6.7}$$

yields only one solution, namely

$$\theta_1 = \bar{\theta}_1 \tag{6.8}$$

$$\theta_2 = \bar{\theta}_2. \tag{6.9}$$

The structural sub-model is therefore structurally globally identifiable. Now consider the addition of a globally identifiable statistical sub-model in which a normal distribution is postulated for parameter θ_1 and a lognormal distribution for parameter θ_2. The mixed-effects model has therefore the following structure

$$\begin{aligned} \dot{x} &= -(\theta_1 + \eta_1)x \\ x(0) &= D \end{aligned} \tag{6.10}$$

with observation

$$y = \theta_2 e^{\eta_2} x \tag{6.11}$$

with the unknown structural parameter vector

$$\boldsymbol{\theta} = (\theta_1, \theta_2). \tag{6.12}$$

The random effects vector $\boldsymbol{\eta}$ is given by

$$\boldsymbol{\eta} = (\eta_1, \eta_2) \tag{6.13}$$

which is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.14}$$

with a diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_1 & 0 \\ 0 & \omega_2 \end{pmatrix}. \tag{6.15}$$

The functions of random variables derived from equations (6.4)–(6.5) are given by

$$Z_1 = \theta_2 e^{\eta_2} D \tag{6.16}$$

$$Z_2 = (\theta_1 + \eta_1)\theta_2 e^{\eta_2} D \tag{6.17}$$

Equating

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.18}$$

$$\mathbb{E}[Z_2^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.19}$$

for $m = 1, 2$ yields the following equations

$$\theta_2 e^{\frac{\omega_2}{2}} = \bar{\theta}_2 e^{\frac{\bar{\omega}_2}{2}} \tag{6.20}$$

$$\theta_2^2 e^{\omega_2}(e^{\omega_2} - 1) = \bar{\theta}_2^2 e^{\bar{\omega}_2}(e^{\bar{\omega}_2} - 1) \tag{6.21}$$

$$\theta_1 \theta_2 e^{\frac{\omega_2}{2}} = \bar{\theta}_1 \bar{\theta}_2 e^{\frac{\bar{\omega}_2}{2}} \tag{6.22}$$

$$\theta_2^2 e^{\omega_2}\left((e^{\omega_2} - 1)\theta_1^2 + \omega_1 e^{\omega_2}\right) = \bar{\theta}_2^2 e^{\bar{\omega}_2}\left((e^{\bar{\omega}_2} - 1)\bar{\theta}_1^2 + \bar{\omega}_1 e^{\bar{\omega}_2}\right) \tag{6.23}$$

for which only one solutions exist, namely

$$\theta_1 = \bar{\theta}_1 \tag{6.24}$$

$$\theta_2 = \bar{\theta}_2 \tag{6.25}$$

$$\omega_1 = \bar{\omega}_1 \tag{6.26}$$

$$\omega_2 = \bar{\omega}_2. \tag{6.27}$$

The mixed-effects version of model (6.1) is therefore structurally globally identifiable, which was expected since both the structural sub-model and the statistical sub-model were identifiable. This result is summarised in Conjecture 1.

Conjecture 1. If a structurally globally identifiable non-mixed-effects model in the form (2.4) is combined with an identifiable statistical sub-model the subsequent mixed-effects model is always structurally globally identiable.

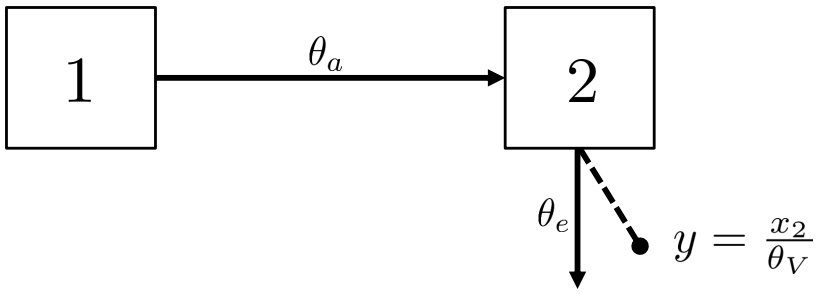


Figure 6.1: The one-compartment absorption model with linear absorption and elimination.

6.3 The effect on structural identifiability of where random effects enter into the structural model

Two different models will be used to exemplify the dependence of where the random effects enter the structural model has on whether the model is structurally identifiable or otherwise. Before considering mixed-effects models, the corresponding non-mixed-effects models will be considered. The following examples are given as case studies.

Example: One-compartment absorption model

The one-compartment absorption model, Figure 6.1, has the following structure

$$\begin{aligned} \dot{x}_1 &= -\theta_a x_1 \\ \dot{x}_2 &= \theta_a x_1 - \theta_e x_2 \\ x_1(0) &= D \\ x_2(0) &= 0 \end{aligned} \tag{6.28}$$

with observation

$$y = \frac{x_2}{\theta_V} \tag{6.29}$$

with the unknown parameter vector

$$\boldsymbol{\theta} = (\theta_e, \theta_a, \theta_V) \tag{6.30}$$

and a known dose parameter D. By taking Laplace transforms and generating the transfer function for this linear system the following system moment invariants can be derived

$$\sigma_1 = \frac{\theta_a}{\theta_V} \tag{6.31}$$

$$\sigma_2 = \theta_a + \theta_e \tag{6.32}$$

$$\sigma_3 = \theta_a \theta_e. \tag{6.33}$$

Equating

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \tag{6.34}$$

for $k = 1, 2, 3$ and solving for $\boldsymbol{\theta}$ results in the following two solutions

$$\theta_a = \bar{\theta}_a \tag{6.35}$$

$$\theta_e = \bar{\theta}_e \tag{6.36}$$

$$\theta_V = \bar{\theta}_V \tag{6.37}$$

and

$$\theta_a = \bar{\theta}_e \tag{6.38}$$

$$\theta_e = \bar{\theta}_a \tag{6.39}$$

$$\theta_V = \frac{\bar{\theta}_a \bar{\theta}_V}{\bar{\theta}_e}. \tag{6.40}$$

The non-mixed-effects one-compartment absorption model (6.28) is therefore structurally locally identifiable with two solutions.

Example: Linear three compartment model

The second example model that will be used to illustrate that it matters where the random effects enter into the structural model with respect to structural identifiability is a linear three-compartment model. The non-mixed-effects model, Figure 6.2A, is known to be locally identifiable with two solutions and has the following structure

$$\begin{aligned} \dot{x}_1 &= -(\theta_{12} + \theta_{13} + \theta_{10})x_1 + \theta_{21}x_2 + \theta_{31}x_3 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ \dot{x}_3 &= \theta_{13}x_1 - \theta_{31}x_3 \\ x_1(0) &= D \\ x_2(0) &= 0 \\ x_3(0) &= 0 \end{aligned} \tag{6.41}$$

with observation

$$y = x_1 \tag{6.42}$$

with known initial conditions and with the unknown model parameter vector

$$\boldsymbol{\theta} = (\theta_{12}, \theta_{13}, \theta_{21}, \theta_{31}, \theta_{10}). \tag{6.43}$$

By taking Laplace transforms the transfer function of the model can be computed

$$G(s) = \frac{s^2 + \beta_1 s + \beta_2}{s^3 + \beta_3 s^2 + \beta_4 s + \beta_5} \tag{6.44}$$

with the macro parameters

$$\beta_1 = \theta_{21} + \theta_{31} \tag{6.45}$$

$$\beta_2 = \theta_{31}\theta_{21} \tag{6.46}$$

$$\beta_3 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31} \tag{6.47}$$

$$\beta_4 = \theta_{21}(\theta_{10} + \theta_{13}) + \theta_{31}(\theta_{10} + \theta_{12} + \theta_{21}) \tag{6.48}$$

$$\beta_5 = \theta_{10}\theta_{21}\theta_{31} \tag{6.49}$$

from which the following system moment invariants can be derived

$$\sigma_1 = \theta_{21}\theta_{31} \tag{6.50}$$

$$\sigma_2 = \theta_{21} + \theta_{31} \tag{6.51}$$

$$\sigma_3 = \theta_{10}\theta_{21}\theta_{31} \tag{6.52}$$

$$\sigma_4 = \theta_{10}\theta_{21} + \theta_{10}\theta_{31} + \theta_{12}\theta_{31} + \theta_{13}\theta_{21} + \theta_{21}\theta_{31} \tag{6.53}$$

$$\sigma_5 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31}. \tag{6.54}$$

By introducing an alternative parameter vector $\bar{\boldsymbol{\theta}}$ and equating the moment invariants as

$$\sigma_k(\boldsymbol{\theta}) = \sigma_k(\bar{\boldsymbol{\theta}}) \tag{6.55}$$

for $k = 1, \ldots, 5$ it can be shown that the system has the following two solutions for the parameters

$$\theta_{12} = \bar{\theta}_{12} \tag{6.56}$$

$$\theta_{13} = \bar{\theta}_{13} \tag{6.57}$$

$$\theta_{21} = \bar{\theta}_{21} \tag{6.58}$$

$$\theta_{31} = \bar{\theta}_{31} \tag{6.59}$$

$$\theta_{10} = \bar{\theta}_{10} \tag{6.60}$$

and

$$\theta_{12} = \bar{\theta}_{13} \tag{6.61}$$

$$\theta_{13} = \bar{\theta}_{12} \tag{6.62}$$

$$\theta_{21} = \bar{\theta}_{31} \tag{6.63}$$

$$\theta_{31} = \bar{\theta}_{21} \tag{6.64}$$

$$\theta_{10} = \bar{\theta}_{10}. \tag{6.65}$$

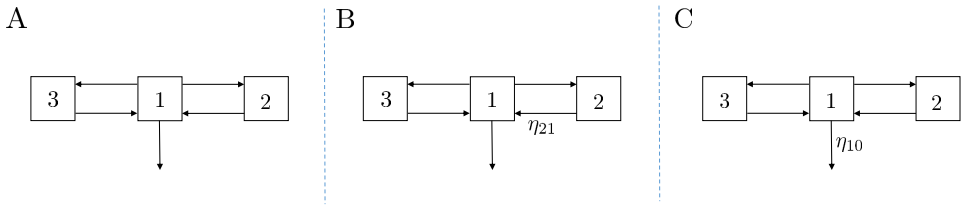


Figure 6.2: The linear three-compartment model. The non-mixed-effects model is A and the mixed-effects models are B and C. In B the random effect has entered via the flow from compartment 2 to 1. In C the random effect has entered via the elimination from compartment 1.

Notice that because of the symmetry (only a labelling difference) in the model structure, the parameters

$$\{\theta_{12}, \theta_{21}, \theta_{13}, \theta_{31}\} \tag{6.66}$$

are locally identifiable with two solutions while the parameter θ_{10} is globally identifiable.

The one-compartment absorption model and the three-compartment model provided in these examples will now be considered in a mixed-effects framework where one random effect will enter the structural model in two different ways.

6.3.1 Random effects render a structurally locally identifiable model to become a globally identifiable mixed-effects model

In this section random effects are added to the structural model of the two examples models such that the models change from being structurally locally identifiable to structurally globally identifiable via their inclusion.

One-compartment absorption model

If two random effects are introduced to the structural parameters θ_V and θ_e the following system of functions of random variables is obtained

$$Z_1 = \frac{\theta_a}{\theta_V e^{\eta_V}} \tag{6.67}$$

$$Z_2 = \theta_a + \theta_e e^{\eta_e} \tag{6.68}$$

$$Z_3 = \theta_a \theta_e e^{\eta_e} \tag{6.69}$$

with the random effects vector $\boldsymbol{\eta}$ which is normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.70}$$

with a diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_V & 0 \\ 0 & \omega_e \end{pmatrix}. \tag{6.71}$$

The first and second statistical moments of Z_1 are

$$\mathbb{E}[Z_1] = \frac{\theta_{\mathrm{a}} e^{\frac{\omega_V}{2}}}{\theta_V} \tag{6.72}$$

$$\mathbb{E}[Z_1^2] = \frac{\theta_{\mathrm{a}}^2 e^{\omega_V} \left(e^{\omega_V} - 1\right)}{\theta_V^2}. \tag{6.73}$$

Equating

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.74}$$

for $m = 1, 2$ yields the following equations

$$\frac{\theta_{\mathrm{a}} e^{\frac{\omega_V}{2}}}{\theta_V} = \frac{\bar{\theta}_{\mathrm{a}} e^{\frac{\bar{\omega}_V}{2}}}{\bar{\theta}_V} \tag{6.75}$$

$$\frac{\theta_{\mathrm{a}}^2 e^{\omega_V} \left(e^{\omega_V} - 1\right)}{\theta_V^2} = \frac{\bar{\theta}_{\mathrm{a}}^2 e^{\bar{\omega}_V} \left(e^{\bar{\omega}_V} - 1\right)}{\bar{\theta}_V^2} \tag{6.76}$$

for which there is only one solution, namely

$$\frac{\theta_a}{\theta_V} = \frac{\bar{\theta}_a}{\bar{\theta}_V} \tag{6.77}$$

$$\omega_V = \bar{\omega}_V. \tag{6.78}$$

The first and second statistical moments of Z_3 are

$$\mathbb{E}[Z_3] = \theta_{\mathrm{a}}\theta_{\mathrm{e}} e^{\frac{\omega_{\mathrm{e}}}{2}} \tag{6.79}$$

$$\mathbb{E}[Z_3^2] = \theta_{\mathrm{a}}^2\theta_{\mathrm{e}}^2 e^{\omega_{\mathrm{e}}} \left(e^{\omega_{\mathrm{e}}} - 1\right) \tag{6.80}$$

Equating

$$\mathbb{E}[Z_3^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_3^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.81}$$

for $m = 1, 2$ yields the following equations

$$\theta_{\mathrm{a}}\theta_{\mathrm{e}} e^{\frac{\omega_{\mathrm{e}}}{2}} = \bar{\theta}_{\mathrm{a}}\bar{\theta}_{\mathrm{e}} e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}} \tag{6.82}$$

$$\theta_{\mathrm{a}}^2\theta_{\mathrm{e}}^2 e^{\omega_{\mathrm{e}}} \left(e^{\omega_{\mathrm{e}}} - 1\right) = \bar{\theta}_{\mathrm{a}}^2\bar{\theta}_{\mathrm{e}}^2 e^{\bar{\omega}_{\mathrm{e}}} \left(e^{\bar{\omega}_{\mathrm{e}}} - 1\right) \tag{6.83}$$

for which the following solution can be derived

$$\theta_a\theta_e = \bar{\theta}_a\bar{\theta}_e \tag{6.84}$$

$$\omega_e = \bar{\omega}_e \tag{6.85}$$

The first and second statistical moments of Z_2 are given by

$$\mathbb{E}[Z_2] = \theta_{\mathrm{a}} + \theta_{\mathrm{e}} e^{\frac{\omega_{\mathrm{e}}}{2}} \tag{6.86}$$

$$\mathbb{E}[Z_2^2] = \theta_{\mathrm{e}}^2 e^{\omega_{\mathrm{e}}} \left(e^{\omega_{\mathrm{e}}} - 1\right). \tag{6.87}$$

Equating

$$\mathbb{E}[Z_2^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.88}$$

for $m = 1, 2$ yields the following equations

$$\theta_{\mathrm{a}} + \theta_{\mathrm{e}} e^{\frac{\omega_{\mathrm{e}}}{2}} = \bar{\theta}_{\mathrm{a}} + \bar{\theta}_{\mathrm{e}} e^{\frac{\bar{\omega}_{\mathrm{e}}}{2}} \tag{6.89}$$

$$\theta_{\mathrm{e}}^2 e^{\omega_{\mathrm{e}}} \left(e^{\omega_{\mathrm{e}}} - 1\right) = \bar{\theta}_{\mathrm{e}}^2 e^{\bar{\omega}_{\mathrm{e}}} \left(e^{\bar{\omega}_{\mathrm{e}}} - 1\right) \tag{6.90}$$

which has, using previous results, only one solution, namely

$$\theta_e = \bar{\theta}_e \tag{6.91}$$

$$\theta_a = \bar{\theta}_a. \tag{6.92}$$

Using (6.91) with (6.75) it is also clear that

$$\theta_V = \bar{\theta}_V. \tag{6.93}$$

All model parameters including the statistical ones have thus been shown to be uniquely determined by the distribution of Z_1, Z_2 and Z_3, i.e.,

$$\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}. \tag{6.94}$$

The structurally locally identifiable non-mixed-effects one-compartment absorption model (6.28) is therefore structurally globally identifiable if two lognormally distributed random effects η_e and η_V are associated with the structural parameters θ_e and θ_V.

Linear three-compartment model

By introducing a random effect η_{21}, with a lognormal distribution to ensure positivity, for the locally identifiable parameter θ_{21} we now have a mixed-effects model, Figure 6.2B. Using the system's moment invariants from the corresponding non-

mixed-effects model, Figure 6.2A, we obtain the following set of functions of random variables

$$Z_1 = \theta_{21} e^{\eta_{21}} \theta_{31} \tag{6.95}$$

$$Z_2 = \theta_{21} e^{\eta_{21}} + \theta_{31} \tag{6.96}$$

$$Z_3 = \theta_{10} \theta_{21} e^{\eta_{21}} \theta_{31} \tag{6.97}$$

$$Z_4 = \theta_{10} \theta_{21} e^{\eta_{21}} + \theta_{10} \theta_{31} + \theta_{12} \theta_{31} + + \theta_{13} \theta_{21} e^{\eta_{21}} + \theta_{21} e^{\eta_{21}} \theta_{31} \tag{6.98}$$

$$Z_5 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} e^{\eta_{21}} + \theta_{31} \tag{6.99}$$

where

$$\eta_{21} \sim N(0, \sqrt{\omega_{21}}). \tag{6.100}$$

The first two statistical moments of Z_1 are given by

$$\mathbb{E}[Z_1] = \theta_{21} e^{\frac{\omega_{21}}{2}} \theta_{31} \tag{6.101}$$

$$\mathbb{E}[Z_1^2] = \theta_{21}^2 e^{\omega_{21}} \left(e^{\omega_{21}} - 1\right) \theta_{31}^2. \tag{6.102}$$

By equating and solving

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.103}$$

for $m = 1, 2$ the following equations are obtained

$$\theta_{21} e^{\frac{\omega_{21}}{2}} \theta_{31} = \bar{\theta}_{21} e^{\frac{\bar{\omega}_{21}}{2}} \bar{\theta}_{31} \tag{6.104}$$

$$\theta_{21}^2 e^{\omega_{21}} \left(e^{\omega_{21}} - 1\right) \theta_{31}^2 = \bar{\theta}_{21}^2 e^{\bar{\omega}_{21}} \left(e^{\bar{\omega}_{21}} - 1\right) \bar{\theta}_{31}^2 \tag{6.105}$$

from which we can derive that

$$\omega_{21} = \bar{\omega}_{21} \tag{6.106}$$

$$\theta_{21} \theta_{31} = \bar{\theta}_{21} \bar{\theta}_{31}. \tag{6.107}$$

The second statistical moment of Z_2 is given by

$$\mathbb{E}[Z_2^2] = \theta_{21}^2 e^{\omega_{21}} \left(e^{\omega_{21}} - 1\right). \tag{6.108}$$

Equating and solving

$$\mathbb{E}[Z_2^2(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_2^2(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.109}$$

yields the following equation

$$\theta_{21}^2 e^{\omega_{21}} \left(e^{\omega_{21}} - 1\right) = \bar{\theta}_{21}^2 e^{\bar{\omega}_{21}} \left(e^{\bar{\omega}_{21}} - 1\right) \tag{6.110}$$

which has, using previous results (6.106), only one solution, namely

$$\theta_{21} = \bar{\theta}_{21}. \tag{6.111}$$

Using equation (6.111) it can be concluded from equation (6.107) that

$$\theta_{31} = \bar{\theta}_{31}. \tag{6.112}$$

The first statistical moment of Z_3 is given by

$$\mathbb{E}[Z_3] = \theta_{10}\theta_{21} e^{\frac{\omega_{21}}{2}} \theta_{31}. \tag{6.113}$$

Equating

$$\mathbb{E}[Z_3(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_3(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.114}$$

yields the following equation

$$\theta_{10}\theta_{21} e^{\frac{\omega_{21}}{2}} \theta_{31} = \bar{\theta}_{10}\bar{\theta}_{21} e^{\frac{\bar{\omega}_{21}}{2}} \bar{\theta}_{31} \tag{6.115}$$

which has, using previous results, only one solution, namely

$$\theta_{10} = \bar{\theta}_{10}. \tag{6.116}$$

The first and second statistical moments for Z_4 and Z_5 are given by

$$\mathbb{E}[Z_4] = \theta_{31}\left(\theta_{10} + \theta_{12}\right) + \theta_{21} e^{\frac{\omega_{21}}{2}}\left(\theta_{10} + \theta_{13} + \theta_{31}\right) \tag{6.117}$$

$$\mathbb{E}[Z_4^2] = \theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right)\left(\theta_{10} + \theta_{13} + \theta_{31}\right)^2 \tag{6.118}$$

$$\mathbb{E}[Z_5] = \theta_{10} + \theta_{12} + \theta_{21}\left(e^{\frac{\omega_{21}}{2}} + 1\right) + \theta_{31} \tag{6.119}$$

$$\mathbb{E}[Z_5^2] = \theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right). \tag{6.120}$$

Equating and solving

$$\mathbb{E}[Z_k^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_k^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.121}$$

for $k = 1, 2$ and $m = 1, 2$ yields the following equations

$$\theta_{31}\left(\theta_{10} + \theta_{12}\right) + \theta_{21} e^{\frac{\omega_{21}}{2}}\left(\theta_{10} + \theta_{13} + \theta_{31}\right) = \bar{\theta}_{31}\left(\bar{\theta}_{10} + \bar{\theta}_{12}\right) + \bar{\theta}_{21} e^{\frac{\bar{\omega}_{21}}{2}}\left(\bar{\theta}_{10} + \bar{\theta}_{13} + \bar{\theta}_{31}\right) \tag{6.122}$$

$$\theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right)\left(\theta_{10} + \theta_{13} + \theta_{31}\right)^2 = \bar{\theta}_{21}^2 e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}} - 1\right)\left(\bar{\theta}_{10} + \bar{\theta}_{13} + \bar{\theta}_{31}\right)^2 \tag{6.123}$$

$$\theta_{10} + \theta_{12} + \theta_{21}\left(e^{\frac{\omega_{21}}{2}} + 1\right) + \theta_{31} = \bar{\theta}_{10} + \bar{\theta}_{12} + \bar{\theta}_{21}\left(e^{\frac{\bar{\omega}_{21}}{2}} + 1\right) + \bar{\theta}_{31} \tag{6.124}$$

$$\theta_{21}^2 e^{\omega_{21}}\left(e^{\omega_{21}} - 1\right) = \bar{\theta}_{21}^2 e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}} - 1\right) \tag{6.125}$$

for which, using previous results, only one solution, namely

$$\theta_{12} = \bar{\theta}_{12} \tag{6.126}$$

$$\theta_{13} = \bar{\theta}_{13}. \tag{6.127}$$

It has been shown that all model parameters can be uniquely determine, i.e.,

$$\{\boldsymbol{\theta}, \boldsymbol{\Omega}\} = \{\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\Omega}}\}. \tag{6.128}$$

The mixed-effects model is therefore structurally globally identifiable. This is because by introducing a random effect on a locally identifiable parameter the symmetry for this particular model is broken and the two states x_2 and x_3 become distinguishable.

6.3.2 Random effects on globally identifiable parameters do not affect the structural identifiability of the mixed-effects model

In this section random effects are added to the structural model of the two example models in such a way that the structural identifiability of the models is not affected.

One-compartment absorption model

If only one random effect is added the structural parameter θ_V in the one-compartment absorption model (6.28) the following functions of random variable can be derived

$$Z_1 = \frac{\theta_a}{\theta_V e^{\eta_V}} \tag{6.129}$$

$$Z_2 = \theta_a + \theta_e \tag{6.130}$$

$$Z_3 = \theta_a \theta_e. \tag{6.131}$$

The first and second statistical moments of Z_1 are given by

$$\mathbb{E}[Z_1] = \frac{\theta_{\mathrm{a}} e^{\frac{\omega_V}{2}}}{\theta_V} \tag{6.132}$$

$$\mathbb{E}[Z_1^2] = \frac{\theta_{\mathrm{a}}^2 e^{\omega_V} \left(e^{\omega_V} - 1\right)}{\theta_V^2}. \tag{6.133}$$

Equating

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.134}$$

for $m = 1, 2$ yields the following equations

$$\frac{\theta_{\mathrm{a}} e^{\frac{\omega_V}{2}}}{\theta_V} = \frac{\bar{\theta}_{\mathrm{a}} e^{\frac{\bar{\omega}_V}{2}}}{\bar{\theta}_V} \tag{6.135}$$

$$\frac{\theta_{\mathrm{a}}^2 e^{\omega_V} \left(e^{\omega_V} - 1\right)}{\theta_V^2} = \frac{\bar{\theta}_{\mathrm{a}}^2 e^{\bar{\omega}_V} \left(e^{\bar{\omega}_V} - 1\right)}{\bar{\theta}_V^2} \tag{6.136}$$

for which there is only one solution, namely

$$\frac{\theta_a}{\theta_V} = \frac{\bar{\theta}_a}{\bar{\theta}_V} \tag{6.137}$$

$$\omega_V = \bar{\omega}_V. \tag{6.138}$$

Since neither Z_2 or Z_3 contain a random variable all statistical moments higher than one are equal to zero. The remaining expressions are therefore in the same form as for the non-mixed-effects case, i.e., there are still two solutions for the structural parameters. Therefore, with a lognormally distributed random effect only on the structural parameter θ_V the mixed-effects model is still structurally locally identifiable.

Linear three-compartment model

Here a random effect η_{10} is added to the structural parameter θ_{10}, which is globally identifiable in the non-mixed-effects case. From the mixed-effects model, Figure 6.2C, the following functions of random variables can be derived

$$Z_1 = \theta_{21}\theta_{31} \tag{6.139}$$

$$Z_2 = \theta_{21} + \theta_{31} \tag{6.140}$$

$$Z_3 = \theta_{10} e^{\eta_{10}} \theta_{21}\theta_{31} \tag{6.141}$$

$$Z_4 = \theta_{10} e^{\eta_{10}} \theta_{21} + \theta_{10} e^{\eta_{10}} \theta_{31} + \theta_{12}\theta_{31} + \theta_{13}\theta_{21} + \theta_{21}\theta_{31} \tag{6.142}$$

$$Z_5 = \theta_{10} e^{\eta_{10}} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31} \tag{6.143}$$

where

$$\eta_{10} \sim N(0, \sqrt{\omega_{10}}). \tag{6.144}$$

The first statistical moments of Z_1 and Z_3 are given by

$$\mathbb{E}[Z_1] = \theta_{21}\theta_{31} \tag{6.145}$$

$$\mathbb{E}[Z_1^2] = 0 \tag{6.146}$$

$$\mathbb{E}[Z_3] = \theta_{10} e^{\frac{\omega_{10}}{2}} \theta_{21}\theta_{31} \tag{6.147}$$

$$\mathbb{E}[Z_3^2] = \theta_{10}^2 e^{\omega_{10}} \left(e^{\omega_{10}} - 1\right) \theta_{21}^2 \theta_{31}^2. \tag{6.148}$$

Equating

$$\mathbb{E}[Z_1(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_1(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.149}$$

and

$$\mathbb{E}[Z_4^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z_4^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.150}$$

for $m = 1, 2$ yields the following equations

$$\theta_{21}\theta_{31} = \bar{\theta}_{21}\bar{\theta}_{31} \tag{6.151}$$

$$\theta_{10} e^{\frac{\omega_{10}}{2}} \theta_{21}\theta_{31} = \bar{\theta}_{10} e^{\frac{\bar{\omega}_{10}}{2}} \bar{\theta}_{21}\bar{\theta}_{31} \tag{6.152}$$

$$\theta_{10}^2 e^{\omega_{10}} \left(e^{\omega_{10}} - 1\right) \theta_{21}^2 \theta_{31}^2 = \bar{\theta}_{10}^2 e^{\bar{\omega}_{10}} \left(e^{\bar{\omega}_{10}} - 1\right) \bar{\theta}_{21}^2 \bar{\theta}_{31}^2 \tag{6.153}$$

from which it can be concluded that

$$\theta_{10} = \bar{\theta}_{10} \tag{6.154}$$

$$\omega_{10} = \bar{\omega}_{10} \tag{6.155}$$

$$\theta_{21}\theta_{31} = \bar{\theta}_{21}\bar{\theta}_{31}. \tag{6.156}$$

Since the parameters θ_{10} and ω_{10} can be determined uniquely, and since no other random effects are present in the model, the structure of the remaining expressions for the parameters in the equations is the same as in the non-mixed-effects case with the moment invariants. This means that the remaining parameters

$$\{\theta_{12}, \theta_{13}, \theta_{21}, \theta_{31}\} \tag{6.157}$$

are locally identifiable with two solutions. It can therefore be concluded that if there is a single random effect on the structural parameter θ_{10} the mixed-effects model is still structurally locally identifiable. This is because by introducing the random effect on an already globally identifiable parameter the symmetry is not broken leaving the two states x_2 and x_3 to be still indistinguishable.

Conjecture 2. Let $\Sigma^{l_n}(\boldsymbol{\theta})$ denote a structurally locally identifiable non-mixed effects system of the form (2.4) with n possible solutions. Let $\boldsymbol{\theta}_{gi}$ denote a vector of all globally identifiable parameters in $\Sigma^{l_n}(\boldsymbol{\theta})$. For all n, if any distribution is postulated for any member of $\boldsymbol{\theta}_{gi}$ by introducing random effects then the corresponding mixed-effects model will still be structurally locally identifiable.

6.4 The effect on structural identifiability of the form of the random effects

In this section two examples are provided which show how different forms for the random effects may affect whether or not a mixed-effects model is structurally identifiable. The example model used here is the one-compartment absorption model, Figure 6.3 with a bioavailability parameter θ_F which is used to model the uptake and elimination of a pharmaceutical drug with, for instance, an oral administration. The non-mixed-effects version of the one-compartment absorption model has the

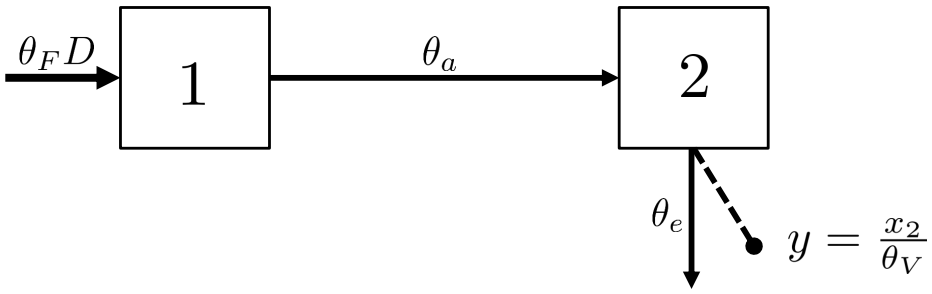


Figure 6.3: The one-compartment absorption model with linear absorption and elimination and a bioavailability parameter θ_F.

following structure

$$\dot{x}_1 = -\theta_a x_1 \tag{6.158}$$

$$\dot{x}_2 = \theta_a x_1 - \theta_e x_2 \tag{6.159}$$

$$x_1(0) = \theta_F D \tag{6.160}$$

$$x_2(0) = 0 \tag{6.161}$$

with observation

$$y = \frac{x_2}{\theta_V} \tag{6.162}$$

with unknown parameter vector

$$\boldsymbol{\theta} = (\theta_a, \theta_e, \theta_F, \theta_V) \tag{6.163}$$

and with the parameter D known. With some constraint on θ_a and θ_e, such as $\theta_a > \theta_e$, it has been shown, for instance in Lavielle and Aarons [2015], that the following model parameters

$$\{\frac{\theta_V}{\theta_F}, \theta_a, \theta_e\} \tag{6.164}$$

are globally identifiable, but the model itself is structurally unidentifiable since only the ratio $\frac{\theta_V}{\theta_F}$ is identifiable. We will now examine what happens to the structural identifiability of the mixed-effects model when different forms of random effects are introduced to the parameters θ_V and θ_F.

6.4.1 Single random effect on unidentifiable parameter

In this first example, a single random effect η_V will be added to the one-compartment model resulting in a lognormal distribution of the volume parameter. In the mixed-effects setting the identifiable ratio $\frac{\theta_V}{\theta_F}$ then becomes the following random variable function Z

$$Z = \frac{\theta_V e^{\eta_V}}{\theta_F} \tag{6.165}$$

where the random effect η_V is normally distributed as

$$\eta_V \sim N(0, \sqrt{\omega_V}) \tag{6.166}$$

The first two statistical moments of Z are

$$\mathbb{E}[Z] = \frac{\theta_V e^{\frac{\omega_V}{2}}}{\theta_F} \tag{6.167}$$

$$\mathbb{E}[Z^2] = \frac{\theta_V^2 e^{\omega_V} \left(e^{\omega_V} - 1\right)}{\theta_F^2}. \tag{6.168}$$

By substituting the population parameters as

$$\beta_\theta = \frac{\theta_V}{\theta_F} \tag{6.169}$$

and equating the statistical moments as

$$\mathbb{E}[Z^m(\boldsymbol{\theta}, \eta_V)] = \mathbb{E}[Z^m(\bar{\boldsymbol{\theta}}, \bar{\eta}_V)] \tag{6.170}$$

for $m = 1, 2$ the following equations are obtained

$$\beta_\theta e^{\frac{\omega_V}{2}} = \bar{\beta}_{\bar{\theta}} e^{\frac{\bar{\omega}_V}{2}} \tag{6.171}$$

$$\beta_V^2 e^{\omega_V}(e^{\omega_V} - 1) = \bar{\beta}_V^2 e^{\bar{\omega}_V}(e^{\bar{\omega}_V} - 1) \tag{6.172}$$

which have the following solutions

$$\beta_\theta = \bar{\beta}_\theta \tag{6.173}$$

$$\omega_V = \bar{\omega}_V. \tag{6.174}$$

The following parameter combinations are therefore uniquely determined

$$\{\frac{\theta_V}{\theta_F}, \omega_V\} \tag{6.175}$$

and the mixed-effects model is therefore structurally unidentifiable since still only the ratio can be determined.

6.4.2 With two lognormal random effects the mixed-effects model remains unidentifiable

In the second example the one-compartment absorption model with both random effects being lognormally distributed is considered. The identifiable ratio $\frac{\theta_V}{\theta_F}$ in a mixed-effects setting becomes the following random variable function Z

$$Z = \frac{\theta_V e^{\eta_V}}{\theta_F e^{\eta_F}} \tag{6.176}$$

with the random effects vector

$$\boldsymbol{\eta} = (\eta_V, \eta_F) \tag{6.177}$$

being normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.178}$$

with a diagonal covariance matrix $\boldsymbol{\Omega}$, i.e., there is no covariance between η_V and η_F. The first two statistical moments of Z are

$$\mathbb{E}[Z] = \frac{\theta_V e^{\frac{\omega_F}{2} + \frac{\omega_V}{2}}}{\theta_F} \tag{6.179}$$

$$\mathbb{E}[Z^2] = \frac{\theta_V^2 e^{\omega_F + \omega_V} \left(e^{\omega_F + \omega_V} - 1\right)}{\theta_F^2}. \tag{6.180}$$

The function Z is a ratio of two lognormally distributed random variables. Because of this, Z is also lognormally distributed. Therefore, only the first two statistical moments need to be considered as they fully characterise the distribution of Z (Borovkov [2013]). This results in two equations with four unknowns. By substituting the population parameters and the variance parameters as

$$\beta_\theta = \frac{\theta_V}{\theta_F} \tag{6.181}$$

$$\beta_\omega = \omega_V + \omega_F \tag{6.182}$$

and equating the statistical moments as

$$\mathbb{E}[Z^m(\boldsymbol{\theta}, \boldsymbol{\eta})] = \mathbb{E}[Z^m(\bar{\boldsymbol{\theta}}, \bar{\boldsymbol{\eta}})] \tag{6.183}$$

for $m = 1, 2$ the following equations are obtained

$$\beta_\theta e^{\frac{\beta_\omega}{2}} = \bar{\beta}_\theta e^{\frac{\bar{\beta}_\omega}{2}} \tag{6.184}$$

$$\beta_\theta^2 e^{\beta_\omega} \left(e^{\beta_\omega} - 1\right) = \bar{\beta}_\theta^2 e^{\bar{\beta}_\omega} \left(e^{\bar{\beta}_\omega} - 1\right) \tag{6.185}$$

which has only one solution, namely

$$\beta_\theta = \bar{\beta}_\theta \tag{6.186}$$

$$\beta_\omega = \bar{\beta}_\omega. \tag{6.187}$$

It has therefore been shown that still only the ratio of the population parameters and the sum of the variance parameters

$$\{\frac{\theta_V}{\theta_F}, \omega_V + \omega_F\} \tag{6.188}$$

are identifiable. The one-compartment absorption model (6.158) with two lognormally distributed random effects associated with θ_V and θ_F is therefore structurally unidentifiable.

To illustrate this analytical result numerically, the one-compartment absorption model (6.158) with two lognormally distributed random effects was simulated using two different parameter sets, see Table 6.1 for the particular values that were used.

Table 6.1: Parameter values that were used for the one-compartment absorption mixed-effects model (6.158) with two lognormally distributed random effects.

Parameter set	θ_a	θ_e	θ_F	θ_V	ω_F	ω_V
A	1	0.5	0.8	1.2	0.8	0.2
B	1	0.5	0.48	0.72	0.2	0.8

Note that the parameter values in Table 6.1 were chosen so that the ratio of $\frac{\theta_V}{\theta_F}$ and the sum $\omega_V + \omega_F$ are equal for the two parameter sets respectively following from the results in (6.188), i.e.,

$$\frac{1.2}{0.8} = \frac{0.72}{0.48} = \frac{3}{2} \tag{6.189}$$

$$0.8 + 0.2 = 0.2 + 0.8 = 1. \tag{6.190}$$

The simulation of the model using these particular parameter values can be seen in

Figure 6.4. As expected from the analytical analysis, both the simulation for the typical subject and the distribution of the output for parameter set A and B are identical.

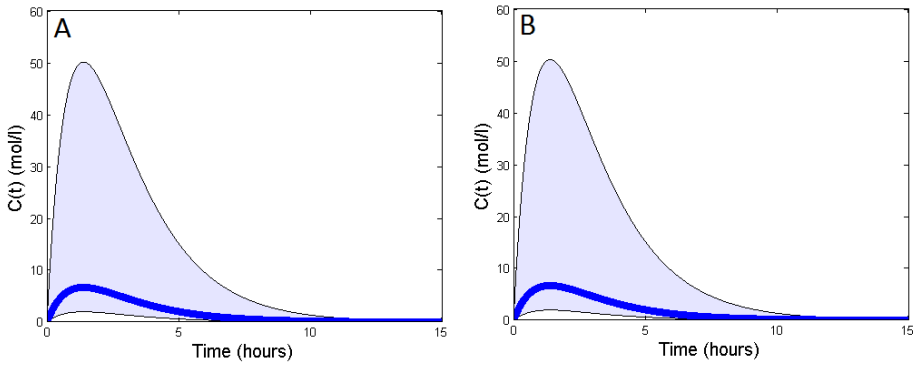


Figure 6.4: Two simulations of the one-compartment absorption model with two lognormally distributed random effects on θ_V and θ_F using 50000 subjects. The parameter values that were used are given in Table 6.1. The thick line is the simulation for the typical subject and the shaded area is the 95-percentile, both of which are identical in A and B and thus illustrating the analytical result.

6.4.3 Lognormal and logit-normal random effects renders the mixed-effects model identifiable

In this example, the one-compartment absorption model (6.158) is considered with a lognormally distributed random effect on θ_V and a logit-normally distributed random effect on θ_F. Postulating a logit-normal distribution of the biodistribution parameter θ_F makes more sense in a biological context since bioavailability is defined between $0 \leq \theta_F \leq 1$. The corresponding function of random variables is

$$Z = \frac{\theta_V e^{\eta_V}}{\frac{1}{1+\frac{1-\theta_F}{\theta_F e^{\eta_F}}}} \tag{6.191}$$

with the random effects vector

$$\boldsymbol{\eta} = (\eta_V, \eta_F) \tag{6.192}$$

being normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.193}$$

with a diagonal covariance matrix $\boldsymbol{\Omega}$.

In contrast to (6.176), the function of random variables (6.191) is not log-normally distributed. We therefore consider the first four statistical moments of (6.191) since we have four unknown parameters

$$\boldsymbol{\gamma} = (\theta_V, \theta_F, \omega_V, \omega_F). \tag{6.194}$$

The first four statistical moments of Z_4 are given by

$$\mathbb{E}[Z] = \theta_V e^{\frac{\omega_V}{2}} \left(\frac{(1-\theta_F)\, e^{\frac{\omega_F}{2}}}{\theta_F} + 1 \right) \tag{6.195}$$

$$\mathbb{E}[Z^2] = \theta_V^2 e^{2\omega_V} \left(\frac{(\theta_F - 1)^{\,2} e^{2\omega_F}}{\theta_F^2} - \frac{2\,(\theta_F - 1)\, e^{\frac{\omega_F}{2}}}{\theta_F} + 1 \right) \tag{6.196}$$

$$\mathbb{E}[Z^3] = \theta_V^3 e^{\frac{9\omega_V}{2}} \left(-\frac{(\theta_F - 1)^{\,3} e^{\frac{9\omega_F}{2}}}{\theta_F^3} + \frac{3\,(\theta_F - 1)^{\,2} e^{2\omega_F}}{\theta_F^2} - \frac{3\,(\theta_F - 1)\, e^{\frac{\omega_F}{2}}}{\theta_F} + 1 \right) \tag{6.197}$$

$$\mathbb{E}[Z^4] = \frac{\theta_V^4 e^{8\omega_V} \left((\theta_F - 1)^{\,4} e^{8\omega_F} - 4\theta_F\, (\theta_F - 1)^{\,3} e^{\frac{9\omega_F}{2}} \right)}{\theta_F^4} + \frac{\theta_V^4 e^{8\omega_V} \left(6\theta_F^2\, (\theta_F - 1)^{\,2} e^{2\omega_F} - 4\theta_F^3\, (\theta_F - 1)\, e^{\frac{\omega_F}{2}} + \theta_F^4 \right))}{\theta_F^4}. \tag{6.198}$$

The structural identifiability of the model was considered by generating the Jacobian matrix for the first four statistical moments

$$J = \begin{pmatrix} \frac{\partial \mathbb{E}[Z]}{\partial \theta_F} & \frac{\partial \mathbb{E}[Z]}{\partial \theta_V} & \frac{\partial \mathbb{E}[Z]}{\partial \omega_F} & \frac{\partial \mathbb{E}[Z]}{\partial \omega_V} \\ \frac{\partial \mathbb{E}[Z^2]}{\partial \theta_F} & \frac{\partial \mathbb{E}[Z^2]}{\partial \theta_V} & \frac{\partial \mathbb{E}[Z^2]}{\partial \omega_F} & \frac{\partial \mathbb{E}[Z^2]}{\partial \omega_V} \\ \frac{\partial \mathbb{E}[Z^3]}{\partial \theta_F} & \frac{\partial \mathbb{E}[Z^3]}{\partial \theta_V} & \frac{\partial \mathbb{E}[Z^3]}{\partial \omega_F} & \frac{\partial \mathbb{E}[Z^3]}{\partial \omega_V} \\ \frac{\partial \mathbb{E}[Z^4]}{\partial \theta_F} & \frac{\partial \mathbb{E}[Z^4]}{\partial \theta_V} & \frac{\partial \mathbb{E}[Z^4]}{\partial \omega_F} & \frac{\partial \mathbb{E}[Z^4]}{\partial \omega_V} \end{pmatrix}. \tag{6.199}$$

The explicit expressions for the elements in the Jacobian matrix have been omitted

due to the expressions being too large. The Mathematica code for generating the statistical moments and the Jacobian (6.199) and the subsequent analysis can be found in Appendix E. It follows however that J has full rank, i.e., the columns are linearly independent, meaning that the mixed-effects model with lognormally and logit-normally distributed random effects is at least locally identifiable (Pohjanpalo and Wahlström [1982]). This is an analytical confirmation of the results presented in Lavielle and Aarons [2015] where the same one-compartment absorption model is considered with the same parameter distributions assumed, but is analysed using a numerical approach.

In general, it has been shown in this example that two unidentifiable model parameters will never become identifiable by postulating a lognormal distribution of both parameters when they appear as a fraction. However, by postulating one lognormal distribution and one logit-normal distribution the symmetry is broken and the model becomes structurally identifiable.

Conjecture 3. Let $\Sigma^u(\boldsymbol{\theta})$ denote a structurally unidentifiable non-mixed effects system in the form (2.4). Let $\boldsymbol{\theta}_u$ denote a vector of all unidentifiable parameters in $\Sigma^u(\boldsymbol{\theta})$. If a distribution is postulated for only one of the members in $\boldsymbol{\theta}_u$ using some random effect η then the corresponding mixed-effects model is still structurally unidentifiable. If more than one member of $\boldsymbol{\theta}_u$ is postulated to have some distribution using random effects $\boldsymbol{\eta}$ then the corresponding mixed-effects model can either remain unidentifiable, or become structurally locally/globally identifiable.

6.5 The covariance matrix

In this section the impact of what form the covariance matrix has on structural identifiability will be studied. It is easy to show that if two variance parameters are globally identifiable it follows that any parameter representing the covariance between them is also identifiable.

A perhaps more interesting question is what happens with the structural identifiability of a model when otherwise unidentifiable variance parameters have

a non-zero covariance with some other structurally globally identifiable variance parameter. It will be shown that the parameter space for an unidentifiable variance parameter is restricted by its covariance with an identifiable variance parameter.

6.5.1 Covariance restricts the parameter space for the variance parameters

Suppose there exists a model structure from which the following two functions of random variables can be derived

$$Z_1 = \theta_1 e^{\eta_1} \theta_2 e^{\eta_2} \tag{6.200}$$

$$Z_2 = \theta_3 e^{\eta_3} \tag{6.201}$$

with the random effects vector

$$\boldsymbol{\eta} = (\eta_1, \eta_2, \eta_3) \tag{6.202}$$

normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.203}$$

and a non-zero off-diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_1 & 0 & \omega_{13} \\ 0 & \omega_2 & 0 \\ \omega_{13} & 0 & \omega_3 \end{pmatrix}, \tag{6.204}$$

i.e., there is a covariance between η_1 and η_3 represented with the covariance parameter ω_{13}. The first two statistical moments of Z_2 are given by

$$\mathbb{E}[Z_2] = \theta_3 e^{\frac{\omega_3}{2}} \tag{6.205}$$

$$\mathbb{E}[Z_2^2] = \theta_3^2 e^{\omega_3} \left(e^{\omega_3} - 1\right). \tag{6.206}$$

By equating

$$\mathbb{E}[Z_2^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_2^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{6.207}$$

for $m = 1, 2$ the following two equations are obtained

$$\theta_3 e^{\frac{\omega_3}{2}} = \bar{\theta}_3 e^{\frac{\bar{\omega}_3}{2}} \tag{6.208}$$

$$\theta_3^2 e^{\omega_3}\left(e^{\omega_3} - 1\right) = \bar{\theta}_3^2 e^{\bar{\omega}_3}\left(e^{\bar{\omega}_3} - 1\right) \tag{6.209}$$

which have only one solution, namely

$$\theta_3 = \bar{\theta}_3 \tag{6.210}$$

$$\omega_3 = \bar{\omega}_3. \tag{6.211}$$

The statistical moments of Z_1 are given by

$$\mathbb{E}[Z_1] = \theta_1\theta_2 e^{\frac{\omega_1}{2}+\frac{\omega_2}{2}} \tag{6.212}$$

$$\mathbb{E}[Z_1^2] = \theta_1^2\theta_2^2 e^{\omega_1+\omega_2}\left(e^{\omega_1+\omega_2} - 1\right). \tag{6.213}$$

Using the following substitution

$$\beta_\theta = \theta_1\theta_2 \tag{6.214}$$

$$\beta_\omega = \omega_1 + \omega_2 \tag{6.215}$$

and equating the statistical moments of Z_1 as

$$\mathbb{E}[Z_1^m(\boldsymbol{\theta},\boldsymbol{\eta})] = \mathbb{E}[Z_1^m(\bar{\boldsymbol{\theta}},\bar{\boldsymbol{\eta}})] \tag{6.216}$$

for $m = 1, 2$ the following equations are obtained

$$\beta_\theta e^{\frac{\beta_\omega}{2}} = \bar{\beta}_\theta e^{\frac{\bar{\beta}_\omega}{2}} \tag{6.217}$$

$$\beta_\theta^2 e^{\beta_\omega}(e^{\beta_\omega} - 1) = \bar{\beta}_\theta^2 e^{\bar{\beta}_\omega}(e^{\bar{\beta}_\omega} - 1) \tag{6.218}$$

which have only one solution, namely

$$\beta_\theta = \bar{\beta}_\theta \tag{6.219}$$

$$\beta_\omega = \bar{\beta}_\omega. \tag{6.220}$$

This means that only the product

$$\theta_1\theta_2 \tag{6.221}$$

and the sum

$$\omega_1 + \omega_2 \tag{6.222}$$

are identifiable. The covariance between Z_1 and Z_2 is given by

$$\mathrm{Cov}(Z_1(\boldsymbol{\theta},\boldsymbol{\eta})Z_2(\boldsymbol{\theta},\boldsymbol{\eta})) = \mathbb{E}[Z_1Z_2] - \mathbb{E}[Z_1]\mathbb{E}[Z_2] = \theta_1\theta_2\theta_3 e^{\frac{1}{2}(\omega_1+\omega_2+\omega_3)}\left(e^{\omega_{13}} - 1\right). \tag{6.223}$$

It has previously been shown in (6.216)–(6.222) that the product $\theta_1\theta_2$ and the sum $\omega_1 + \omega_2$ are uniquely determined by the distribution of Z_1. Since θ_3 and ω_3 can be determined uniquely, it follows that also both the product $\theta_1\theta_2\theta_3$ and the sum $\omega_1 + \omega_2 + \omega_3$ can be determined uniquely. Therefore, it follows directly from (6.223) that the covariance parameter ω_{13} is globally identifiable.

Now the covariance matrix $\boldsymbol{\Omega}$ itself is considered. By definition, a covariance matrix is always positive semi-definite. A well-known property of a positive semi-definite matrix is that the principal minors are greater than or equal to zero (Woerdeman [1962]). From this property the following relations hold

$$\omega_1\omega_2 \geq 0 \tag{6.224}$$

$$\omega_2\omega_3 \geq 0 \tag{6.225}$$

$$\omega_1\omega_3 \geq \omega_{13}^2 \tag{6.226}$$

where the last relation $\omega_1\omega_3 \geq \omega_{13}^2$ can be rewritten as

$$-\sqrt{\omega_1} \leq \frac{\omega_{13}}{\sqrt{\omega_3}} \leq \sqrt{\omega_1}. \tag{6.227}$$

An alternative route to deriving (6.227) is to consider the determinant of the covariance matrix $\mathbf{\Omega}$ which is greater than or equal to zero for all positive semi-definite matrices. To summarise, the following equality and inequality constraints for the variance and covariance parameters hold for this case

$$\frac{\omega_{13}}{\sqrt{\omega_3}} \leq \sqrt{\omega_1} \tag{6.228}$$

$$0 \leq \omega_2 \tag{6.229}$$

$$0 \leq \omega_3 \tag{6.230}$$

$$0 \leq \omega_{13} \tag{6.231}$$

$$C = \omega_1 + \omega_2 \tag{6.232}$$

where C, ω_{13} and ω_3 are globally identifiable and ω_1 and ω_2 are unidentifiable. This means that, even though both ω_1 and ω_2 are unidentifiable, upper and lower bounds for the values that they can take exist.

6.5.2 Covariance restricts the parameter space for the variance parameters to unique values

In the previous section it was shown how general properties of the covariance matrix $\mathbf{\Omega}$ can be used to derive restrictions on the parameter space for the variance parameters. In this section, a special case of parameter space restriction will be considered, namely when the variance parameters are restricted to unique values.

Assume that a model structure exists from which the following functions of

random variables can be derived

$$Z_1 = \theta_1 e^{\eta_1} \theta_2 e^{\eta_2} \tag{6.233}$$

$$Z_2 = \theta_3 e^{\eta_3} \tag{6.234}$$

$$Z_3 = \theta_4 e^{\eta_4} \tag{6.235}$$

with the random effects vector

$$\boldsymbol{\eta} = (\eta_1, \eta_2, \eta_3, \eta_4) \tag{6.236}$$

normally distributed as

$$\boldsymbol{\eta} \sim N(\mathbf{0}, \boldsymbol{\Omega}) \tag{6.237}$$

with the off-diagonal covariance matrix

$$\boldsymbol{\Omega} = \begin{pmatrix} \omega_1 & 0 & \omega_{13} & 0 \\ 0 & \omega_2 & 0 & \omega_{24} \\ \omega_{13} & 0 & \omega_3 & \\ 0 & \omega_{24} & 0 & \omega_4 \end{pmatrix}. \tag{6.238}$$

In this case, there is covariance between the variance parameters ω_1 and ω_3 represented by the covariance parameter ω_{13}, and covariance between the variance parameters ω_2 and ω_4 represented by ω_{24}. Using the same approach as in the previous section it can be shown that the parameters in the vector

$$\boldsymbol{\gamma} = (\theta_3, \theta_4, \omega_3, \omega_4, \omega_{13}, \omega_{24}) \tag{6.239}$$

are globally identifiable, and that the sum $\omega_1 + \omega_2$ is globally identifiable.

By computing the principal minors of the covariance matrix $\boldsymbol{\Omega}$, and using the previous results (6.228)–(6.232), the following equality and inequality relations

for the variance and covariance parameters are obtained

$$\frac{\omega_{13}^2}{\omega_3} \leq \omega_1 \tag{6.240}$$

$$\frac{\omega_{24}^2}{\omega_4} \leq \omega_2 \tag{6.241}$$

$$0 \leq \omega_3 \tag{6.242}$$

$$0 \leq \omega_4 \tag{6.243}$$

$$0 \leq \omega_{13} \tag{6.244}$$

$$0 \leq \omega_{24} \tag{6.245}$$

$$C = \omega_1 + \omega_2. \tag{6.246}$$

where C, ω_3, ω_4, ω_{13} and ω_{24} are globally identifiable.

This particular semialgebraic set has a single solution if and only if the identifiable sum of $\omega_1 + \omega_2$ is in a special case equal to the sum of the lower bounds of ω_1 and ω_2 while $\omega_3 \neq 0$ and $\omega_4 \neq 0$. Whether or not this equality holds depends on the particular data set that is used. By equating the sum of the lower bounds with the variance parameters the following equality is obtained

$$\frac{\omega_{13}^2}{\omega_3} + \frac{\omega_{24}^2}{\omega_4} = \omega_1 + \omega_2. \tag{6.247}$$

By subtracting (6.247) from (6.240) and (6.241) the following two inequalities are obtained

$$-\frac{\omega_{24}^2}{\omega_4} \leq -\omega_2 \tag{6.248}$$

$$-\frac{\omega_{13}^2}{\omega_3} \leq -\omega_1. \tag{6.249}$$

By combining (6.240) and (6.241) with (6.248) and (6.249) it can be shown that

$$\omega_1 = \frac{\omega_{13}^2}{\omega_3} \tag{6.250}$$

$$\omega_2 = \frac{\omega_{24}^2}{\omega_4}. \tag{6.251}$$

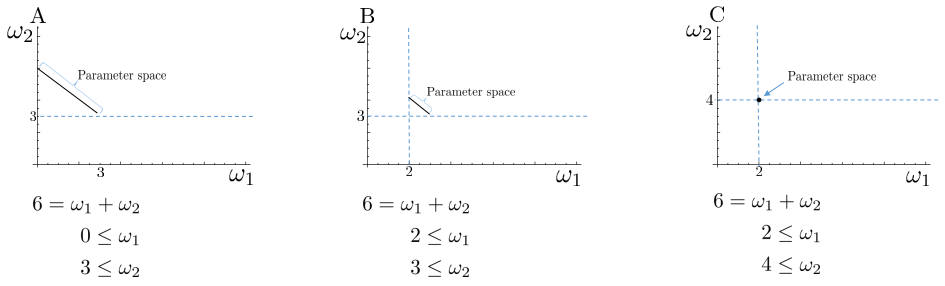


Figure 6.5: The parameter space of unidentifiable variance parameters ω_1 and ω_2 is restricted by related identifiable variance and covariance parameters.

Since the parameters

$$\{\omega_{13}, \omega_{24}, \omega_3\omega_4\} \tag{6.252}$$

are globally identifiable then so are ω_1 and ω_2 for this particular case. Figure 6.5 exemplifies the extent of how different numerical values of the identifiable variance parameters restricts the parameter space of the unidentifiable parameters in this case.

6.6 Summary

In this chapter some of the methods developed for studying structural identifiability in mixed-effects models that have been developed within this thesis and presented in Chapter 4 have been used to explore how structural identifiability analysis results for non-mixed-effects model translate to the mixed-effects case.

In particular, the question regarding whether structural identifiability results for a non-mixed-effects model also hold for a mixed-effects model have been explored. This has been done by using examples with the same structural model, but with different statistical sub-models.

From the provided examples it is clear that structural identifiability of mixed-effects models is dependent on the statistical sub-model. It has been shown that the structural identifiability of a mixed-effects model is dependent on which structural

parameters have associated random effects. It has also been shown that for some forms for the distribution of the random effects, a mixed-effects model is structurally unidentifiable while for other forms of distribution for the random effects the model is structurally identifiable. In addition, it has been shown that covariance between unidentifiable variance parameters and an identifiable variance parameter restricts the parameter space for the unidentifiable variance parameter.

To conclude, structural identifiability results for non-mixed-effects models do not necessarily translate to the mixed-effects case. It has been shown using examples that, depending on where the random effects enter the structural model, the postulated form of the distribution of the random effects and the structure of the covariance matrix, introducing random effects to a non-mixed-effects model may render a locally identifiable model to become globally identifiable, or even an unidentifiable model to become either locally or globally identifiable, see Figure 6.6. The conditions for these different scenarios have been summarised in Conjectures 1–3.

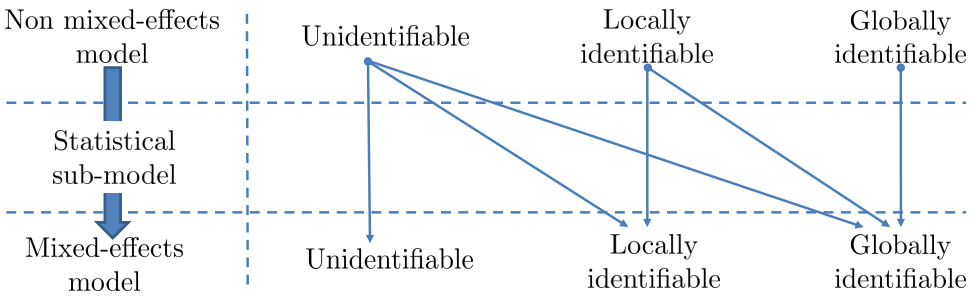


Figure 6.6: Flowchart of how structural identifiability changes when introducing an identifiable statistical sub-model to a non mixed-effects model. An unidentifiable non mixed-effects model can become locally/globally identifible, or remain unidentifiable depending on the statistical sub-model. A locally identifiable non mixed-effects model can become globally identifiable, or remain locally identifiable, depending on the statistical sub-model. A globally identifiable model will remain globally identifiable.

If the data contains a large variability between subjects it is often necessary to use mixed-effects modelling. There are several advantages of using such models over a distinct number of non-mixed-effects model such as obtaining individual

parameter estimates, estimation of population variability and better handling of sparsely sampled and noisy data. In this chapter it has been shown that the issue of structural identifiability can now also be added as a potential advantage of using mixed-effects models since it may in some cases solve structural identifiability issues.

Chapter 7

Structural indistinguishability analysis of mixed-effects models: Case studies

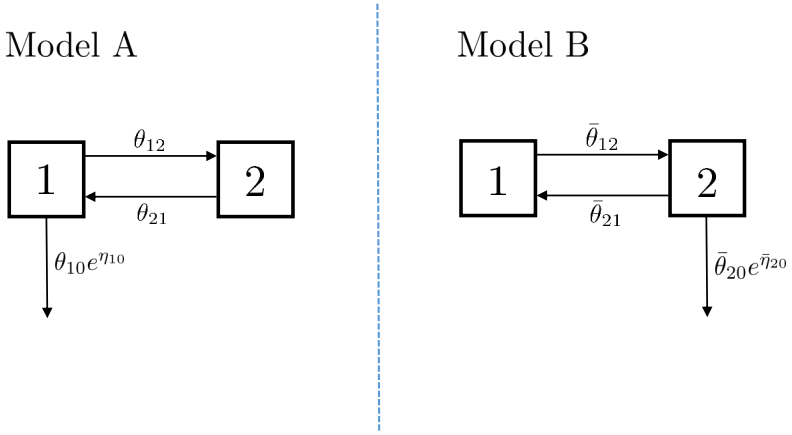
7.1 Introduction

As discussed in Chapter 2, structural identifiability and structural indistinguishability are closely related. Because of this, some of the techniques to study structural identifiability of non-mixed-effects models can be modified in order to study structural indistinguishability. In this thesis it has been shown that this holds true for mixed-effects models as well.

In this chapter structural indistinguishability analysis of a set of mixed-effects models is performed to show how the methods developed work in practice. Two examples of pair-wise mixed-effects models will be analysed from a structural indistinguishability perspective. In both examples each of the three methods Laplace transform, Taylor series expansion and the input-output form approach previously presented in Chapter 4 are applied.

First the model structures of the example models are given in Section 7.1.1–7.1.2. These models are then analysed in the subsequent sections.

Figure 7.1: Model structures of the two structurally indistinguishable linear two-compartment models: model A and model B.



7.1.1 Example 1: Two two-compartment models

In this first example two linear two-compartment models denoted by model A and model B, Figure 7.1, are considered. The only difference in a structural sense between the two models is from which compartment the elimination occurs. The statistical sub-model is the same for models A and B in the sense that there is only one random effect, η_{10} or $\bar{\eta}_{20}$, and this random effect is associated only with the elimination parameter, θ_{10} or $\bar{\theta}_{20}$, in the two models.

In model A, the drug is eliminated from compartment 1 and has the following model structure

$$\begin{aligned} \dot{x}_1 &= -(\theta_{12} + \theta_{10}e^{\eta_{10}})x_1 + \theta_{21}x_2 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ x_1(0) &= D \\ x_2(0) &= 0 \end{aligned} \tag{7.1}$$

with observation

$$y = x_1 \tag{7.2}$$

with known initial conditions and with the following unknown structural parameter vector

$$\boldsymbol{\theta} = (\theta_{10}, \theta_{12}, \theta_{21}) \tag{7.3}$$

with the random effect η_{10} being normally distributed as

$$\eta_{10} \sim N(0, \sqrt{\omega_{10}}) \tag{7.4}$$

where ω_{10} is the unknown variance parameter.

In model B (7.5) the drug is instead eliminated from compartment 2 and has the following model structure

$$\begin{aligned} \dot{x}_1 &= -\bar{\theta}_{12}x_1 + \bar{\theta}_{21}x_2 \\ \dot{x}_2 &= \bar{\theta}_{12}x_1 - (\bar{\theta}_{21} + \bar{\theta}_{20}e^{\bar{\eta}_{20}})x_2. \\ x_1(0) &= D \\ x_2(0) &= 0 \end{aligned} \tag{7.5}$$

with observation

$$y = x_1 \tag{7.6}$$

with known initial conditions and with the following unknown structural parameter vector

$$\bar{\boldsymbol{\theta}} = (\bar{\theta}_{10}, \bar{\theta}_{12}, \bar{\theta}_{21}) \tag{7.7}$$

and with the random effect $\bar{\eta}_{20}$ being normally distributed as

$$\bar{\eta}_{20} \sim N(0, \sqrt{\bar{\omega}_{20}}) \tag{7.8}$$

where $\bar{\omega}_{20}$ is the unknown variance parameter. In Section 7.2 it will be shown that

these two non-mixed-effects are structurally indistinguishable but that the mixed-effects versions analysed are structurally distinguishable..

7.1.2 Example 2: Two three-compartment models

In the first example, the two models had different structural sub-models but the same statistical sub-model. In this second example the two models in Figure 7.2, model C and model D are mathematically equivalent where the only difference between them is the labelling of compartment 2 and 3. It is therefore expected that the subsequent analysis would show that model C and model D are indistiguishable given that the methods applied are sound. For model C, a random effect η_{31} is associated with the structural parameter θ_{31}, while for model D the random effect $\bar{\eta}_{21}$ is associated with the structural parameter $\bar{\theta}_{21}$. The model structure for model C is therefore given by

$$\begin{aligned} \dot{x}_1 &= -(\theta_{12} + \theta_{13} + \theta_{10})x_1 + \theta_{21}x_2 + \theta_{31}e^{\eta_{31}}x_3 \\ \dot{x}_2 &= \theta_{12}x_1 - \theta_{21}x_2 \\ \dot{x}_3 &= \theta_{13}x_1 - \theta_{31}e^{\eta_{31}}x_3 \\ x_1(0) &= D \\ x_2(0) &= 0 \\ x_3(0) &= 0 \end{aligned} \tag{7.9}$$

with observation

$$y = x_1 \tag{7.10}$$

with known initial conditions and with the unknown structural model parameter vector

$$\boldsymbol{\theta} = (\theta_{12}, \theta_{13}, \theta_{21}, \theta_{31}, \theta_{10}) \tag{7.11}$$

with the random effect η_{31} being normally distributed as

$$\eta_{31} \sim N(0, \sqrt{\omega_{31}}) \tag{7.12}$$

where ω_{31} is the unknown variance parameter. The structure for model D is given by

$$\begin{aligned}
\dot{x}_1 &= -(\bar{\theta}_{12} + \bar{\theta}_{13} + \bar{\theta}_{10})x_1 + \bar{\theta}_{21}e^{\bar{\eta}_{21}}x_2 + \bar{\theta}_{31}x_3 \\
\dot{x}_2 &= \bar{\theta}_{12}x_1 - \bar{\theta}_{21}e^{\bar{\eta}_{21}}x_2 \\
\dot{x}_3 &= \bar{\theta}_{13}x_1 - \bar{\theta}_{31}x_3 \\
x_1(0) &= D \\
x_2(0) &= 0 \\
x_3(0) &= 0
\end{aligned} \tag{7.13}$$

with the observation

$$y = x_1 \tag{7.14}$$

with known initial conditions and with the unknown model parameter vector

$$\bar{\boldsymbol{\theta}} = (\bar{\theta}_{12}, \bar{\theta}_{13}, \bar{\theta}_{21}, \bar{\theta}_{31}, \bar{\theta}_{10}). \tag{7.15}$$

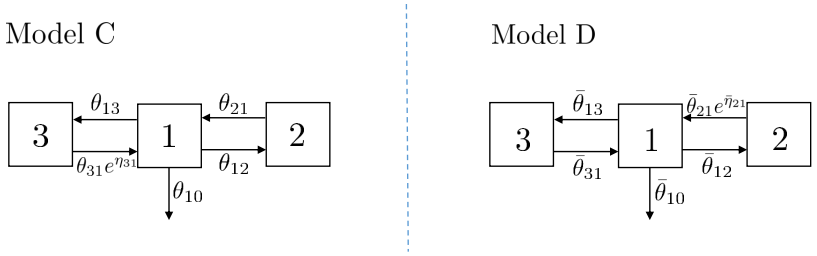
with the random effect $\bar{\eta}_{21}$ being normally distributed as

$$\bar{\eta}_{21} \sim N(0, \sqrt{\bar{\omega}_{21}}) \tag{7.16}$$

where $\bar{\omega}_{21}$ is the unknown variance parameter.

These two linear mixed-effects models will now be analysed from a structural indistinguishability perspective using the following three methods: The Laplace transform approach, the Taylor series approach and the input-output form approach.

Figure 7.2: Model structures of the two linear three-compartment mixed-effects models C and model D.



7.2 Laplace transform approach

In this section the Laplace transform approach, outlined in section 4.4.1, will be applied to analyse whether the two pairs of mixed-effects models A-B and C-D are structurally indistinguishable respectively. In order for two mixed-effects models to be structurally indistinguishable the distribution of the system moment invariants must be equal.

7.2.1 Example 1

The transfer function for the structural sub-model of model A (7.1) is given by

$$G(s) = \frac{s + \theta_{21}}{s^2 + (\theta_{10} + \theta_{12} + \theta_{21})s + \theta_{10}\theta_{21}}. \tag{7.17}$$

The transfer function for the structural sub-model of model B (7.5) is given by

$$\bar{G}(s) = \frac{s + \bar{\theta}_{21} + \bar{\theta}_{20}}{s^2 + (\bar{\theta}_{20} + \bar{\theta}_{21} + \bar{\theta}_{12})s + \bar{\theta}_{12}\bar{\theta}_{20}}. \tag{7.18}$$

From the transfer functions $G(s)$ and $\bar{G}(s)$ in (7.17) and (7.18) the system moment invariants can be derived from their respective coefficients. Model A (7.1) has the

following system moment invariants:

$$\sigma_1 = \theta_{21} \tag{7.19}$$

$$\sigma_2 = \theta_{21}\theta_{10} \tag{7.20}$$

$$\sigma_3 = \theta_{10} + \theta_{12} + \theta_{21}. \tag{7.21}$$

The moment invariants for model B (7.5) are given by

$$\bar{\sigma}_1 = \bar{\theta}_{20} + \bar{\theta}_{21} \tag{7.22}$$

$$\bar{\sigma}_2 = \bar{\theta}_{12}\bar{\theta}_{20} \tag{7.23}$$

$$\bar{\sigma}_3 = \bar{\theta}_{12} + \bar{\theta}_{21} + \bar{\theta}_{20}. \tag{7.24}$$

To analyse whether the non-mixed-effects versions of model A and model B are structurally indistinguishable or otherwise their system moment invariants are equated as

$$\sigma_1 = \bar{\sigma}_1 \tag{7.25}$$

$$\sigma_2 = \bar{\sigma}_2 \tag{7.26}$$

$$\sigma_3 = \bar{\sigma}_3 \tag{7.27}$$

and thus generating the following equation system

$$\theta_{21} = \bar{\theta}_{20} + \bar{\theta}_{21} \tag{7.28}$$

$$\theta_{21}\theta_{10} = \bar{\theta}_{12}\bar{\theta}_{20} \tag{7.29}$$

$$\theta_{10} + \theta_{12} + \theta_{21} = \bar{\theta}_{12} + \bar{\theta}_{21} + \bar{\theta}_{20}. \tag{7.30}$$

Combining equation (7.28) with (7.29) yields

$$\theta_{10} = \frac{\bar{\theta}_{12}\bar{\theta}_{20}}{\bar{\theta}_{20} + \bar{\theta}_{21}}. \tag{7.31}$$

Combining equations (7.28)–(7.29) yields

$$\theta_{12} = \bar{\theta}_{21} - \frac{\bar{\theta}_{12}\bar{\theta}_{20}}{\bar{\theta}_{20} + \bar{\theta}_{21}} = \frac{\bar{\theta}_{12}\bar{\theta}_{21}}{\bar{\theta}_{20} + \bar{\theta}_{21}}. \tag{7.32}$$

A generic relation, equations (7.28),(7.31)–(7.32), between the parameters from the two non-mixed-effects version of model A and model B can be derived and the two models are therefore structurally indistinguishable.

Now the mixed-effects version model A (7.1) will be considered. The corresponding functions of random variables are given by

$$Z_1 = \theta_{21} \tag{7.33}$$

$$Z_2 = \theta_{21}\theta_{10}e^{\eta_{10}} \tag{7.34}$$

$$Z_3 = \theta_{10}e^{\eta_{10}} + \theta_{12} + \theta_{21}. \tag{7.35}$$

The functions of random variables for model B (7.5) are given by

$$\bar{Z}_1 = \bar{\theta}_{20}e^{\bar{\eta}_{20}} + \bar{\theta}_{21} \tag{7.36}$$

$$\bar{Z}_2 = \bar{\theta}_{12}\bar{\theta}_{20}e^{\bar{\eta}_{20}} \tag{7.37}$$

$$\bar{Z}_3 = \bar{\theta}_{12} + \bar{\theta}_{21} + \bar{\theta}_{20}e^{\bar{\eta}_{20}}. \tag{7.38}$$

By equating the second statistical moments of Z_1 and $\bar{Z}_1$ as

$$\mathbb{E}[Z_1^2] = \mathbb{E}[\bar{Z}_1^2] \tag{7.39}$$

the following relation is obtained:

$$0 = \bar{\theta}_{20}^2 e^{\bar{\omega}_{20}} \left(e^{\bar{\omega}_{20}} - 1\right) \tag{7.40}$$

which only holds if at least one of the following relations is true

$$\bar{\omega}_{20} = 0 \tag{7.41}$$

or

$$\bar{\theta}_{20} = 0. \tag{7.42}$$

In other words, since at least one of the above relations must hold in order for the distributions of Z_1 and Z_2 to be equal, no non-zero generic parameter relation between model A (7.1) and model B (7.5) can be established for this particular choice of statistical sub-model. Therefore, it can be concluded that the two mixed-effects models A (7.1) and B (7.5) are structurally distinguishable.

7.2.2 Example 2

Since the structural sub-model is the same for model C (7.9) and model D (7.13), the transfer function for both models is also the same, namely

$$G(s) = \frac{s^2 + \beta_1 s + \beta_2}{s^3 + \beta_3 s^2 + \beta_4 s + \beta_5}. \tag{7.43}$$

with the macro parameters

$$\beta_1 = \theta_{21} + \theta_{31} \tag{7.44}$$

$$\beta_2 = \theta_{31}\theta_{21} \tag{7.45}$$

$$\beta_3 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31} \tag{7.46}$$

$$\beta_4 = \theta_{21}(\theta_{10} + \theta_{13}) + \theta_{31}(\theta_{10} + \theta_{12} + \theta_{21}) \tag{7.47}$$

$$\beta_5 = \theta_{10}\theta_{21}\theta_{31} \tag{7.48}$$

The system moment invariants are given by

$$\sigma_1 = \theta_{21}\theta_{31} \tag{7.49}$$

$$\sigma_2 = \theta_{21} + \theta_{31} \tag{7.50}$$

$$\sigma_3 = \theta_{10}\theta_{21}\theta_{31} \tag{7.51}$$

$$\sigma_4 = \theta_{10}\theta_{21} + \theta_{10}\theta_{31} + \theta_{12}\theta_{31} + +\theta_{13}\theta_{21} + \theta_{21}\theta_{31} \tag{7.52}$$

$$\sigma_5 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31}. \tag{7.53}$$

By including the statistical sub-model, the functions of random variables for model C (7.9) are given by

$$Z_1 = \theta_{21}\theta_{31}e^{\eta_{31}} \tag{7.54}$$

$$Z_2 = \theta_{21} + \theta_{31}e^{\eta_{31}} \tag{7.55}$$

$$Z_3 = \theta_{10}\theta_{21}\theta_{31}e^{\eta_{31}} \tag{7.56}$$

$$Z_4 = \theta_{10}\theta_{21} + \theta_{10}\theta_{31}e^{\eta_{31}} + \theta_{12}\theta_{31}e^{\eta_{31}} + \theta_{13}\theta_{21} + \theta_{21}\theta_{31}e^{\eta_{31}} \tag{7.57}$$

$$Z_5 = \theta_{10} + \theta_{12} + \theta_{13} + \theta_{21} + \theta_{31}e^{\eta_{31}} \tag{7.58}$$

and for model D (7.13) the functions of random variables are given by

$$\bar{Z}_1 = \bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{31} \tag{7.59}$$

$$\bar{Z}_2 = \bar{\theta}_{21}e^{\bar{\eta}_{21}} + \bar{\theta}_{31} \tag{7.60}$$

$$\bar{Z}_3 = \bar{\theta}_{10}\bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{31} \tag{7.61}$$

$$\bar{Z}_4 = \bar{\theta}_{10}\bar{\theta}_{21}e^{\eta_{21}} + \bar{\theta}_{10}\bar{\theta}_{31} + \bar{\theta}_{12}\bar{\theta}_{31} + +\bar{\theta}_{13}\bar{\theta}_{21}e^{\bar{\eta}_{21}} + \bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{31} \tag{7.62}$$

$$\bar{Z}_5 = \bar{\theta}_{10} + \bar{\theta}_{12} + \bar{\theta}_{13} + \bar{\theta}_{21}e^{\bar{\eta}_{21}} + \bar{\theta}_{31}. \tag{7.63}$$

By equating the first and second statistical moments of Z_1 and $\bar{Z}_1$ as

$$\mathbb{E}[Z_1] = \mathbb{E}[\bar{Z}_1] \tag{7.64}$$

$$\mathbb{E}[Z_1^2] = \mathbb{E}[\bar{Z}_1^2] \tag{7.65}$$

the following two equations are obtained

$$\theta_{21}\theta_{31}e^{\frac{\omega_{31}}{2}} = \bar{\theta}_{21}\bar{\theta}_{31}e^{\frac{\bar{\omega}_{21}}{2}} \tag{7.66}$$

$$\theta_{21}^2\theta_{31}^2e^{\omega_{31}}\left(e^{\omega_{31}}-1\right) = \bar{\theta}_{21}^2\bar{\theta}_{31}^2e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}}-1\right) \tag{7.67}$$

from which it can be shown that the two variance parameters must be equal, i.e.,

$$\omega_{31} = \bar{\omega}_{21} \tag{7.68}$$

and that the following relation must hold

$$\theta_{21}\theta_{31} = \bar{\theta}_{21}\bar{\theta}_{31}. \tag{7.69}$$

By equating the second statistical moments of Z_2 and $\bar{Z}_2$ as

$$\mathbb{E}[Z_2^2] = \mathbb{E}[\bar{Z}_2^2] \tag{7.70}$$

the following equation is obtained

$$\theta_{31}^2e^{\omega_{31}}(e^{\omega_{31}}-1) = \bar{\theta}_{21}^2e^{\bar{\omega}_{21}}(e^{\bar{\omega}_{21}}-1) \tag{7.71}$$

which has, using the previous result (7.68), the following unique solution

$$\theta_{31} = \bar{\theta}_{21}. \tag{7.72}$$

Combining the two equations (7.72) and (7.69) yields

$$\theta_{21} = \bar{\theta}_{31}. \tag{7.73}$$

Equating the first statistical moments of Z_3 and $\bar{Z}_3$ as

$$\mathbb{E}[Z_3] = \mathbb{E}[\bar{Z}_3] \tag{7.74}$$

yields the following equation

$$\theta_{10}\theta_{21}\theta_{31}e^{\frac{\omega_{31}}{2}} = \bar{\theta}_{10}\bar{\theta}_{21}\bar{\theta}_{31}e^{\frac{\bar{\omega}_{21}}{2}} \tag{7.75}$$

which by using the previous results (7.68), (7.72) and (7.73) can be shown to have the following unique solution

$$\theta_{10} = \bar{\theta}_{10}. \tag{7.76}$$

Equating the second statistical moment of Z_4 and $\bar{Z}_4$

$$\mathbb{E}[Z_4^2] = \mathbb{E}[\bar{Z}_4^2] \tag{7.77}$$

yields the following equation

$$\left(\theta_{10}+\theta_{12}+\theta_{21}\right)^2\theta_{31}^2e^{\omega_{31}}\left(e^{\omega_{31}}-1\right) = \bar{\theta}_{21}^2e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}}-1\right)\left(\bar{\theta}_{10}+\bar{\theta}_{13}+\bar{\theta}_{31}\right)^2 \tag{7.78}$$

which, using the previous results (7.68), (7.72), (7.73) and (7.76) can be shown to have the following unique solution

$$\theta_{12} = \bar{\theta}_{13}. \tag{7.79}$$

Lastly, equating the first statistical moment of Z_5 and $\bar{Z}_5$ as

$$\mathbb{E}[Z_5^2] = \mathbb{E}[\bar{Z}_5^2] \tag{7.80}$$

yields the following equation

$$\theta_{10}+\theta_{12}+\theta_{13}+\theta_{21}+\theta_{31}e^{\frac{\omega_{31}}{2}} = \bar{\theta}_{10}+\bar{\theta}_{12}+\bar{\theta}_{13}+\bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}}+\bar{\theta}_{31} \tag{7.81}$$

which, using the previous results (7.68), (7.72), (7.73), (7.76) and (7.79) can be

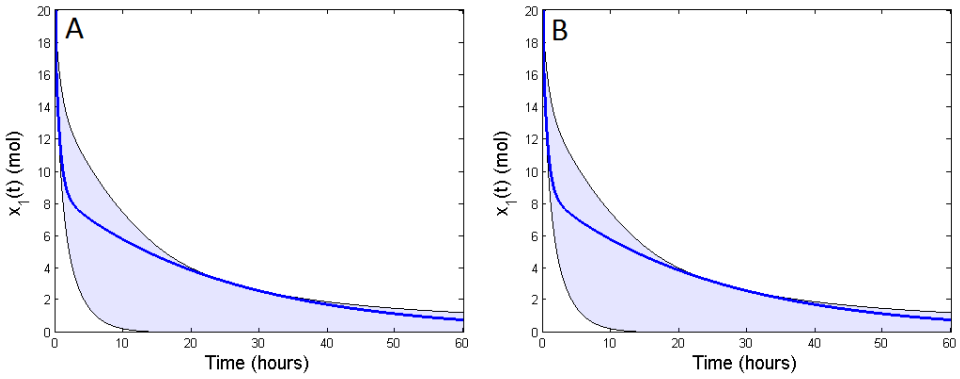


Figure 7.3: Simulation of model (7.9) and (7.13) using the parameter values in Table 7.1. The thick line is the simulation for the typical subject and the shaded area is the 95-percentile, both of which are identical in A and B and thus illustrating the analytical result that the two mixed-effects models are only different in terms of labelling and therefore structurally indistinguishable.

shown to have the following unique solution

$$\theta_{13} = \bar{\theta}_{12}. \tag{7.82}$$

In summary, if the following generic parameter relations hold

$$\theta_{10} = \bar{\theta}_{10} \tag{7.83}$$

$$\theta_{12} = \bar{\theta}_{13} \tag{7.84}$$

$$\theta_{21} = \bar{\theta}_{31} \tag{7.85}$$

$$\theta_{13} = \bar{\theta}_{12} \tag{7.86}$$

$$\theta_{31} = \bar{\theta}_{21} \tag{7.87}$$

$$\omega_{31} = \bar{\omega}_{21}. \tag{7.88}$$

then the output function from the two models C (7.9) and D (7.13) are identical and they are therefore structurally indistinguishable. A numerical example of this analytical result can be seen in Figure 7.3 using the parameter values in Table 7.1.

Table 7.1: Parameter values used when simulating the two mixed-effects models (7.9) and (7.13). The simulations can be seen in Figure 7.3.

Model	θ_{10}	θ_{12}	θ_{13}	θ_{21}	θ_{31}	ω_{31}	ω_{21}
A	0.09	0.3	0.5	0.9	0.6	5	-
B	0.09	0.5	0.3	0.6	0.9	-	5

7.3 Taylor series expansion approach

In this section the Taylor series expansion approach, outlined in Section 4.4.2, will be applied to analyse whether the two pairs of mixed-effects models A-B and C-D are structurally indistinguishable respectively.

For two non-mixed-effects models to be structurally indistinguishable all coefficients in the Taylor series expansion must be equal. For two mixed-effects models this means that the distribution of the coefficients in the Taylor series expansion from two models must be equal in order for them to be structurally indistinguishable.

7.3.1 Example 1

The first two coefficients in the Taylor series expansion for Model A (7.1) are given by

$$y(0) = D \tag{7.89}$$

$$\dot{y}(0) = D\left(-\theta_{10} - \theta_{12}\right). \tag{7.90}$$

The first two coefficients in the Taylor series expansion for model B (7.5) are given by

$$y(0) = D \tag{7.91}$$

$$\dot{y}(0) = -D\bar{\theta}_{12}. \tag{7.92}$$

The corresponding functions of random variables are therefore given by

$$Z_1 = D \tag{7.93}$$

$$Z_2 = D\left(-\theta_{10}e^{\eta_{10}} - \theta_{12}\right) \tag{7.94}$$

$$\bar{Z}_1 = D \tag{7.95}$$

$$\bar{Z}_2 = -D\bar{\theta}_{12}. \tag{7.96}$$

By computing the second statistical moments of Z_2 and $\bar{Z}_2$ and equating them as

$$\mathbb{E}[Z_2^2] = \mathbb{E}[\bar{Z}_2^2] \tag{7.97}$$

the following relation is obtained:

$$D^2\theta_{10}^2 e^{\omega_{10}}\left(e^{\omega_{10}} - 1\right) = 0 \tag{7.98}$$

which is only true if at least one of the following relations

$$\omega_{10} = 0 \tag{7.99}$$

$$\theta_{12} = 0 \tag{7.100}$$

hold given that $D \neq 0$. Therefore no non-zero generic parameter relations can be derived between the two mixed-effects models and they are therefore structurally distinguishable.

7.3.2 Example 2

Since the structural sub-model is the same for models C and D the Taylor series expansion coefficients are the same for both models. The first three coefficients are

given by

$$y(0) = D \tag{7.101}$$

$$\dot{y}(0) = D\left(-\theta_{10} - \theta_{12} - \theta_{13}\right) \tag{7.102}$$

$$\ddot{y}(0) = D\left(-\theta_{10} - \theta_{12} - \theta_{13}\right)^2 + D\theta_{12}\theta_{21} + D\theta_{13}\theta_{31}. \tag{7.103}$$

The functions of random variables derived from the first three coefficients in the Taylor series expansion of model C are given by

$$Z_1 = D \tag{7.104}$$

$$Z_2 = D\left(-\theta_{10} - \theta_{12} - \theta_{13}\right) \tag{7.105}$$

$$Z_3 = D\left(-\theta_{10} - \theta_{12} - \theta_{13}\right)^2 + D\theta_{12}\theta_{21} + D\theta_{13}\theta_{31}e^{\eta_{31}}. \tag{7.106}$$

The functions of random variables derived from the first three coefficients in the Taylor series expansion of model D (7.13) are given by

$$\bar{Z}_1 = D \tag{7.107}$$

$$\bar{Z}_2 = D\left(-\bar{\theta}_{10} - \bar{\theta}_{12} - \bar{\theta}_{13}\right) \tag{7.108}$$

$$\bar{Z}_3 = D\left(-\bar{\theta}_{10} - \bar{\theta}_{12} - \bar{\theta}_{13}\right)^2 + D\bar{\theta}_{12}\bar{\theta}_{21}e^{\bar{\eta}_{21}} + D\bar{\theta}_{13}\bar{\theta}_{31}. \tag{7.109}$$

By equating the second statistical moments of Z_3 and $\bar{Z}_3$ as

$$\mathbb{E}[Z_3^2] = \mathbb{E}[\bar{Z}_3^2] \tag{7.110}$$

the following equation is obtained

$$D^2\theta_{13}^2\theta_{31}^2e^{\omega_{31}}\left(e^{\omega_{31}} - 1\right) = D^2\bar{\theta}_{12}^2\bar{\theta}_{21}^2e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}} - 1\right). \tag{7.111}$$

It has been previously shown that the linear three compartment model is structurally locally identifiable where one of the solutions is given by

$$\theta_{13} = \bar{\theta}_{12} \tag{7.112}$$

$$\theta_{31} = \bar{\theta}_{21}. \tag{7.113}$$

Since the structural sub-model is the same in both cases a structural identifiability result is the same as a structural indistinguishability result. Using this relation together with (7.111) it is clear that the two variance parameters must be equal, i.e.,

$$\omega_{31} = \bar{\omega}_{21}. \tag{7.114}$$

A generic parameter relation between model C and D exists and it can therefore be concluded that they are structurally indistinguishable.

7.4 Input-Output approach

In this section the input-output approach, outlined in Section 4.4.3, is applied to analyse whether the two pairs of mixed-effects models A-B and C-D are structurally indistinguishable respectively. For two mixed-effects models to be structurally indistinguishable the distribution of all coefficients in the input-output relation must be equal.

7.4.1 Example 1

Model A (7.1) rewritten in an input-output form together with the initial conditions yields the following expressions

$$\ddot{y} + (\theta_{12} + \theta_{10} + \theta_{21})\dot{y} + \theta_{21}\theta_{10}y = 0 \tag{7.115}$$

$$y(0) = D \tag{7.116}$$

and

$$\dot{y}(0) = -D(\theta_{12} + \theta_{10}). \tag{7.117}$$

Model B (7.5) rewritten in an input-output form together with the initial conditions yields the following expressions

$$\ddot{y} + (\bar{\theta}_{12} + \bar{\theta}_{21} + \bar{\theta}_{20})\dot{y} + \bar{\theta}_{20}\bar{\theta}_{12}y = 0 \tag{7.118}$$

$$y(0) = D \tag{7.119}$$

$$\dot{y}(0) = -D\bar{\theta}_{12}. \tag{7.120}$$

The corresponding functions of random variables for model A are given by

$$Z_1 = \theta_{12} + \theta_{10}e^{\eta_{10}} + \theta_{21} \tag{7.121}$$

$$Z_2 = \theta_{21}\theta_{10}e^{\eta_{10}} \tag{7.122}$$

$$Z_3 = \theta_{12} + \theta_{10}e^{\eta_{10}}. \tag{7.123}$$

The corresponding functions of random variables for model B are given by

$$\bar{Z}_1 = \bar{\theta}_{12} + \bar{\theta}_{21} + \bar{\theta}_{20}e^{\bar{\eta}_{20}} \tag{7.124}$$

$$\bar{Z}_2 = \bar{\theta}_{20}e^{\bar{\eta}_{20}}\bar{\theta}_{12} \tag{7.125}$$

$$\bar{Z}_3 = \bar{\theta}_{12}. \tag{7.126}$$

Computing the second statistical moments of Z_3 and $\bar{Z}_3$ and equating them as

$$\mathbb{E}[Z_3^2] = \mathbb{E}[\bar{Z}_3^2] \tag{7.127}$$

yields the following relation

$$\theta_{10}^2 e^{\omega_{10}} \left(e^{\omega_{10}} - 1\right) = 0 \tag{7.128}$$

which only holds if either

$$\omega_{10} = 0 \tag{7.129}$$

or

$$\theta_{10} = 0 \tag{7.130}$$

hold. Again, since no generic parameter relationship can be derived between model A (7.1) and model B (7.5) it can be concluded that the two models are structurally distinguishable.

7.4.2 Example 2

Model C (7.9) and model D (7.13) have the same structural sub-model and therefore also the same input-output form, namely

$$y^{(3)} + (\theta_{21} + \theta_{10} + \theta_{12} + \theta_{13} + \theta_{31})\ddot{y} + (\theta_{21}\theta_{13} + \theta_{21}\theta_{10} + \theta_{21}\theta_{31} + \theta_{31}\theta_{12} + \theta_{31}\theta_{10})\dot{y} + \theta_{21}\theta_{31}\theta_{10}y = 0. \tag{7.131}$$

The initial conditions for both model C (7.9) and model D (7.13) are given by

$$y(0) = D \tag{7.132}$$

$$\dot{y}(0) = -D(\theta_{12} + \theta_{10} + \theta_{13}) \tag{7.133}$$

$$\ddot{y}(0) = D\left((\theta_{12} + \theta_{10} + \theta_{13})^2 + \theta_{12}\theta_{21} + \theta_{13}\theta_{31}\right). \tag{7.134}$$

The functions of random variables for model C (7.9) are given by

$$Z_1 = \theta_{21} + \theta_{10} + \theta_{12} + \theta_{13} + \theta_{31}e^{\eta_{31}} \tag{7.135}$$

$$Z_2 = \theta_{21}\theta_{13} + \theta_{21}\theta_{10} + \theta_{21}\theta_{31}e^{\eta_{31}} + \theta_{31}e^{\eta_{31}}\theta_{12} + \theta_{31}e^{\eta_{31}}\theta_{10} \tag{7.136}$$

$$Z_3 = \theta_{21}\theta_{31}e^{\eta_{31}}\theta_{10} \tag{7.137}$$

$$Z_4 = \theta_{12} + \theta_{10} + \theta_{13} \tag{7.138}$$

$$Z_5 = (\theta_{12} + \theta_{10} + \theta_{13})^2 + \theta_{12}\theta_{21} + \theta_{13}\theta_{31}e^{\eta_{31}}. \tag{7.139}$$

The functions of random variables for model D (7.13) are given by

$$\bar{Z}_1 = \bar{\theta}_{21}e^{\bar{\eta}_{21}} + \bar{\theta}_{10} + \bar{\theta}_{12} + \bar{\theta}_{13} + \bar{\theta}_{31} \tag{7.140}$$

$$\bar{Z}_2 = \bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{13} + \bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{10} + \bar{\theta}_{21}e^{\eta_{21}}\bar{\theta}_{31} + \bar{\theta}_{31}\bar{\theta}_{12} + \bar{\theta}_{31}\bar{\theta}_{10} \tag{7.141}$$

$$\bar{Z}_3 = \bar{\theta}_{21}e^{\bar{\eta}_{21}}\bar{\theta}_{31}\bar{\theta}_{10} \tag{7.142}$$

$$\bar{Z}_4 = \bar{\theta}_{12} + \bar{\theta}_{10} + \bar{\theta}_{13} \tag{7.143}$$

$$\bar{Z}_5 = (\bar{\theta}_{12} + \bar{\theta}_{10} + \bar{\theta}_{13})^2 + \bar{\theta}_{12}\bar{\theta}_{21}e^{\bar{\eta}_{21}} + \bar{\theta}_{13}\bar{\theta}_{31}. \tag{7.144}$$

Computing the second statistical moments of Z_1 and $\bar{Z}_1$ and equating them yields the following relationships

$$\theta_{31}^2 e^{\omega_{31}} \left(e^{\omega_{31}} - 1\right) = \bar{\theta}_{21}^2 e^{\bar{\omega}_{21}} \left(e^{\bar{\omega}_{21}} - 1\right). \tag{7.145}$$

Computing the second statistical moments of Z_5 and $\bar{Z}_5$ and equating them yields the following relationships

$$\theta_{13}^2\theta_{31}^2 e^{\omega_{31}} \left(e^{\omega_{31}} - 1\right) = \bar{\theta}_{12}^2\bar{\theta}_{21}^2 e^{\bar{\omega}_{21}} \left(e^{\bar{\omega}_{21}} - 1\right). \tag{7.146}$$

Combining (7.145) and (7.146) yields the following solution

$$\theta_{13} = \bar{\theta}_{12}. \tag{7.147}$$

Computing the first and second statistical moments of Z_3 and $\bar{Z}_3$ and equating them yields the following relationship

$$\theta_{10}\theta_{21}\theta_{31}e^{\frac{\omega_{31}}{2}} = \bar{\theta}_{10}\bar{\theta}_{21}\bar{\theta}_{31}e^{\frac{\omega_{21}}{2}} \tag{7.148}$$

$$\theta_{10}^2\theta_{21}^2\theta_{31}^2e^{\omega_{31}}\left(e^{\omega_{31}}-1\right) = \bar{\theta}_{10}^2\bar{\theta}_{21}^2\bar{\theta}_{31}^2e^{\bar{\omega}_{21}}\left(e^{\bar{\omega}_{21}}-1\right). \tag{7.149}$$

which has the following single solution

$$\omega_{31} = \bar{\omega}_{21} \tag{7.150}$$

Combining (7.146), (7.147) and (7.150) yields

$$\theta_{31} = \bar{\theta}_{21}. \tag{7.151}$$

Computing the first statistical moments of Z_4 and $\bar{Z}_4$ and equating them yields the following relationship

$$\theta_{12}+\theta_{10}+\theta_{13} = \bar{\theta}_{12}+\bar{\theta}_{10}+\bar{\theta}_{13}. \tag{7.152}$$

Computing the first statistical moments of Z_1 and $\bar{Z}_1$ and equating them yields the following relationship

$$\theta_{21}+\theta_{10}+\theta_{12}+\theta_{13}+\theta_{31}e^{\frac{\omega_{31}}{2}} = \bar{\theta}_{21}e^{\frac{\bar{\omega}_{21}}{2}}+\bar{\theta}_{10}+\bar{\theta}_{12}+\bar{\theta}_{13}+\bar{\theta}_{31}. \tag{7.153}$$

Combining the previous results (7.150), (7.151), (7.147) and (7.152) yields the following solution

$$\theta_{21} = \bar{\theta}_{31} \tag{7.154}$$

Combining (7.150), (7.151) and (7.154) in (7.148) yields

$$\theta_{10} = \bar{\theta}_{10}. \tag{7.155}$$

Combining (7.147) and (7.155) in (7.152) yields

$$\theta_{12} = \bar{\theta}_{13}. \tag{7.156}$$

Therefore, the following generic parameter relations between model C (7.9) and model D (7.13) hold

$$\theta_{12} = \bar{\theta}_{13} \tag{7.157}$$

$$\theta_{13} = \bar{\theta}_{12} \tag{7.158}$$

$$\theta_{21} = \bar{\theta}_{31} \tag{7.159}$$

$$\theta_{31} = \bar{\theta}_{21} \tag{7.160}$$

$$\theta_{10} = \bar{\theta}_{10} \tag{7.161}$$

$$\omega_{31} = \bar{\omega}_{21} \tag{7.162}$$

and the two mixed-effects models are structurally indistinguishable.

7.5 Summary

Three methods developed within this thesis to study the structural indistinguishability of mixed-effects models have been applied to two example cases where a pairwise indistinguishability analysis has been performed. Although both structural sub-models that were analysed are linear, the methods can be applied to nonlinear structural sub-models as well. In a non-mixed-effects case, it is possible to exhaustively generate all linear models that are structructurally indistinguishable. However in the mixed-effects case it is not known whether this is possible for mixed-effects models where the structural sub-model is linear.

In the first example, the structural sub-model is different for model A (7.1) and model B (7.5) while the statistical model is the same in the sense that the single random effect is associated with the structural parameter representing the rate of elimination in both models. A structural indistinguishability analysis was

performed with the three developed methods which all yielded the same conclusion, namely that model A (7.1) and model B (7.5) are structurally distinguishable since no generic parameter relation exists between the two models.

In the second example, the structural sub-models in model C (7.9) and model D (7.13) are identical while the statistical sub-models are different. The structural indistinguishability analysis with all three methods concluded that model C (7.9) and model D (7.13) are structurally indistinguishable as a generic parameter relation between them could be derived.

From the examples presented, it is clear that structural indistinguishability results do not necessarily translate from the non-mixed-effects case to the mixed-effects case. In the non-mixed-effects case model A (7.1) and model B (7.5) are structurally indistinguishable. However, by introducing a random effect to the elimination parameter in the two models the two models become structurally distinguishable, i.e., the two models have transformed from being structurally indistinguishable to structurally distinguishable. This is an important insight as it illustrates the importance of performing structural indistinguishability analysis on the whole mixed-effects model and not only on the structural sub-model.

Finally, similar to non-mixed-effects models, depending on the method used the difficulty of showing whether or not two mixed-effects models are structurally indistinguishable may vary in complexity and tractability. For some models analysed by the Taylor series approach, it may become apperent early in the analysis that no non-zero generic relation between the parameters from the analysed models can be established, e.g., it is apperant already in the first or second coefficient in the Taylor series expansion. But for the same set of models but applying the input-output approach might give an inconclusive result if the generating the input-output form of the different models is computionally intractable. However, depending on the structure of the analysed models, it could also be the other way around, i.e., computing the necessary coefficients in the Taylor series expansion approach is computionally intractable but the input-output form can be generated. In addition, the Laplace transform approach is limited to analyse structural indistinguishability between lin-

ear models.

Chapter 8

Conclusions

8.1 Introduction

As has been discussed previously, structural identifiability is an important concept when modelling dynamical systems. In a biological system the majority of the different parts often interact with each other in a highly nonlinear way. This makes mathematical modelling a highly appropriate and often necessary approach in order to be able to characterise the key components within the system of interest. In a PKPD context, such characteristics often include numerical estimates of the model parameters following parameter estimation when fitting the model to experimental data. These numerical estimates often have a biological interpretation such as bioavailability, saturation levels, the clearance rate of a drug from the body etc. In PKPD modelling projects, it is not uncommon to use such parameter values as a guide to further improve the formulation of a pharmaceutical drug under development. Unfortunately, structural identifiability is often overlooked when making, or attempting to make, such interpretations of the model parameters even though there are numerous published methods on the topic. The main reason why this is the case is most likely because performing such an analysis often requires a different skill set than those necessary to undertake the modelling.

For mixed-effects models, no analytical approaches to study structural identifiability prior to this thesis existed. In other words, it has not been possible to

study whether the unknown parameters in a mixed-effects model can be uniquely determined or otherwise. The lack of such methods in turn means that there has been no way of knowing, except for possibly including some prior biological knowledge about the system, e.,g., upper/lower limits or relations between some of the model parameters, whether parameters in a given mixed-effects model have valid biological interpretation or not.

The following bullet points summarise the outcomes and insights from this thesis in relation to the aims and objectives as declared in Section 1.1.

- Formal, more general, definitions of structural identifiability and structural indistinguishability have been introduced which include mixed-effects models formulations.

- Analytical methods applicable to the study of structural identifiability and structural indistinguishability of mixed-effects models have been developed.

- A set of mixed-effects models has for the first time been analysed analytically in both a structural identifiability and structural indistinguishability context using the developed methods.

- By analysing mixed-effects models using the developed methods it has been shown that neither structural identifiability nor indistinguishability results necessarily translate from the non-mixed-effects case to the mixed-effects case.

- It has been shown that the identifiability of a mixed-effects model is dependent on all three components of the statistical sub-model, i.e., where the random effects enter into the structural sub-model, the form of the distribution of the random effects and the structure of the corresponding covariance matrix.

- A set of non-mixed-effects models has been analysed using previously established structural identifiability analysis techniques. This was done in collaboration with the other IMPACT EU projects in which the models were then used.

In this thesis, the concepts of structural identifiability and indistinguishability have been generalised to include mixed-effects models. Following on from these new, more general definitions, a set of analytical methods applicable to the study of mixed-effects models has been developed. The strengths and limitations of these methods will be discussed in detail below. In addition, suggestions for future work will be provided.

8.2 Methods introduced

The repeated measurement approach presented in Section 4.3.1 and Theorems 1–4 relies on certain structures or combinations of model parameters appearing in the model identifiability analysis. In models where these structures do not exist the repeated measurement approach can not be used to study structural identifiability. It is also necessary that the parameter estimation includes parallel models with some parameters shared, such as in (5.1) and (5.3) in Section 5.2.1. In modelling patient variability, another scenario could be, for instance, a difference in the distribution of the bioavailability parameter F between males or females while the remaining model parameters belong to the same distribution for both males and females. Finally, Theorems 1–4 assume that the random variables are independent and therefore offer no insight into how to handle covariance between the model parameters.

Augmenting the original system to a random differential equation system form is in some sense a more general approach to the structural identifiability problem for mixed-effects models compared to the repeated measurement approach. This is due to the following three reasons:

i) In the augmented system approach the problem of structural identifiability can instead be regarded as an observability problem. If the augmented system is observable, then the original system is structurally identifiable since all of the model parameters are included in the initial conditions.

ii) In contrast to the repeated measurement approach, any model structure may be considered. This includes any form of covariance between any two random

effects.

iii) In contrast to the repeated measurement approach, if the model structure is unidentifiable the augmented system approach still informs on which parameters are identifiable/unidentifiable and also on the parameter combinations that are structurally identifiable. However, it is worth noting that, even for very simple model structures, the expressions to be evaluated quickly grow in complexity. In addition, this method requires finding the system solution $\boldsymbol{q}(\cdot)$ which may become computationally very expensive.

The Laplace transform, the Taylor series expansion and the input-output form approaches for the study of the structural identifiability of mixed-effects models are similar in the sense that they are all used to generate functions of random variables via generating an exhaustive summary. However, there are a few differences between the approaches that should be mentioned.

The Laplace transform approach is only applicable to linear systems while the Taylor series expansion and the input-output form approaches are applicable to both linear and nonlinear systems. The Taylor series expansion suffers from computational problems even for relatively simple model structures. The input-output approach can handle more complex model structures than the Taylor series approach, but there is still a limit as to how complex the models can be in order for an analytical approach to be feasible. With the input-output approach it is necessary to check for linear independence among the terms in the input-output relationship. This can be done by computing the relevant Wronskian determinant (Denis-Vidal et al. [2001]), a potentially computationally demanding task. A structural identifiability analysis for a mixed-effects system is often more computationally demanding than its corresponding non-mixed-effects system since the use of random effects introduces the covariance matrix $\boldsymbol{\Omega}$ with unknown variance parameters, and with a non-diagonal covariance matrix, additional unknown covariance parameters. Nevertheless, the presented methods are still useful since many mixed-effects models used within the pharmaceutical industry are relatively simple in structure and dimension as illustrated by the many examples presented in this thesis.

All methods presented in this thesis analyzes mixed-effects model analytically. The advantage with an analytical approach, compareed to a numerical approach, is that the outcome from a structural identifiability or indistinguishability analysis is proven mathematically. With a numerical approach, there is always a potential risk of generating false results both due to approximations and introduction of small error in matrices computations as well as the fact that only a finite number of observations can be considered, compared to the analytical case where an infite number of observations is assumed. In Shivva et al. [2013], a numerical approach to study structural identifiability in mixed-effects models is presented. Their method is based on an information theoretic approach applicable to mixed-effects models centered around the Fisher information matrix (Mentre et al. [1997]; Retout and Mentre [2003]). In order to compare and contrast this numerical approach to analytical approaches presented in this thesis, the Bateman model analyzed in Shivva et al. [2013] was considered. However, the analysis was inconclusive since one of the functions of random variables generated from the exhaustive summary had a non-standard distribution, meaning that no upper limit on how many higher orders of statistical moments are necessary to prove structural local identifiability or unidentifiability was known. A number of higher orders of statistical moments were derived but the subsequent algebraic computation was too demanding computer memory wise. This is therefore a good example of where a numerical approach could be of use if analytical approaches prove to be computationally infeasible. It should still be noted that, in contrast to the analytical approaches presented in this thesis, in the approach presented in Shivva et al. [2013] there is no obvious way in the case of unidentifiability to *i)* Show what additional measurements and/or inputs are required to achieve identifiability *ii)* Derive the functional form of identifiable combinations of parameters.

8.3 Structural identifiability and indistinguishability does not necessarily translate from non-mixed-effects models to mixed-effects models

An important insight regarding both structural identifiability and structural indistinguishability that has come out of this thesis is the fact that results from such analysis of non-mixed-effects models do not necessarily translate to the mixed-effects case.

In the simplest case, i.e., combining a structurally globally identifiable structural sub-model and an identifiable statistical sub-model, intuition tells us that the resulting mixed-effects model must also be structurally globally identifiable. This follows directly from the fact that if an infinite number of subjects have a unique parameter solution, i.e., a structurally globally identifiable structural sub-model, then there exists a unique distribution that describes all of the individual parameters for which the parameterisation is uniquely identifiable. However, if a structurally locally or unidentifiable structural sub-model is combined with an identifiable statistical sub-model then the question as to whether the resulting mixed-effects models is structurally globally, locally or unidentifiable becomes non-trivial. By exploiting the methods developed in this thesis it has been possible to show using examples that even if a structural sub-model is locally identifiable or unidentifiable the resulting mixed-effects model can be structurally globally or locally identifiable depending on the statistical sub-model used. These insights are collected in Conjectures 1–3 in Chapter 6.

Similarily, by using the methods developed in this thesis it was possible to analyse mixed-effects models in an indistinguishability context. By using examples, it was shown that structural sub-models which are structurally indistinguishable may become distinguishable in a mixed-effects framework depending on where the random effects enter the system. It was also shown that two mixed-effects models with identical structural sub-models, but with different statistical sub-models, can still be structurally indistinguishable.

8.4 Future potential impact

The new, more general, definition of structural identifiability and indistinguishability and the developed methods introduced in this thesis open up the possibility of studying the structural identifiability of mixed-effects models analytically. This could potentially have a big impact on modelling in the pharmaceutical industry since such types of models are routinely used. Knowing whether the structural and variance parameters are structurally identifiable or otherwise increases the confidence in the model parameter estimates, leaving only the experimental data as a source of uncertainty for the model parameters. This is perhaps especially important when modelling PKPD-relationships since parameters in such models are often subject to biological interpretations, e.g., clearance (CL) or potency (EC_{50}) of a pharmaceutical drug. The presented methods should thus ideally also be integrated into the model development process as well as experimental design in order to offer support while answering various experimental questions, e.g., "what to measure?" or "what administration routes to use in order to ensure structural identifiability?".

The methods presented in this thesis are generic by nature, i.e., not limited to population modelling in pharmacology. The methods can be applied to models used in any field where mixed-effects modelling is used, e.g., ecology.

8.4.1 Experimental design and model building

As mentioned in the introduction of the thesis and in Figure 2.4, structural identifiability and indistinguishability analysis should be an integrated part of the experimental design and model building.

This is because a structural identifiability analysis shows whether the model parameters can be uniquely determined or otherwise from a particular experiment design. If the model parameters can be determined uniquely then it is theoretically sound to draw biological interpretations and conclusions from the parameter estimates. If an identifiability analysis has shown that some of the model parameters cannot be determined given a particular experimental setup, alternative input and

output functions can be considered in a new identifiability analysis and the results from such an analysis could then inform the experimental design. Alternatively, such an analysis could also inform the model building in order to achieve identifiability, e.g., showing that some of the model parameters must be fixed to literature values if they cannot be determined in that particular model structure, or that the complexity of the model needs to be reduced. In practice, since there is a limitation on how both the input and output can be designed, the outcome from an identifiability analysis could potentially affect both the experimental design and the model building.

For structural indistinguishability the analysis includes a set of candidate models all representing the biological system of interest. Often, the candidate models corresponds to the different hyphothesis of the underlying structure of the system. Such an analysis informs whether a particular experimental setup is sufficient in a structural sense in order to be able to distinguish between the different candidate models.

How structural identifiability and indistinguishability can be used for both experimental design and model building for non-mixed-effects models holds true also for mixed-effects models. In terms of model building, it has been shown in this thesis that unidentifiable non-mixed-effects models can become identifiable as mixed-effects models. Taking the one-compartment absorption model as an example, the mixed-effects version with a logit-distribution of the bioavailability parameter should be chosen over the non-mixed-effects model. In addition to what structural model to use, a modeller needs to in a mixed-effects framework decide on how to design the statistical sub-model; what population parameters should have an associated random effect, what postulated form of distribution should the different random effects have and what structure of the covariance matrix should be used. The approaches presented in this thesis are to be considered as a tool for model building of the statistical model as well as the structural model. Different combinations of structural models and statistical sub-models together with different sets of input and output functions can be considered using the methods presented in this thesis

and they can thus help influence and decide on both experimental design and the model building in such a way that experiments are done in an optimal way with regards to learning as much as possible on the system of interest.

8.5 Future work

A future research topic that has arisen out of this work is with regard to the possible existence of upper and lower bounds on the order of the statistical moments of the functions of random variables needed in order to determine structural identifiability. Consider the case of some function of random variables Z_i being either normally, or lognormally distributed. The first two moments fully characterise the distribution and the upper and lower bound is therefore two. This was the case in the last example in Section 5.2.5 where both of the functions of random variables (5.102)–(5.103) are lognormally distributed and therefore only the first two statistical moments were considered. As the required relevant upper bound for structural identifiability was reached it was possible to conclude that the model is structurally unidentifiable. For a non-standard distribution for Z_i, the lower bound is the same as the number of unknown parameters in Z_i, given that those unknown parameters do not appear in any other Z_j where $j \neq i$. However, deriving an upper bound for non-standard distributions will almost certainly require dividing them up into different groups of distributions, a task for ongoing research. A consequence of having unknown upper bounds is that it makes proving that a model is structurally unidentifiable impossible, since the upper bound for the required number of moments is not known. A natural topic for future work following from this thesis is therefore to further study the potential existence of upper bounds of different distributions, in order to be able to prove structural unidentifiability of mixed-effects models.

Regarding the translatability of identifiability and indistinguishability results when going from a non-mixed-effects model to a mixed-effects model three conjectures, Conjectures 1–3, summarised the insights of the work on this topic in this thesis. However, these conjectures are based on the examples provided and therefore

providing a more general mathematical proof for these is a natural topic for future work.

For linear non-mixed-effects models, there are approaches available to generate all model structures that are structurally indistinguishable (Bonate [2011]). Future work related to this might be to explore whether or not it is possible to generate all linear mixed-effects models that are structurally indistinguishable. Such research could perhaps be first limited to only consider models where the random effect η_i enters linearly into the structural sub-model, i.e.,

$$\phi_i = \theta + \eta_i \tag{8.1}$$

and once that special case is understood then to start considering when the random effects η_i enter into the structural sub-model in a nonlinear fashion, e.g.,

$$\phi_i = \theta e^{\eta_i}. \tag{8.2}$$

In Chapter 4.3.2 the augmented system approach was presented as a way of analysing mixed-effects models in a structural identifiability context. A potential avenue that was left unexplored in this thesis which is related to this approach is observability. For non-mixed-effects models written in an extended state-space form, i.e., the model parameters are written as states with zero time-derivatives, observability implies identifiability. In the augmented system approach all model parameters are written in such an extended state-space form. Due to this, expanding the observability concept to mixed-effects models could therefore potentially offer yet another approach to study the structural identifiability of mixed-effects models.

In the augmented system approach presented in this thesis in Chapter 4.3.2, the system is rewritten in an extended state-space form. Therefore, although no explicit approach is presented, it is still worth mentioning that an alternative to considering the moments of the output function $y(t)$ is to instead combine existing observability tests such as the *Observability Rank Criterion* (Hermann and Krener [1977]) together with the joint density function of the initial conditions $p_0(\boldsymbol{x}_0)$ to

determine whether the system is observable or not and therefore determine whether the system is structurally identifiable or otherwise.

Appendix A

Pharmacodynamic model

The following Maple-code was used to analyse the 16 pharmacodynamic models in Chapter 3.

with(*LinearAlgebra*) : *with*(*Groebner*) :

Forsman Code

```
lieDer :=proc(H, F, vars)
  local V :
  V := map((a, b)→diff(b, a), vars, H) :
  DotProduct(Vector(F), Vector(V), conjugate = false)
 end:

listLieDer := proc(H, F, k)
  local L, i, tmp, N, vars :
  L := [y[0] − H] : tmp := H :
  N := nops(F) ;
  vars := [seq(x[t], t = 1 .. N)] ;
  for i to k do
    tmp := lieDer(tmp, F, vars) :
    L := [op(L), y[i]-tmp]
  od;
 end:

xlistLieDer := proc(H, F, k, uvars)
  local L, i, f, h, tmp, N, var, vars, duvars ;
  N := nops(F) ;
  f := F ;
  h := H ;
  for var in uvars do
    f := subs(var = var[0], f) ;
    h := subs(var = var[0], h) ;
  od;
  duvars := [ ] ;
  vars := [seq(x[t], t = 1..N)] ;
  for var in uvars do
    vars := [op(vars), seq(var[i], i = 0..10)] ;
    duvars := [op(duvars), seq(var[i], i = 1..11)] ;
  od;
  f := [op(f), op(duvars)] ;
  L := [y[0]-h]; tmp := h ;
  i := 1 ;
  for i to k do
    tmp := lieDer(tmp, f, vars) ;
    L := [op(L), y[i]-tmp] ;
   od;
 end:

iorel := proc(f, h, uvars)
  local n, L :
  n := nops(f) :
  if _params['uvars'] = NULL then
    L := listLieDer(h, f, n) :
```

```
  else
    L := xlistLieDer(h, f, n, uvars) :
  fi;
  L := map(expand, numer(L)) :
  UnivariatePolynomial(y[n], L, [seq(x[t], t = 1..n), y[n]]) ;
end:
```

Model 2 (sigmoid, augmented, m is here the parameter in the exponent in the original system.)

Defining the system as:

$$F := \left[-x[1],\ \frac{m \cdot k[d] \cdot x[2]}{k[d] + x[1]} \right]$$

$$\left[-x_1,\ \frac{m\, k_d\, x_2}{k_d + x_1} \right] \tag{1}$$

$$H := \frac{E[maxx]}{x[2] \cdot RC[50] + 1} ;$$

$$\frac{E_{maxx}}{x_2\, RC_{50} + 1} \tag{2}$$

$$outptEqn := iorel(F, H)$$

$$-m\, y_1\, y_0\, E_{maxx} + m\, E_{maxx}\, y_0\, y_2 - m\, E_{maxx}\, y_1^2 + m\, y_1\, y_0^2 - m\, y_2\, y_0^2 + 2\, m\, y_0\, y_1^2 - E_{maxx}\, y_1^2 \tag{3}$$

Collecting the coefficients and setting up expression with alternative parameter vector

$$uA := \{coeffs(collect(outptEqn, [y[0], y[1], y[2]], 'distributed'), [y[0], y[1], y[2]])\}$$

$$\{m,\ m\, E_{maxx},\ -m,\ 2\, m,\ -m\, E_{maxx},\ -m\, E_{maxx} - E_{maxx}\} \tag{4}$$

$$uB := eval(uA, [m = mb, E[maxx] = Eb[maxx], RC[50] = RCb[50], k[d] = kb[d]])$$

$$\{mb,\ mb\, Eb_{maxx},\ -mb,\ 2\, mb,\ -mb\, Eb_{maxx},\ -mb\, Eb_{maxx} - Eb_{maxx}\} \tag{5}$$

$$eqns := convert(uA, list) - convert(uB, list)$$

$$[-mb + m,\ -mb\, Eb_{maxx} + m\, E_{maxx},\ mb - m,\ -2\, mb + 2\, m,\ mb\, Eb_{maxx} - m\, E_{maxx},\ mb\, Eb_{maxx} + Eb_{maxx} - m\, E_{maxx} - E_{maxx}] \tag{6}$$

Setting up the equations for the inital conditions

$$n := 2 :$$

Calculating the derivative of output function y, then evaluating the expressions for the initial conditions

$$icsnoteval := eval(-1 * listLieDer(H, F, n - 1), [seq(y[i] = 0, i = 0 .. n - 1)])$$

$$\left[\frac{E_{maxx}}{x_2\, RC_{50} + 1},\ -\frac{m\, k_d\, x_2\, E_{maxx}\, RC_{50}}{(k_d + x_1)\,(x_2\, RC_{50} + 1)^2} \right] \tag{7}$$

$$icseval := eval(icsnoteval, \{x[1] = Dose, x[2] = a0\})$$

$$\left[\frac{E_{maxx}}{a0\,RC_{50}+1},\ -\frac{m\,k_d\,a0\,E_{maxx}\,RC_{50}}{\left(k_d+Dose\right)\,\left(a0\,RC_{50}+1\right)^2}\right] \tag{8}$$

Introducing alternative parameter vector and finalizing the expression for the initial conditions

$icsA := Vector(icseval);$
$icsB := eval(icsA, [m = mb, E[maxx] = Eb[maxx], RC[50] = RCb[50], k[d] = kb[d]]);$

$$\left[\begin{array}{c}\frac{E_{maxx}}{a0\,RC_{50}+1}\\ -\frac{m\,k_d\,a0\,E_{maxx}\,RC_{50}}{\left(k_d+Dose\right)\,\left(a0\,RC_{50}+1\right)^2}\end{array}\right] \tag{9}$$

$ics := [icsA(1) - icsB(1), icsA(2) - icsB(2)]$

$$\left[\frac{E_{maxx}}{a0\,RC_{50}+1}-\frac{Eb_{maxx}}{a0\,RCb_{50}+1},\ -\frac{m\,k_d\,a0\,E_{maxx}\,RC_{50}}{\left(k_d+Dose\right)\,\left(a0\,RC_{50}+1\right)^2}+\frac{mb\,kb_d\,a0\,Eb_{maxx}\,RCb_{50}}{\left(kb_d+Dose\right)\,\left(a0\,RCb_{50}+1\right)^2}\right] \tag{10}$$

$solve([op(ics), op(eqns)], [m, E[maxx], RC[50], k[d]])$

$$[[m = mb, E_{maxx} = Eb_{maxx}, RC_{50} = RCb_{50}, k_d = kb_d]] \tag{11}$$

Summary: The model is globally identifiable.

Appendix B

Five-compartment lung model

The Mathematica code used for analysing the five-compartment lung PK model can be found below.

Structural Identifiability Analysis of a 5-compartment PK lung model

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IV dosing

$$
\begin{aligned}
\text{deqIV} = \{ & \\
& A1'[t] == \frac{A2[t]}{V2} * fu2 * CLD12 + \frac{A4[t] * fu4}{V4} * CLD14 - \\
& \quad \frac{A1[t]}{V1} * CL - \frac{A1[t]}{V1} * fu1 * CLD12 - \frac{A1[t]}{V1} * fu1 * CLD14 + u[t], \\
& A2'[t] == K32 * A3[t] + CLD12 * fu1 * \frac{A1[t]}{V1} - \\
& \quad \frac{A2[t]}{V2} * fu2 * CLD12 - \frac{A2[t]}{V2} * fu2 * CLD23, \\
& A3'[t] == fu2 * CLD23 * \frac{A2[t]}{V2} - K32 * A3[t], \\
& A4'[t] == \\
& \quad K32 * A5[t] + CLD14 * fu1 * \frac{A1[t]}{V1} - \frac{A4[t] * fu4}{V4} * CLD14 - \frac{A4[t] * fu4}{V4} * CLD45, \\
& A5'[t] == CLD45 * \frac{A4[t] * fu4}{V4} - K32 * A5[t] \\
\}; &
\end{aligned}
$$

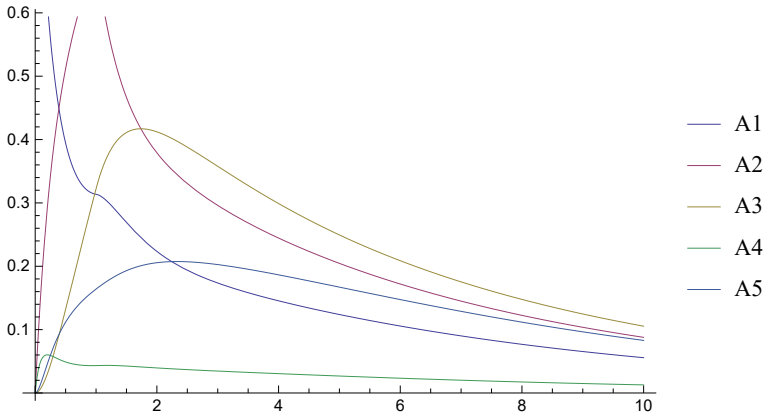
IT dosing

```
deqIT = {
    A1'[t] == A2[t]/V2 * fu2 * CLD12 + A4[t] * fu4/V4 * CLD14 -
      A1[t]/V1 * CL - A1[t]/V1 * fu1 * CLD12 - A1[t]/V1 * fu1 * CLD14,
    A2'[t] == K32 * A3[t] + CLD12 * fu1 * A1[t]/V1 - A2[t]/V2 * fu2 * CLD12 -
      A2[t]/V2 * fu2 * CLD23 + u[t],
    A3'[t] == fu2 * CLD23 * A2[t]/V2 - K32 * A3[t],
    A4'[t] ==
     K32 * A5[t] + CLD14 * fu1 * A1[t]/V1 - A4[t] * fu4/V4 * CLD14 - A4[t] * fu4/V4 * CLD45,
    A5'[t] == CLD45 * A4[t] * fu4/V4 - K32 * A5[t],
  };
```

Solving and simulating the system using arbitrary chosen model parameters

```
params = {V1 → 1, V2 → 1, V4 → 2, fu4 → 11, CL → 1, fu1 → 1,
    fu2 → 1, CLD12 → 1, CLD14 → 1, CLD45 → 1, K32 → 1, CLD23 → 1};
ic = {A1[0] == 1, A2[0] == 0, A3[0] == 0, A4[0] == 0, A5[0] == 0};
sol = NDSolve[{deqIV, ic} /. params /. u[t] → Piecewise[{{1, t < 1}}],
     {A1, A2, A3, A4, A5}, {t, 0, 10}][[1]];

Plot[Evaluate[{A1[t], A2[t], A3[t], A4[t], A5[t]} /. sol],
 {t, 0, 10}, PlotLegends → {A1, A2, A3, A4, A5}]
```



The system seems to be properly defined.

Defining output function, model states, initial conditions and model parameters

```
modelStates = {A1, A2, A3, A4, A5};
ic = {A1[0] == 0, A2[0] == 0, A3[0] == 0, A4[0] == 0, A5[0] == 0};
observationVectorReal = {(A2[t] + A3[t]) / V2, A1[t] / V1};
modelParameters =
  {V1, V2, V4, fu4, CL, fu1, fu2, CLD12, CLD14, CLD45, K32, CLD23};
```

Structural Identifiability Analysis

IV dosing

```
iad = IdentifiabilityAnalysis[
  {{deqIV, ic}, observationVectorReal}, modelStates, modelParameters, t, u]
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
summaryIV = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{CLD12, CLD14, CLD23, CLD45, fu1, fu2, fu4, V4}, 2}
```

This shows that the model is structurally unidentifiable with IV dosing.

IT dosing

```
iad = IdentifiabilityAnalysis[
  {{deqIT, ic}, observationVectorReal}, modelStates, modelParameters, t, u]
summaryIT = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
{{CLD12, CLD14, CLD23, CLD45, fu1, fu2, fu4, V4}, 2}
```

This shows that the model is structurally unidentifiable with IT dosing.

A direct inspection of the model equations shows that the parameters fu4 and V4 always appears as a ratio fu4/V4 and should therefore be replaced with a new parameter fu4V4.

IV dosing : reperametrised

```
deqReparamIV = {
   A1'[t] == A2[t]/V2 * fu2 * CLD12 + A4[t] * fu4V4 * CLD14 -
     A1[t]/V1 * CL - A1[t]/V1 * fu1 * CLD12 - A1[t]/V1 * fu1 * CLD14 + u[t],
   A2'[t] == K32 * A3[t] + CLD12 * fu1 * A1[t]/V1 -
     A2[t]/V2 * fu2 * CLD12 - A2[t]/V2 * fu2 * CLD23 ,
   A3'[t] == fu2 * CLD23 * A2[t]/V2 - K32 * A3[t],
   A4'[t] ==
    K32 * A5[t] + CLD14 * fu1 * A1[t]/V1 - A4[t] * fu4V4 * CLD14 - A4[t] * fu4V4 * CLD45,
   A5'[t] == CLD45 * A4[t] * fu4V4 - K32 * A5[t]
  };
```

IT dosing : reperametrised

```
deqReparamIT = {
   A1'[t] == A2[t]/V2 * fu2 * CLD12 + A4[t] * fu4V4 * CLD14 -
     A1[t]/V1 * CL - A1[t]/V1 * fu1 * CLD12 - A1[t]/V1 * fu1 * CLD14,
   A2'[t] == K32 * A3[t] + CLD12 * fu1 * A1[t]/V1 - A2[t]/V2 * fu2 * CLD12 -
     A2[t]/V2 * fu2 * CLD23 + u[t],
   A3'[t] == fu2 * CLD23 * A2[t]/V2 - K32 * A3[t],
   A4'[t] ==
    K32 * A5[t] + CLD14 * fu1 * A1[t]/V1 - A4[t] * fu4V4 * CLD14 - A4[t] * fu4V4 * CLD45,
   A5'[t] == CLD45 * A4[t] * fu4V4 - K32 * A5[t],
  };
```

Defining output function, model states, initial conditions and reparameterized model

```
modelStates = {A1, A2, A3, A4, A5};
ic = {A1[0] == 0, A2[0] == 0, A3[0] == 0, A4[0] == 0, A5[0] == 0};
observationVectorReal = {(A2[t] + A3[t])/V2, A1[t]/V1};
modelParameters2 =
  {V1, V2, fu4V4, CL, fu1, fu2, CLD12, CLD14, CLD45, K32, CLD23};
```

Structural Identifiability Analysis

IV dosing

```
iad = IdentifiabilityAnalysis[{{deqReparamIV, ic}, observationVectorReal},
  modelStates, modelParameters2, t, u]
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
summaryReparamIV = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{CLD12, CLD14, CLD23, CLD45, fu1, fu2, fu4V4}, 1}
```

This shows that the reparametrized model is structurally unidentifiable with IV dosing.

IT dosing

```
iad = IdentifiabilityAnalysis[{{deqReparamIT, ic}, observationVectorReal},
  modelStates, modelParameters2, t, u]
summaryIT = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
{{CLD12, CLD14, CLD23, CLD45, fu1, fu2, fu4V4}, 1}
```

This shows that the reparametrized model is structurally unidentifiable with IT dosing.

If any of the parameters {CLD12,CLD14,CLD23,CLD45,fu1,fu2,fu4V4} is fixed then the model is at least locally structurally identifiable. For instance, if the parameter fu1 is set to 1 we get the following system

IV dosing, fu1 = 1

```
deqReparamIVfu1fixed = {
   A1'[t] == A2[t]/V2 * fu2 * CLD12 + A4[t] * fu4V4 * CLD14 -
     A1[t]/V1 * CL - A1[t]/V1 * CLD12 - A1[t]/V1 * CLD14 + u[t],
   A2'[t] == K32 * A3[t] + CLD12 * A1[t]/V1 - A2[t]/V2 * fu2 * CLD12 - A2[t]/V2 * fu2 * CLD23 ,
   A3'[t] == fu2 * CLD23 * A2[t]/V2 - K32 * A3[t],
   A4'[t] ==
    K32 * A5[t] + CLD14 * A1[t]/V1 - A4[t] * fu4V4 * CLD14 - A4[t] * fu4V4 * CLD45,
   A5'[t] == CLD45 * A4[t] * fu4V4 - K32 * A5[t]
  };
```

IT dosing

```
deqReparamITfu1fixed = {
    A1'[t] == A2[t]/V2 * fu2 * CLD12 +
      A4[t] * fu4V4 * CLD14 - A1[t]/V1 * CL - A1[t]/V1 * CLD12 - A1[t]/V1 * CLD14,
    A2'[t] == K32 * A3[t] + CLD12 * A1[t]/V1 - A2[t]/V2 * fu2 * CLD12 -
      A2[t]/V2 * fu2 * CLD23 + u[t],
    A3'[t] == fu2 * CLD23 * A2[t]/V2 - K32 * A3[t],
    A4'[t] ==
     K32 * A5[t] + CLD14 * A1[t]/V1 - A4[t] * fu4V4 * CLD14 - A4[t] * fu4V4 * CLD45,
    A5'[t] == CLD45 * A4[t] * fu4V4 - K32 * A5[t],
   };
```

Defining output function, model states, initial conditions and reparameterized model

```
modelStates = {A1, A2, A3, A4, A5};
ic = {A1[0] == 0, A2[0] == 0, A3[0] == 0, A4[0] == 0, A5[0] == 0};
observationVectorReal = {(A2[t] + A3[t])/V2, A1[t]/V1};
modelParameters2fu1fixed =
  {V1, V2, fu4V4, CL, fu2, CLD12, CLD14, CLD45, K32, CLD23};
```

Structural Identifiability Analysis

IV dosing

```
iad = IdentifiabilityAnalysis[
  {{deqReparamIVfu1fixed, ic}, observationVectorReal},
  modelStates, modelParameters2fu1fixed, t, u]
```

```
IdentifiabilityAnalysisData[True, <>]
```

```
summaryReparamIVfu1fixed =
 {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{}, 0}
```

This shows that the reparametrized model with fu1 fixed is at least structurally locally identifiable with IV dosing.

IT dosing

```
iad = IdentifiabilityAnalysis[
  {{deqReparamITfu1fixed, ic}, observationVectorReal},
  modelStates, modelParameters2fu1fixed, t, u]
summaryIT = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
IdentifiabilityAnalysisData[True, <>]
```

```
{{}, 0}
```

This shows that the reparametrized model with fu1 fixed is at least structurally locally identifiable with IT dosing.

Appendix C

Example code

The following Mathematica code is an example of how a structural identifiability analysis of a mixed-effects model can done.

Two-compartment model in a mixed-effect framework

This model is known to be globally identifiable in a deterministic form.

```
Am = {{-(θk12 + θk1e), θk21}, {θk12, -θk21}};
Bm = {{1}, {0}};
Cm = {θc1, 0};

nmb = 2;
G = Simplify[Cm.Inverse[s * IdentityMatrix[nmb] - Am].Bm];
momentInvariantsnumerator = CoefficientList[Numerator[G], s];
momentInvariantsdenominator = CoefficientList[Denominator[G], s];

parametervector = {θc1, θk1e, θk12, θk21};
replacevector = {θc1 → θc1b, θk1e → θk1eb, θk12 → θk12b, θk21 → θk21b};
momentInvariantsnumeratorb = momentInvariantsnumerator /. replacevector;
momentInvariantsdenominatorb = momentInvariantsdenominator /. replacevector;

Solve[{momentInvariantsnumerator[[]], momentInvariantsdenominator[[]]} ==
  {momentInvariantsnumeratorb[[]],
   momentInvariantsdenominatorb[[]]}, parametervector]
```

$$\{\{\theta_{c1} \to \theta_{c1b},\ \theta_{k1e} \to \theta_{k1eb},\ \theta_{k12} \to \theta_{k12b},\ \theta_{k21} \to \theta_{k21b}\}\}$$

The population parameters are structurally globally identifiable. The moment invariants, as calculated above, are

```
momentInvariant1 = θc1;
momentInvariant2 = θc1 θk21;
momentInvariant3 = θk1e θk21;
momentInvariant4 = θk12 + θk1e + θk21;

Z1 = TransformedDistribution[θc1 * Exp[ηc1],
    {ηc1} é MultinormalDistribution[{0}, {{ωc1}}]];
Z2 = TransformedDistribution[θc1 * Exp[ηc1] * θk21 * Exp[ηk21],
    {ηc1, ηk21} é MultinormalDistribution[{0, 0}, {{ωc1, 0}, {0, ωK21}}]];
Z3 = TransformedDistribution[θk1e * Exp[ηk1e] * θk21 * Exp[ηk21],
    {ηk1e, ηk21} é MultinormalDistribution[{0, 0}, {{ωK1e, 0}, {0, ωK21}}]];
Z4 = TransformedDistribution[θk1e * Exp[ηk1e] + θk12 * Exp[ηk12] + θk21 * Exp[ηk21],
    {ηk1e, ηk12, ηk21} é MultinormalDistribution[
      {0, 0, 0}, {{ωK1e, 0, 0}, {0, ωK12, 0}, {0, 0, ωK21}}]];

ExpectedValueZ1 = Mean[Z1];

Assuming[{ωbarc1 > 0}, Simplify[
  Solve[ExpectedValueZ1 == {ExpectedValueZ1 /. {ωc1 → ωbarc1}}, ωc1, Reals]]]
```

$$\{\{\omega_{c1} \to \omega bar_{c1}\}\}$$

Conclusion: The variance of parameter c1 is globally identifiable as only positive values are allowed.

Note: Not solving for ω_{C1} as it has been shown above to be globally identifiable.

```
ExpectedValueZ2 = Mean[Z2];
```

```
Solve[ExpectedValueZ2 == {ExpectedValueZ2 /. {ω_K21 → ωbar_K21}}, ω_K21, Reals]
```

```
{{ω_K21 → ωbar_K21}}
```

Conclusion: The variance of parameter k21 is globally identifiable as only positive values are allowed.

Note: Not solving for ω_{k21} as it has been shown above to be globally identifiable.

```
ExpectedValueZ3 = Mean[Z3];
```

```
Solve[ExpectedValueZ3 == {ExpectedValueZ3 /. {ω_K1e → ωbar_K1e}}, ω_K1e, Reals]
```

```
{{ω_K1e → ωbar_K1e}}
```

Conclusion: The variance of parameter k1e is globally identifiable as only positive values are allowed.

Note: Not solving for ω_{k1e} or ω_{k21} as they have been shown above to be globally identifiable.

```
ExpectedValueZ4 = Mean[Z4];
```

```
Assuming[{ωbar_K12 > 0}, Simplify[
  Solve[ExpectedValueZ4 == {ExpectedValueZ4 /. {ω_K12 → ωbar_K12}}, ω_K12, Reals]]]
```

```
{{ω_K12 → ωbar_K12}}
```

Conclusion: The variance of parameter k12 is globally identifiable as only positive values are allowed.

Summary: All population parameters and their variances are structurally globally identifiable.

Appendix D

Input estimation

This is the Mathematica code used to generate the identifiability results for the input estimation section in Chapter 3.

Structural Identifiability Analysis of PK-model Jusko et al.

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Structural Identifiability Analysis: Bolus dose

Defining model structure, output function, model states, initial conditions and model parameters

```
deqJuskoBolusIV = {
    Cc'[t] == -(kel + kpt) * Cc[t] + ktp * AT[t]/Vc - kon * (Rtot - RC[t]) * Cc[t] + koff * RC[t],
    AT'[t] == kpt * Cc[t] * Vc - ktp * AT[t],
    RC'[t] == kon * (Rtot - RC[t]) * Cc[t] - (koff - kint) * RC[t]
   };
modelStates = {Cc, AT, RC};
icBolusIV = {Cc[0] == Dose/Vc, AT[0] == 0, RC[0] == 0};
modelParameters = {kel, kpt, ktp, Vc, kon, koff, kint, Rtot};
observationVectorReal = {Cc[t]};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[
  {{deqJuskoBolusIV, icBolusIV}, observationVectorReal} /.
   {Dose → RandomReal[{0, 50}]}, modelStates, modelParameters, t, u]
```

```
IdentifiabilityAnalysisData[True, <>]
```

Conclusion: The model is at least structurally locally identifiable.

Structural Identifiability Analysis: IV infusion

Defining model structure, output function, model states, initial conditions and model parameters

```
deqJuskoIV = {
    Cc'[t] ==
     u[t]/Vc - (kel + kpt) * Cc[t] + ktp * AT[t]/Vc - kon * (Rtot - RC[t]) * Cc[t] + koff * RC[t],
    AT'[t] == kpt * Cc[t] * Vc - ktp * AT[t],
    RC'[t] == kon * (Rtot - RC[t]) * Cc[t] - (koff - kint) * RC[t]
   };
modelStates = {Cc, AT, RC};
icIV = {Cc[0] == 0, AT[0] == 0, RC[0] == 0};
modelParameters = {kel, kpt, ktp, Vc, kon, koff, kint, Rtot};
observationVectorReal = {Cc[t]};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[{{deqJuskoIV, icIV}, observationVectorReal} /.
    {Dose → RandomReal[{0, 50}]}, modelStates, modelParameters, t, u]
```

```
IdentifiabilityAnalysisData[True, <>]
```

```
summaryIVnobolus = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{}, 0}
```

Conclusion: The model is at least structurally locally identifiable.

Structural Identifiability Analysis: SC

Defining model structure, output function, model states, initial conditions and model parameters

```
deqJuskoSC = {
    Cc'[t] == ka * xsc[t]/Vc - (kel + kpt) * Cc[t] +
      ktp * AT[t]/Vc - kon * (Rtot - RC[t]) * Cc[t] + koff * RC[t],
    AT'[t] == kpt * Cc[t] * Vc - ktp * AT[t],
    RC'[t] == kon * (Rtot - RC[t]) * Cc[t] - (koff - kint) * RC[t],
    xsc'[t] == -ka * xsc[t]
   };
modelStatesSC = {Cc, AT, RC, xsc};
icSC = {Cc[0] == 0, AT[0] == 0, RC[0] == 0, xsc[0] == F * Dose};
modelParametersSC = {kel, kpt, ktp, Vc, kon, koff, kint, Rtot, F, ka};
observationVectorReal = {Cc[t]};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[
  {{deqJuskoSC, icSC}, observationVectorReal} /. {Dose → RandomReal[{0, 50}]},
  modelStatesSC, modelParametersSC, t, u]
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
summaryIVnobolus = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{F, Vc}, 1}
```

Conclusion: The parameter F and V are unidentifiable and the model is therefore unidentifiable.

If either F or V are fixed, the model is at least structurally locally identifiable. Since all model parameters are shared between the IV and SC experiments the volume parameter V can be fixed, see below

```
modelParametersSCFfix = {kel, kpt, ktp, kon, koff, kint, F, Rtot, ka};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[{{deqJuskoSC, icSC}, observationVectorReal} /.
    {Dose → RandomReal[{0, 50}], Vc → RandomReal[{0, 50}]},
  modelStatesSC, modelParametersSCFfix, t, u]
```

```
IdentifiabilityAnalysisData[True, <>]
```

Conclusion: With parameter V known from the IV experiment all model parameters are at least structurally locally identifiable.

Structural Identifiability Analysis of extended release model Li et al.

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Case 1: All model parameters assumed unknown

Defining output function, model states, initial conditions and model parameters

```
deqLi = {
    a1'[t] == -ktr * a1[t],
    a2'[t] == ktr * (a1[t] - a2[t]) ,
    a3'[t] == ktr * (a2[t] - a3[t]),
    a4'[t] == ktr * (a3[t] - a4[t]),
    a5'[t] == ktr * a4[t] - ka * a5[t],
    a6'[t] == ka * a5[t] - Q/V1 * a6[t] -
      CL/V1 * a6[t] + Q/V2 * a7[t] - kon * a8[t] * a6[t] + koff * a9[t] * V1,
    a7'[t] == Q/V1 * a6[t] - Q/V2 * a7[t],
    a8'[t] == ksyn - kdeg * a8[t] - kon * a8[t] * a6[t]/V1 + koff * a9[t],
    a9'[t] == kon * a8[t] * a6[t]/V1 - koff * a9[t] - kint * a9[t]
  };

modelStates = {a1, a2, a3, a4, a5, a6, a7, a8, a9};
ic = {a1[0] == (1 - F1 - F2 - F3) * F * amtDose,
    a2[0] == F1 * F * amtDose, a3[0] == F2 * F * amtDose, a4[0] == F3 * F * amtDose,
    a5[0] == 0, a6[0] == 0, a7[0] == 0, a8[0] == ksyn/kdeg 0, a9[0] == 0};

observationVectorReal = {a6[t]/V1};

modelParametersAllUnknown =
  {F, F1, F2, F3, ktr, ka, V1, V2, Q, CL, ksyn, kdeg, koff, kon, kint};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[
  {{deqLi, ic}, observationVectorReal} /. {amtDose → RandomInteger[{1, 100}]},
  modelStates, modelParametersAllUnknown, t, u]
```

```
IdentifiabilityAnalysisData[False, <>]
```

```
summary = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{CL, F, F1, F2, F3, Q, V1, V2}, 1}
```

Conclusion: The model is unidentifiable with one degree of freedom.

Case 2: Fixing known parameters

Defining output function, model states, initial conditions and model parameters

```
modelStates = {a1, a2, a3, a4, a5, a6, a7, a8, a9};
ic = {a1[0] == (1 - F1 - F2 - F3) * F * amtDose,
   a2[0] == F1 * F * amtDose, a3[0] == F2 * F * amtDose, a4[0] == F3 * F * amtDose,
   a5[0] == 0, a6[0] == 0, a7[0] == 0, a8[0] == ksyn/kdeg 0, a9[0] == 0};
observationVectorReal = {a6[t]/V1};
fixedParam = {V1 → RandomReal[], V2 → RandomReal[], Q → RandomReal[],
   CL → RandomReal[], ksyn → RandomReal[], kdeg → RandomReal[],
   koff → RandomReal[], kon → RandomReal[], kint → RandomReal[]};
modelParameters = {F, F1, F2, F3, ktr, ka};
```

Structural Identifiability Analysis

```
iad = IdentifiabilityAnalysis[
  {{deqLi, ic}, observationVectorReal} /. fixedParam,
  modelStates, modelParameters, t, u]
```

```
IdentifiabilityAnalysisData[True, <>]
```

```
summary = {iad["NonIdentifiableParameters"], iad["DegreesOfFreedom"]}
```

```
{{}, 0}
```

Conclusion: The model is at least structurally locally identifiable.

Appendix E

Structural identifiability for mixed-effects models and its dependency on the statistical sub-model

This is the Mathematica code used for generating the identifiability results for Chapter 6 to show that the form of the distribution of the random effects affects the structural identifiability of the mixed-effects model.

The form of the random effects matter

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One-compartment absorption model

```
Z1 = TransformedDistribution[θV * Exp[ηV] / (θF * Exp[ηF]),
    {ηF, ηV} ∈ MultinormalDistribution[{0, 0}, {{ωF, 0}, {0, ωV}}]];
```

Computing the first two statistical moments of Z1

```
Mean[Z1]
```

$$\frac{e^{\frac{1}{2}(\omega_F+\omega_V)}\,\theta_V}{\theta_F}$$

```
Variance[Z1]
```

$$\frac{e^{\omega_F+\omega_V}\,(-1+e^{\omega_F+\omega_V})\,\theta_V^2}{\theta_F^2}$$

The ratio $\frac{F}{V}$ and the sum ωF+ωV are structurally globally identifiable but the model is structurally unidentifiable. An alternative way of showing that the system Z1 is structurally unidentifiable is to compute the Jacobian of the system and show that the Jacobian matrix is rank deficient.

Computing the first four statistical moments of Z1

```
moment1Z1 = Moment[Z1, 1]
```

$$\frac{e^{\frac{1}{2}(\omega_F+\omega_V)}\,\theta_V}{\theta_F}$$

```
moment2Z1 = Moment[Z1, 2]
```

$$\frac{e^{2\,(\omega_F+\omega_V)}\,\theta_V^2}{\theta_F^2}$$

```
moment3Z1 = Moment[Z1, 3]
```

$$\frac{e^{\frac{9}{2}(\omega_F+\omega_V)}\,\theta_V^3}{\theta_F^3}$$

```
moment4Z1 = Moment[Z1, 4]
```

$$\frac{e^{8(\omega_F+\omega_V)}\,\theta_V^4}{\theta_F^4}$$

Computing the Jacobian of the above system (Z1) and checking its rank

```
jacZ1 = D[{moment1Z1, moment2Z1, moment3Z1, moment4Z1}, {{θV, θF, ωV, ωF}}];

MatrixRank[jacZ1]
```

2

Conclusion: The Jacobian does not have full rank, meaning that the model is structurally unidentifiable. Next the identifiability of the model is studied when using a logit-normal distribution of parameter F by checking the rank of the Jacobian of the system.

```
Z2 = TransformedDistribution[ θV * Exp[ηV] / (1 + (1 - θF)/(θF * Exp[ηF])),
    {ηV, ηF} é MultinormalDistribution[{0, 0}, {{ωV, 0}, {0, ωF}}]];
```

Computing the first four statistical moments of Z2

```
moment1Z2 = Moment[Z2, 1]
```

$$e^{\frac{\omega_V}{2}}\left(1+\frac{e^{\frac{\omega_F}{2}}(1-\theta_F)}{\theta_F}\right)\theta_V$$

```
moment2Z2 = Moment[Z2, 2]
```

$$e^{2\omega_V}\left(1+\frac{e^{2\omega_F}(-1+\theta_F)^2}{\theta_F^2}-\frac{2\,e^{\frac{\omega_F}{2}}(-1+\theta_F)}{\theta_F}\right)\theta_V^2$$

```
moment3Z2 = Moment[Z2, 3]
```

$$e^{\frac{9\omega_V}{2}}\left(1-\frac{e^{\frac{9\omega_F}{2}}(-1+\theta_F)^3}{\theta_F^3}+\frac{3\,e^{2\omega_F}(-1+\theta_F)^2}{\theta_F^2}-\frac{3\,e^{\frac{\omega_F}{2}}(-1+\theta_F)}{\theta_F}\right)\theta_V^3$$

```
moment4Z2 = Moment[Z2, 4]
```

$$\frac{1}{\theta_F^4}e^{8\omega_V}\left(e^{8\omega_F}(-1+\theta_F)^4-4\,e^{\frac{9\omega_F}{2}}(-1+\theta_F)^3\theta_F+6\,e^{2\omega_F}(-1+\theta_F)^2\theta_F^2-4\,e^{\frac{\omega_F}{2}}(-1+\theta_F)\theta_F^3+\theta_F^4\right)\theta_V^4$$

Computing the Jacobian of the above system (Z2) and checking its

rank

```
jacZ2 = D[{moment1Z2, moment2Z2, moment3Z2, moment4Z2}, {{θV, θF, ωV, ωF}}];

MatrixRank[jacZ2]
```

```
4
```

Conclusion: The Jacobian has full rank,meaning that the model (Z2) is at least locally identifiable.

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