Simultaneous Estimation of States and Parameters in Metabolic Networks
from Filtering Techniques to Systems Biology

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Theory without fact is fantasy, but fact without theory is chaos

C.O. Whitman, 1894
Acknowledgments

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Abstract

The aim of this work is to estimate simultaneously states and parameters in metabolic networks with filtering techniques. The biochemical pathway studied was glycolysis, to which data from NMR experiments in *Lactococcus lactis* of some metabolites concentrations is available. The methods used for both states and parameter estimation were EKF and UKF. The task of parameter estimation revealed to be a harder task, that is justified by the fact that certain parameters are constants whose individual variations do not conditioned the system evolution strongly. It is important to mentioned that for certain parameters their variation can be compensated by other parameters variation, keeping the systems dynamics, but with a different numerical set of parameters, thereby suggesting that this model has an identifiability problem. Based on structural analysis, where the system sensibility of the system was studied in relation to each parameter, one can have an idea about which parameters would be more difficult to estimate, due to the fact that when these parameters change the system behavior does not have large alterations or because these parameters influence more the non directly measured states. This analysis was also important to conclude that the system is stable in the parameter value range considered. As summary, the techniques used revealed to be adequate to the problem in question and the results are promising to an investigation line following this direction.

Keywords

Nonlinear state-space models, Extended Kalman filter, Unscented Kalman filter, parameter estimation, structural analysis, metabolic networks
Resumo

O objectivo deste trabalho prende-se com a estimação simultânea de estados e parâmetros em redes metabólicas, com recurso a técnicas de filtragem. A via bioquímica estudada foi a glicólise, para a qual se têm dados experimentais de NMR em *Lactococcus lactis* relativos às concentrações de alguns metabolitos. Os métodos utilizados foram o Filtro de Kalman Extendido e o Filtro de Kalman Unscented, ambos para estimação de estados e parâmetros. A estimação dos parâmetros revelou ser uma tarefa mais complicada, o que se atribui ao facto de serem constantes cujas variações individuais não condicionam fortemente a evolução do sistema. De referir que a variação de um parâmetro poder ser compensada pela variação de outros, mantendo-se a dinâmica do sistema, mas com um conjunto de parâmetros diferente em valor numérico, o que sugere a existência de um problema de identificabilidade associado a este modelo. Através da análise estrutural do sistema, na qual se estudou a sensibilidade deste em relação a cada um dos parâmetros do modelo, pôde ter-se uma ideia *a priori* de quais seriam os parâmetros mais difíceis de estimar, pelo facto de serem os parâmetros que quando variam o seu valor exercem menor influência no comportamento do sistema ou porque influenciam sobretudo estados que não são directamente medidos. Esta análise permitiu ainda concluir que o sistema é estável na gama de valores considerada para os parâmetros. Em suma, os métodos utilizados revelaram ser adequados ao problema em questão, sendo os resultados promissores para o desenvolvimento de uma linha de investigação nesta direcção.

Palavras Chave

Modelos de estado-espaco não-lineares, Filtro de Kalman Extendido, Filtro de Kalman "Unscented", estimação de parâmetros, análise estrutural, redes metabólicas
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>EKF</td>
<td>Extended Kalman filter</td>
</tr>
<tr>
<td>FBP</td>
<td>Fructose 1,6-Biphosphate</td>
</tr>
<tr>
<td>G6P</td>
<td>Glucose-6-Phosphate</td>
</tr>
<tr>
<td>GLU</td>
<td>Glucose</td>
</tr>
<tr>
<td>KF</td>
<td>Kalman filter</td>
</tr>
<tr>
<td>MSE</td>
<td>Mean Square Error</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>pdf</td>
<td>Probability Density Function</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary Differential Equation</td>
</tr>
<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
</tr>
<tr>
<td>PGA</td>
<td>3-Phosphoglycerate</td>
</tr>
<tr>
<td>UKF</td>
<td>Unscented Kalman filter</td>
</tr>
<tr>
<td>UT</td>
<td>Unscented Transform</td>
</tr>
</tbody>
</table>
1
Introduction

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1. Introduction

The present work addresses the problem of estimation of states and parameters in metabolic networks models, along with nonlinear Kalman filter formulations, namely the Extended Kalman Filter (EKF) and the Unscented Kalman Filter (UKF). It explores a recent work on structural analysis of metabolic networks, whose case study is Lactococcus lactis metabolism, in particular the glycolysis pathway.

A fundamental point that one should have present when working on biochemical systems modeling is that it cannot be guaranteed that a representation of a metabolic network is the exact one. In fact, all models are approximations of real process and one needs to be aware that this scientific field is constantly changing.

Chapter 1 motivates and formulates the problem, briefly revises the state of the art and presents thesis contribution and structure.

1.1 Motivation

Systems biology is an emergent area which intends to integrate several different sciences (Mathematics, Biology, Chemistry and Control, among others) in order to achieve a better understanding of a biological system.

Biological systems are very complex. This is a direct consequence not only of the size, but mainly of the high-level regulation involved in their organization [1]. Cells are continuously exposed to a huge diversity of stimulus, to which they need to answer adequately [2]. Their ability to give coordinated physiological responses depends on their behavior, that is hierarchical organized and controlled [2].

Biological systems are rich in diversity; their dynamics, regulations and adaptation are the result of biological reactions presented along with genetic and metabolic pathways, besides others regulatory networks [2]. Metabolic control studies, specially dynamic modeling of metabolic pathways, is one of the main topics in Systems Biology, due to the increasing amount of experimental data and laboratorial techniques, along with modeling tools for mathematical representation and system analysis. Both complementary areas are continuously improving, leading to a point where it becomes possible to explore biological pathways, not only with the intention to understand their normal functioning and importance, but specially with the goal of manipulating and optimizing the production of some intermediated or final metabolite, defining new targets with biotechnological interest.

1.2 The Problem

The glycolysis network can be reduced to a simple version and graphically translated into a flux diagram, without loss of crucial information. This is represented in the Figure 1.1 where
each vertix represents a metabolite and each edge corresponds to either a flux of mass or activation/inhibitory signals.

![Metabolic pathway of glycolysis in *L. lactis*](image.png)

**Figure 1.1:** Metabolic pathway of glycolysis in *L. lactis*. In grey are activation and inhibitory signals. In [3].

In [4], Voit *et al* proposed one model for the metabolic pathway of glycolysis in *Lactococcus lactis* with seven non-linear differential equations. Each equation corresponds to a state variable that represents a metabolite concentration. The specimens included in the model are described in Table 1.1.

**Table 1.1:** Correspondence between metabolites and state variables

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>State variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU</td>
<td>$X_1$</td>
</tr>
<tr>
<td>G6P</td>
<td>$X_2$</td>
</tr>
<tr>
<td>FBP</td>
<td>$X_3$</td>
</tr>
<tr>
<td>PGA</td>
<td>$X_4$</td>
</tr>
<tr>
<td>PEP</td>
<td>$X_5$</td>
</tr>
<tr>
<td>PYR</td>
<td>$X_6$</td>
</tr>
<tr>
<td>LAC</td>
<td>$X_7$</td>
</tr>
</tbody>
</table>
1. Introduction

The equations read as follows in (1.1):

\[
\begin{align*}
    f_1 &= -\beta_1 X_1^b X_2^b X_5^b \\
    f_2 &= \beta_1 X_1^b X_2^b X_5^b - \beta_2 X_2^b ATP^{h2ATP} \\
    f_3 &= \beta_2 X_2^b ATP^{h2ATP} - \beta_3 X_3^b Pi^{h3Pi} NAD^{h3NAD} \\
    f_4 &= 2\beta_3 X_3^b Pi^{h3Pi} NAD^{h3NAD} + \alpha_4 X_4^b - \beta_4 X_4^b \\
    f_5 &= \beta_4 X_4^b - \beta_1 X_1^b X_2^b X_5^b - \alpha_4 X_4^b \\
    &\quad - \beta_51 X_3^b X_5^b Pi^{h3Pi} - \beta_52 X_5^b \\
    f_6 &= \beta_1 X_1^b X_2^b X_5^b + \beta_51 X_3^b X_5^b Pi^{h3Pi} \\
    &\quad - \beta_51 X_6^b X_3^b X_5^b NAD^{h3NAD} - \beta_52 X_6^b \\
    f_7 &= \beta_61 X_6^b X_3^b X_5^b NAD^{h3NAD}
\end{align*}
\]

In each equation above, \( f_i \) (\( i = 1...7 \)) corresponds to the respective \( \dot{X}_i \); in other words, each function \( f \) corresponds to the time derivative (flux of mass) of the respective metabolite concentration:

\[
f_i \equiv \dot{X}_i
\]

However, this model was not satisfactory, particularly in two points. The first was related with glucose (GLU), whose sigmoid type decay is not easy to reproduce using this formalism, what implies that this metabolite had to be considered an input signal if the formalism was to be kept [3]. Second, based on a structural analysis [3] it was found that \( X_4 \) and \( X_5 \) could be merged into a unique state variable, due to an extremely fast convergence to chemical equilibrium of PGA and PEP.

To overcome these aspects a new model that intends to describe this pathway was proposed by Vinga et al [3]. It considers six equations characterized by a set of twenty-seven parameters. This model is described in equation (1.3) below and is the starting point of the present work.

\[
\begin{align*}
    f_1 &= -k(1 + \alpha t^\beta) X_1 \\
    f_2 &= \beta_1 X_1^b X_2^b X_5^b - \beta_2 X_2^b ATP^{h2ATP} \\
    f_3 &= \beta_2 X_2^b ATP^{h2ATP} - \beta_3 X_3^b Pi^{h3Pi} NAD^{h3NAD} \\
    f_15 &= 2\beta_3 X_3^b Pi^{h3Pi} NAD^{h3NAD} - \beta_1 X_1^b X_2^b X_5^b \\
    &\quad - \beta_51 X_3^b X_5^b Pi^{h3Pi} - \beta_52 X_5^b \\
    f_6 &= \beta_1 X_1^b X_2^b X_5^b + \beta_51 X_3^b X_5^b Pi^{h3Pi} \\
    &\quad - \beta_51 X_6^b X_3^b X_5^b NAD^{h3NAD} - \beta_52 X_6^b \\
    f_7 &= \beta_61 X_6^b X_3^b X_5^b NAD^{h3NAD}
\end{align*}
\]

The first modification to (1.1) is related to glucose flux \( (f_1) \); attempting to describe more accurately the sigmoid-type extracellular glucose decay, a time dependency was introduced that
guarantees this kind of behavior, as the initial formalism was not appropriated. The second modification was the definition of a new state \( X_{45} \), created by algebraic addition between \( X_4 \) and \( X_5 \), due to the proportionality referred above. As

\[
X_{45} = X_4 + X_5 \quad (1.4)
\]

and

\[
X_4 = k_{45}X_5 \quad (1.5)
\]

it implies that

\[
X_4 = \frac{k_{45}X_{45}}{1 + k_{45}} \quad (1.6)
\]

\[
X_5 = \frac{X_{45}}{1 + k_{45}}
\]

In both models, the other metabolites needed, \( ATP, NAD^+/NADH \) and \( Pi \), are given as input signals from experimental raw data. These metabolites participate in many other reactions within the cells, which severely hampers its mathematical modeling. Since they present some irregularities and need to be extrapolated beyond the defined interval, cubic splines and interpolation were used to infer the parameters and correctly simulate the obtained solution [3].

The parameters obtained by Vinga [3] are summarized in Table 1.2; these values are considered the nominal values of parameters.

Not all states can be directly measured due to experimental limitation, since concentrations go below detection limits - in fact, only four of them (GLU, FBP, PEP/PGA and LAC) can, what implies that the problem in hands is concerned with simultaneous estimation of states and parameters.

1.3 State of The Art

1.3.1 Biochemistry

The biochemical system used as case-study was the glycolytic pathway in \( Lactococcus lactis \). Glycolysis is a sequence of enzymatic reactions during which glucose is oxidized to pyruvate [5]. It is the most primitive pathway and exists in all actual living organisms, suggesting that it is a vital process in cells [5].

\( Lactococcus lactis \) is a gram-(+) bacterium, member of the lactic acid bacteria [1]. \( Lactococcus lactis \) is a homofermentative microorganism that converts glucose (or milk sugar lactose) to lactic acid, via the Embden-Meyerhof glycolytic pathway with an yield upper to 95% and because of it is widely used in industrial milk-fermentations, as starters in the manufacture of fermented foods and beverages, such as buttermilk and cheese [4]. The low pH generated by this activity, as well as the action of other fermentation products, inhibits the spoilage and growth of pathogenic bacteria, and consequently extends the shelf-life of the fermented products [4].

Despite the wealth of metabolic information collected, a comprehensive understanding of sugar metabolism and regulatory pathways in this model organism has not yet been achieved.
1. Introduction

Table 1.2: Nominal values for the parameters. The experience behind is a glucose pulse of 40mM under aerobic conditions. For further information see [3].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0.0530251</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.0419958</td>
</tr>
<tr>
<td>$\beta$</td>
<td>2.68092</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>7.20321</td>
</tr>
<tr>
<td>$h_{11}$</td>
<td>0.997546</td>
</tr>
<tr>
<td>$h_{12}$</td>
<td>-1.48643</td>
</tr>
<tr>
<td>$h_{25}$</td>
<td>0.38576</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>0.345889</td>
</tr>
<tr>
<td>$h_{22}$</td>
<td>1.54399</td>
</tr>
<tr>
<td>$h_{2,ATP}$</td>
<td>1.51599</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.338423</td>
</tr>
<tr>
<td>$h_{33}$</td>
<td>1.08298</td>
</tr>
<tr>
<td>$h_{3,P_i}$</td>
<td>0.258372</td>
</tr>
<tr>
<td>$h_{3,NAD}$</td>
<td>-0.0966562</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>0.134164</td>
</tr>
<tr>
<td>$h_{2,5}$</td>
<td>0.0940446</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>0.862421</td>
</tr>
<tr>
<td>$h_{5,13}$</td>
<td>0.7663</td>
</tr>
<tr>
<td>$h_{5,15}$</td>
<td>0.0382342</td>
</tr>
<tr>
<td>$h_{5,1,P_i}$</td>
<td>0.211149</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.0324743</td>
</tr>
<tr>
<td>$h_{6,16}$</td>
<td>0.675486</td>
</tr>
<tr>
<td>$h_{6,13}$</td>
<td>1.03221</td>
</tr>
<tr>
<td>$h_{6,1,NAD}$</td>
<td>-0.0519436</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>1.74742</td>
</tr>
<tr>
<td>$h_{6,26}$</td>
<td>1.40312</td>
</tr>
<tr>
<td>$h_{4,15}$</td>
<td>2.04035</td>
</tr>
</tbody>
</table>

1.3.2 *In vivo* NMR spectroscopy

NMR is a common noninvasive technique that allows *in vivo* measurements of physical, chemical, electronic and structural properties of intracellular metabolites [6]. NMR gives the possibility to obtain in vivo multivariate time series of metabolite concentrations for several organisms and different pathways and perturbations, allowing thus the real-time observation of these processes [7]. This type of information, in particular the glycolytic pathway in *Lactococcus lactis* is the basis for this modeling study. The most important NMR techniques are $^{13}$C-NMR and $^1$H-NMR.

To study metabolites with $^{13}$C-NMR requires the use of $^{13}$C-enriched compounds; when these compounds are present, one is able to identify metabolic pathways and measuring carbon fluxes [6]. However, the basal metabolite concentration is not possible to measure, because the natural carbon isotope ($^{12}$C) is not magnetically active (nuclei spin is integer), what implies that $^{13}$C-NMR is much less sensitive than $^1$H-NMR [6].

This method was used to monitor the kinetics of the intracellular pools of $NAD^+$, $NADH$ and external and internal metabolites intermediates in the living cells of *Lactococcus lactis*. The NMR experimental data referred in this thesis was obtained by the groups of Professor Helena Santos (Cell Physiology & NMR) and Dr Ana Rute Neves (Physiology of lactic acid bacteria & in vivo
1.3 State of The Art

Figure 1.2: Schematic representation of experimental set-up used for \textit{in vivo} NMR. 1 - pH electrode; 2 - pH controller; 3 - gas lines; 4 - circulation pump; 5 - water bath; 6 - safeguard line. In [7].

NMR) at ITQB - Oeiras.

It does not interfere with the cellular processes and unexpected metabolites from microbian metabolism can be identified, what is a big advantage. \textit{\textsuperscript{13}C} NMR spectra show a single peak for each chemically nonequivalent carbon atom. The major disadvantage is the low sensitivity that hampers measurements in growing cultures [6].

Figure 1.3: Typical aspect of a sequence of \textit{\textsuperscript{13}C} spectra for one metabolite. In [6].

Despite \textit{\textsuperscript{1}H} NMR has a much higher sensitivity, the narrow spectral range and the excessive overlap of resonances due to the strong water signal in biological mediums are the big limitations [7].

1.3.3 Models for Metabolic Networks

Information on biological processes are becoming available in the form of metabolic and genetic time series, but to quantitatively characterize these processes is still a challenge [1].

BST (Biochemical Systems Theory) concepts provides a consistent mathematical framework for representing biological processes, giving the opportunity to subsequent quantitative analysis [4]. According to this theory, all process are represented as products of power-law functions, what can be biologically motivated and mathematically derived from Taylor’s theorem of numerical
1. Introduction

analysis, which is applied to variables in logarithmic space \cite{4}. Each flux of mass $V_i$ involves $n$
dependent (state) variables and $m$ independent variables (including control variables and constant
enzyme activities) and takes the format:

$$V_i = \gamma_i \prod_{j=1}^{n+m} X_j^{f_{ij}}$$

where $\gamma_i$ is the rate constant that describes the turnover rate of the process and the exponent $f_{ij}$
is the kinetic order that quantifies the direct effect of variable $X_j$ on $V_i$ \cite{4}.

The most useful alternative representations offered by BST are the Generalised Mass Action
(GMA) and the S-Systems representations \cite{4}.

GMA models focus on processes and therefore represent each reaction by a product of power-
law functions of the type above that includes all variables that have a direct effect on this process.
The dynamics of each variable is given as a sum of power-law terms describing all influxes and
effluxes \cite{4}; the generic GMA structure is

$$\dot{X}_i = \sum_{p=1}^{P_i} (\pm \gamma_{ip} \prod_{j=1}^n X_j^{f_{ipj}}), i = 1, ..., n$$

The S-system form focuses on metabolic pools and represents the collection of all influxes into
a given pool with a single power-law term and the collection of all effluxes, from this pool with a
second power-law term \cite{4}. The generic S-system structure is

$$\dot{X}_i = \alpha_i \prod_{j=1}^n X_j^{g_{ij}} - \beta_i \prod_{j=1}^n X_j^{h_{ij}}, i = 1, ..., n$$

The coefficients $\alpha$ and $\beta$ are nonnegative rate constants and the exponents $g$ and $h$ are real-
valued kinetic orders \cite{4}.

The advantages and disadvantages of these formalism along with several examples of application
are explored elsewhere (see for instance \cite{2}).

In the particular case of the model (1.3) the formalism used was GMA.

1.3.4 Filtering and Nonlinear Estimation

Filtering can be defined as the problem of recursively estimating the states (parameters or
hidden variables) of a system from a set of observations available on-line \cite{8}. Filtering and esti-
mation are two of the most pervasive tools of engineering \cite{8}. When one is working with nonlinear
systems, the estimation task is really complicated. There is an optimal, Bayesian solution to the
problem, but it requires the propagation of the complete probability density function (pdf) \cite{9}. This
solution is extremely general and can be extended to a panoply of applications \cite{9}; however, as
the pdf is not restricted, it cannot be described using a finite number of parameters what requires,
unfortunately, a potentially unbounded number of parameters to have an exact description \cite{9}.
1.3.4.A Kalman Filter

To overcome the limitations of Bayesian approach, approximation techniques must be used. Despite many attempts, Kalman Filter (KF) variants [10] are the most widely used method for tracking and estimation due to its simplicity, optimality, tractability and robustness, when the system dynamics and observations models are linear. This recursive algorithm is a very general treatment from the “state” point of view to the discrete-data linear filtering problem [10]. It attempts to find an estimative for the state of a process by solving a set of mathematical equations in a way that minimizes the mean of the squared error [10].

The filter supports a modeled system whose precise nature is unknown and is able to infer knowledge about unobservable values in the past (interpolation or smoothing problem), present (filtering) and future (prediction problem); the estimation is the designation attributed to these three problems collectively [10]. Some fundamental assumptions are made in filter design [10]:

1. the system should be linear or, at least, linearizable in a neighborhood of the nominal operation point;
2. the noisy present in the system, and the one associated with the measures, have to be white, Gaussian, with zero-mean and constant standard deviation;
3. the system’s noise and the measurement’s noise are not correlated.

The broad application of KF is mainly due to the fact that it only uses the first two moments of the state, mean and covariance, what offers important advantages [9] such as:

⊙ when the distribution is unknown a Gaussian distribution is assumed and the propagation of the mean and covariance requires only the maintenance of a small and constant amount of information that is sufficient to support most kinds of systems - computational complexity and representational flexibility have a successful compromise;
⊙ the mean and covariance are linearly transformable quantities and can be maintained effectively when subjected to linear and quasilinear transformations;
⊙ sets of mean and covariance estimates can be used to characterize additional features of the state distribution.

Kalman filter equations provide an extremely convenient procedure for computer implementation in a direct manner. The central operation of KF is the propagation of a Gaussian random variable through the systems dynamics [11]. The KF addresses the general problem of trying to estimate the state $x \in \mathbb{R}^n$ of a discrete-time controlled system described by the linear stochastic difference equation [11]

$$x_k = Ax_{k-1} + Bu_{k-1} + w_{k-1}$$ (1.10)
1. Introduction

with a measurement $z \in \mathbb{R}^m$ \[ z_k = H x_k + v_k \] \[ (1.11) \]

The probability distributions for random noise variables $w$ and $v$ are \[ p(w) \sim N(0, Q) \]
\[ p(v) \sim N(0, R) \] \[ (1.12) \]

$A$ is an $n \times n$ matrix that relates the state at the previous time step $k - 1$ to the state at the current step $k$ in the absence of either a driving function or a process noise. The $n \times l$ matrix $B$ relates the optional control input $u \in \mathbb{R}^l$ to the state $x$. Matrix $H$ ($m \times n$) relates the state $x_k$ to the measurement $z_k$. $Q$ is the process noise covariance and $R$ the measurement noise covariance. All these matrices might change with each time step or measurement. \[ \]

The KF can be divided in two steps: prediction and measurement update (or filtering). In the prediction step, the filter propagates the estimate $\hat{x}_{k-1}$ from a previous time step $k - 1$ to the current time step $k$ \[ \hat{x}_k^- = A \hat{x}_{k-1} + B U_{k-1} \] \[ (1.13) \]
and projects the error covariance ahead \[ P_k^- = A P_{k-1} A^T + Q \] \[ (1.14) \]
Here, $\hat{x}_k^-$ is the a priori estimate for $x$ at step $k$ given knowledge of the process prior to step $k$ and $P_k^-$ is the a priori estimate error covariance matrix.

During the measurement update, the filter computes the Kalman gain $K_k$ in that instant \[ K_k = P_k^- H^T (H P_k^- H^T + R)^{-1} \] \[ (1.15) \]
updates the estimate with the measurement $z_k$ \[ \hat{x}_k = \hat{x}_k^- + K_k (z_k - H \hat{x}_k^-) \] \[ (1.16) \]
and updates the error covariance \[ P_k = (I - K_k H) P_k^- \] \[ (1.17) \]
Here, $I$ is the identity matrix, $\hat{x}_k$ an a posteriori estimate of the state at step $k$ given measurement $z_k$ and $P_k$ is the a posteriori estimate error covariance.

This method diverges when the error covariance matrix computed by the filter becomes unjustifiably small compared with the actual error in the estimate, what causes the gain matrix to become too small and new measurement data are given too little weight. When it happens, the plant model becomes more important in determining the estimate than the data and any errors in the model can build up over a period of time and cause a significant degradation in the
1.4 Original Contributions

accuracy of the estimate \cite{12}. To overcome it, a good practice is to include a noise term in the \textit{a priori} system estimates \cite{11,9}. It is important to remark that divergence does not occur because of any fault of the filter - if the system were actually linear and the order correct, Kalman showed that the filter equations are stable under very reasonable conditions \cite{13}. Divergence is a direct consequence of the errors introduced by the linear approximation as well as unmodelled dynamics \cite{8}.

1.3.4.B Parameter Estimation

The task of parameter estimation is crucial for modeling and basically corresponds to find the set of parameters able to minimize the predefined cost function \cite{12}. The Kalman Filter may be used to estimate the parameters treating them as a stationary process with identity state transition matrix. When at least some states are unobserved, coupling both state and parameter estimation is required. When one is using Kalman Filter approaches to work with this problem, there are two main possible strategies \cite{12}.

In the \textbf{Dual Kalman Filter} a separate state-space representation for states and parameters is used in a bootstrap scheme. This means that two Kalman Filters have to be run simultaneously, so that at every time-step there is an exchange of information between the filters. This permits the use of the current estimate of the parameters in the states-filter and the current estimated of the states in the parameters-filter \cite{12}.

Another hypothesis is to concatenate into a single joint state vector the states \((x)\) and parameters \((\theta)\) \cite{12}:

\[
X = \begin{bmatrix} x \\ \theta \end{bmatrix} \quad (1.18)
\]

In this case, the process model can be written as

\[
X_k = F(X_{k-1}) = \begin{bmatrix} f(x_{k-1}, \theta_{k-1}) \\ \theta_{k-1} \end{bmatrix} \quad (1.19)
\]

In this approach, \textbf{Joint Kalman Filter}, the state-space equations are written for the joint state and the estimation is done in the joint state-space, what gives simultaneous estimates for the states \(x\) and parameters \(\theta\) \cite{12}. This will be the approach followed in this dissertation.

1.4 Original Contributions

This work explores the case study presented on \cite{3}, using Kalman filter based approaches (EKF and UKF) in a different context of the one they are commonly used. Structural stability, in terms of sensitivities of the states in function of their characteristic parameters and finite-time escape analysis are also included along the thesis. The thesis shows that the use of filter techniques is adequate to metabolic networks modeling studies.
1. Introduction

1.5 Thesis Structure

The structure of the present thesis is as follows. After Chapter 1 that motivates the problem to be solved and summarizes the overall contributions, Chapter 2 reviews the relationship between KF and EKF for nonlinear systems and address the estimation of synthetic data with EKF. Chapter 3 presents the limitations of EKF and motivates the UKF; algorithm details and an example of synthetic data estimation is also here presented. Chapter 4 is concerned with structural analysis, from sensitivity studies to finite-time escape analysis. Results (state and parameter estimation with spline-reconstructed data and experimental data) are compiled on Chapter 5. Global discussion about the work and summary of principal results are in Chapter 6, as well as future work proposals. Appendix A presents a different case study, concerned with mathematical model of glycolysis in red blood cells.
2

Extended Kalman Filter

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2.3 EKF preliminary results and discussion ......................... 16
2. Extended Kalman Filter

The KF was designed for linear systems, but its major application is on nonlinear systems. The EKF is the most common application of KF to nonlinear systems. This chapter presents the EKF and address the estimation of synthetic data with this method.

2.1 Motivation

The KF assumes systems linearity but its major application is on nonlinear systems analysis \[13\], where the EKF is the most common application. The EKF linearizes the nonlinear transformations about the current mean and covariance by a Taylor series approximation using the partial derivatives of the process and measurement functions to compute estimates \[11\].

In the EKF, the state distribution is approximated by a Gaussian random variable which is then propagated analytically through the first-order linearization of the nonlinear system. Linearization assumes that all second and higher order terms in the Taylor series expansion are negligible \[12\]. These approximations can introduce large errors in the true posterior mean and covariance of the transformed (Gaussian) random variable, which may lead to sub-optimal performance and sometimes divergence of the filter \[9\].

2.2 EKF algorithm

Assume that the process to estimate has a state vector \( x \in \mathbb{R}^n \) and that the process is governed by the non-linear stochastic difference equation \[11\]

\[
x_k = f(x_{k-1}, w_{k-1}) \tag{2.1}
\]

with a measurement \( z \in \mathbb{R}^m \) that is \[11\]

\[
z_k = h(x_k, v_k) \tag{2.2}
\]

where the random variables \( w_k \) and \( v_k \) represent again the process and measurement noise, respectively \[11\] (with normal distribution, see equation \(1.12\)).

As one does not know the individual values of the noise at each time step, the state and measurement vectors can be approximated as \[11\]

\[
\tilde{x}_k = f(\tilde{x}_{k-1}, 0) \tag{2.3}
\]

and

\[
\tilde{z}_k = h(\tilde{x}_k, 0) \tag{2.4}
\]

where \( \tilde{x}_k \) is an a posteriori estimate of the state (from a previous time step \( k \)) \[11\].

The new equations that linearizes the estimate about \(2.3\) and \(2.4\) are \[11\]

\[
x_k \approx \tilde{x}_k + A(x_{k-1} - \tilde{x}_{k-1}) + W w_{k-1} \tag{2.5}
\]
2.2 EKF algorithm

\[ z_k \approx \tilde{z}_k + H(x_k - \tilde{x}_k) + Vv_k \]  

(2.6)

where:

- \( x_k \) and \( z_k \) are the actual state and measurement vectors
- \( \tilde{x}_k \) and \( \tilde{z}_k \) are the approximate state and measurement vectors (2.3) and (2.4)
- \( \hat{x}_k \) is an \textit{a posteriori} estimate of the state at step \( k \)
- the random variables \( w_k \) and \( v_k \) represent the process and measurement noise
- the \( n \times n \) matrix \( A \) is the Jacobian matrix of partial derivatives of \( f \) with respect to \( x \)
  \[ A_{[i,j]} = \frac{\partial f_i}{\partial x_j}(\hat{x}_{k-1}, 0) \]  
  (2.7)
- the \( n \times n \) \( W \) is the Jacobian matrix of partial derivatives of \( f \) with respect to \( w \)
  \[ W_{[i,j]} = \frac{\partial f_i}{\partial w_j}(\hat{x}_{k-1}, 0) \]  
  (2.8)
- the \( m \times n \) \( H \) is the Jacobian matrix of partial derivatives of \( h \) with respect to \( x \)
  \[ H_{[i,j]} = \frac{\partial h_i}{\partial x_j}(\hat{x}_k, 0) \]  
  (2.9)
- the \( m \times m \) \( V \) is the Jacobian matrix of partial derivatives of \( h \) with respect to \( v \)
  \[ V_{[i,j]} = \frac{\partial h_i}{\partial v_j}(\hat{x}_k, 0) \]  
  (2.10)

The prediction error is defined as [11]

\[ \tilde{e}_x_k \equiv x_k - \hat{x}_k \]  

(2.11)

and the measurement residual

\[ \tilde{e}_z_k \equiv z_k - \tilde{z}_k \]  

(2.12)

The true value of \( x_k \) is unknown, but \( z_k \) is known, so the governing equations for the error process could be written as [11]

\[ \tilde{e}_x_k \approx A(x_{k-1} - \hat{x}_{k-1}) + \epsilon_k \tilde{e}_z_k \approx H \tilde{e}_z_k + \eta_k \]  

(2.13)

The random variables in (2.13) have approximately the following probability distributions [11]

\[ p(\tilde{e}_x_k) \sim N(0, E[\tilde{e}_z_k \tilde{e}_x_k^T]) \]  

(2.14)

\[ p(\epsilon_k) \sim N(0, WQ_k W^T) \]  

(2.15)

\[ p(\eta_k) \sim N(0, VR_k V^T) \]  

(2.16)

The \textit{a posteriori} state estimates for the original non-linear process can be compute as [11]

\[ \hat{x}_k = \tilde{x}_k + \tilde{e}_k \]  

(2.17)
2. Extended Kalman Filter

The Kalman filter equation for predict the value of estimate error \( \hat{e}_k \) is \[2.18\]

\[
\hat{e}_k = K_k \tilde{e}_{zk}
\]

Connecting (2.17) and (2.18) one obtains [11]

\[
\hat{x}_k = \tilde{x}_k + K_k \tilde{e}_{zk} = \tilde{x}_k + K_k (z_k - \tilde{z}_k)
\]

The time update equations project the state (\( \hat{x}_k^{-} \)) and covariance (\( P_k^{-} \)) estimates from the previous time step \( k - 1 \) to the current time step \( k \) according with equation (2.20) [11].

\[
\hat{x}_k^{-} = f(\hat{x}_{k-1}, u_{k-1}, 0)
\]

\[
P_k^{-} = A_k P_{k-1} A_k^T + W_k Q_{k-1} W_k^T
\]

The measurement update equations correct the state and covariance estimates with the measurement \( z_k \), using the system of equations (2.21) [11]

\[
K_k = P_k^{-} H_k^T (H_k P_k^{-} H_k^T + V_k R_k V_k^T)^{-1}
\]

\[
\hat{x}_k = \hat{x}_k^{-} + K_k (z_k - h(\hat{x}_k^{-}, 0))
\]

\[
P_k = (I - K_k H_k) P_k^{-}
\]

where \( K_k \) is the Kalman gain, \( \hat{x}_k \) the a posteriori updated estimate with measurement \( z_k \) and \( P_k \) the updated error covariance matrix.

2.3 EKF preliminary results and discussion

A first attempt to test EKF implementation was to estimate synthetic data generated from the integration of the model (1.3) in the presence of noise. This allows to analyze the algorithm accuracy in a controlled and known system.

The system in (1.3) is described in continue time and the aim is to approximate this model in discrete time. To do it, the general formula used to integrate numerically the system was an Euler based method.

\[
x_{i+1}^k = x_i^k + hf_i + w_i^k
\]

The initial conditions needed to integrate the model (1.3) according to (2.22) were chosen in accordance with experimental data. The initial values for states \( X_1, X_2, X_3, X_45, X_6, \) and \( X_7 \) were, respectively, \( x_0 = [40, 0.1, 0.72, 40, 0.1, 0.01] \). Parameters were used with the nominal values \( (p_0 = p_{nv} \) (see table 1.2)). The noise was considered Gaussian zero-mean with variance \( q_s = 1 \times 10^{-6} \).

\[1\] More considerations about the method of integration will be made on Chapter 5.
2.3 EKF preliminary results and discussion

About the EKF, the initial conditions chosen for the states were \( \hat{x}_0 = [50, 0.3, 0.85, 30, 0.01, 0.03] \) and for parameters were \( \hat{p}_0 = 1.1p_{\text{nom}} \).

The variance of measurement noise, also assumed to be Gaussian zero-mean, was set to \( r = 1 \times 10^{-3} \).

As in experiments only four metabolite concentrations are measured, that means, only \( X_1, X_3, X_{45} \) and \( X_7 \) are observable states, the \( H \) matrix (equation (2.9)) was defined in order to simulate the same conditions

\[
H = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 \\
\end{bmatrix} \quad \text{zeros(4,27)} \tag{2.23}
\]

The integration step \( h \) was set to 0.001 min and the limits of integration were defined in accordance with experimental time series. The integrated system and the estimated one are presented on Figure 2.1.

![Figure 2.1](image)

**Figure 2.1:** Simulated system with synthetic data (on the left) and the respective estimated system with EKF (on the right). Synthetic, real states and the corresponding simulation, estimated states are almost the same.

One can easily see on Figure 2.2(a) that the system is accurately estimated, even the non directly measurable states. This is very interesting because the equations are highly nonlinear and the EKF, as discussed before, starts by linearizing them but it does not seem to compromise filter performance. A more detailed analysis, where each state and each parameter are directly compared with the correspondent estimative are presented along Figure 2.2.

However, analyzing Figures 2.2(b), 2.2(c) and 2.2(d) where parameters estimatives are presented, the first conclusion is that parameter estimates are not so good as the state estimates. The values for parameters obtained with the filter are, for the majority of the cases, different from nominal values.

The parameters quickly converge to a constant value, that is kept for the rest of the simulation. It suggests that the set of values found for parameters is equally valid to simulate system dynamics.
2. Extended Kalman Filter

Figure 2.2: Comparison between the nominal values (in blue) and the respective estimated values (in green) with EKF for synthetic data.

In order to have a quantitative analysis that better explains the results, a mean square error (MSE) was computed according to

\[ \varepsilon_{\text{syntheticdata}}^{EKF} = \sqrt{\frac{\sum (X - \hat{X})^2}{\sum X}} \]  

(2.24)

where \( X \) are the expected values for the augmented state and \( \hat{X} \) the respective estimates. The results are compiled in Table 2.1.
2.3 EKF preliminary results and discussion

Table 2.1: MSE (%) for estimation of synthetic data with EKF.

<table>
<thead>
<tr>
<th>State</th>
<th>$\varepsilon_{\text{synthetic data}}$</th>
<th>Parameter</th>
<th>$\varepsilon_{\text{EKF}}$</th>
</tr>
</thead>
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<tr>
<td>$X_1$</td>
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<td>$k$</td>
<td>34.68</td>
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<tr>
<td>$X_2$</td>
<td>36.33</td>
<td>$\alpha$</td>
<td>46.30</td>
</tr>
<tr>
<td>$X_3$</td>
<td>0.61</td>
<td>$\beta$</td>
<td>9.15</td>
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<tr>
<td>$X_7$</td>
<td>0.043</td>
<td>$h_{12}$</td>
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<td></td>
<td></td>
<td>$h_{25}$</td>
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<td></td>
<td></td>
<td>$\beta_2$</td>
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<td></td>
<td></td>
<td>$h_{22}$</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>$h_{2ATP}$</td>
<td>8.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\beta_3$</td>
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<tr>
<td></td>
<td></td>
<td>$h_{33}$</td>
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<td>$h_{3P_i}$</td>
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<td></td>
<td>$h_{5i5}$</td>
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<td>$h_{5iP_i}$</td>
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<td>$h_{616}$</td>
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</tr>
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<td></td>
<td>$h_{626}$</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>$k_{45}$</td>
<td>8.88</td>
</tr>
</tbody>
</table>

For directly measurable states, the maximum error is along the $X_1$ estimate; the other three states are accurately estimated, with MSE’s lower than 1%. The non directly measurable states $X_2$ and $X_6$ are estimated with MSE around 36% and 21% respectively, but it does not seem to conditioned states dynamics, because they are kept, as can be seen in Figure 2.2(a). If these two states were observable certainly their MSE error would be smaller.

In relation to parameters, MSE’s are comprised between 2% and 63%. This is an indicator of the difficulty of the estimation task: it will be certainly easier to estimate the states, even those non directly measurable, than the parameters. The harder task of parameter estimation may be associated with identifiability problems of this model, that means, the influence of one parameter in the whole system cannot be study separately from the other parameters. Parameters can change their values in a coordinated way, keeping the system behavior, giving the possibility to exist another set of parameters, different from the one defined by the nominal parameter values, that may be able to reproduce the same system dynamics. This phenomena must be carefully considered.

As general preliminary conclusion and based on these results, one can expect that EKF will yield a good performance to estimate the system with experimental data measurements.
3 Unscented Kalman Filter

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3.4 UKF preliminary results and discussion ............. 25
The EKF is not the ideal solution to deal with nonlinear systems due to the mandatory linearization of the system that can compromise the performance of the method. A different Kalman-based filter, UKF, that does not need to linearize the system and does not need to calculate the Jacobian matrices is introduced along this chapter and some estimation results of synthetic data are also presented.

### 3.1 Motivation

As seen in the previous chapter, the KF can be applied to nonlinear systems if a consistent set of predicted quantities can be calculated. These quantities are derived by projecting a prior estimate through a nonlinear transformation.

Although the EKF keeps the elegant and computationally efficient recursive update form of the Kalman Filter, there are three major drawbacks that limit its use:

1. linearized transformations are only reliable if the error propagation can be well approximated by a linear function. If this condition does not hold, the linearized approximation can be extremely poor, what, at best, undermines the performance of the filter;

2. linearization can be applied only if the Jacobian matrix is well conditioned; and

3. to compute the Jacobian matrices can be a very difficult and error-prone process because their derivation are non trivial in most applications, such as the one considered in this work, leading to significant implementation difficulties.

There is a strong need for a method more accurate than linearization but which does not incur the implementation nor computational cost of other higher order filtering schemes.

### 3.2 Unscented Transformation

One of the most fundamental tasks in filtering and estimation is to calculate the statistics of a random variable which has undergone a transformation. The UKF is a filter founded on the intuition that it is easier to approximate a probability distribution than it is to approximate an arbitrary nonlinear function or transformation.

The problem of predicting the evolution of states and observations can be simply put in the following terms. Suppose that $x$ is a random variable with mean $\bar{x}$ and covariance $P_{xx}$. Suppose that $y$ is a random variable related to $x$ through the nonlinear function

$$y = g(x)$$

(3.1)

The objective is to calculate the mean ($\bar{y}$) and covariance ($P_{yy}$) of $y$. The statistics of $y$ are calculated by determining the density function of the transformed distribution and evaluating the
3.2 Unscented Transformation

statistics from that distribution \([9]\). Exact, closed form solutions with an acceptable computational load and limited memory do not exist in general, what implies the use of approximate methods. The method chosen should yield consistent statistics, which should be efficient and unbiased \([9]\).

To be consistent, a transformed statistics have to hold \([9]\)

\[
P_{yy} - E[y - \bar{y}y - \bar{y}^T] \geq 0 \tag{3.2}
\]

When the statistics are not consistent, the value of \(P_{yy}\) is under-estimated. Even if the method is consistent, its usefulness is not guaranteed (the value of \(P_{yy}\) might be greatly in excess of the actual mean squared error); to be sure the transformation is efficient, inequation \(3.2\) has to be minimized. At last, the unbiased characteristic of estimator can be expressed as \([9]\)

\[
\bar{y} \approx E[y] \tag{3.3}
\]

The Unscented Transform (UT) is a method for calculating the statistics of a random variable which undergoes a nonlinear transformation \([13]\). A set of points - sigma points - are chosen so that their sample mean and sample covariance are \(\bar{x}\) and \(P_{xx}\), respectively \([12]\). The nonlinear function is applied to each point in turn to yield a cloud of transformed points and \(\bar{y}\) and \(P_{yy}\) are the statistics of the transformed points. The samples are not drawn at random but rather according to a specific, deterministic algorithm, what is an extremely important and fundamental difference to Monte Carlo - type methods \([13]\).

According to UT, the \(L\)-dimensional variable \(x\), with mean \(\bar{x}\) and covariance \(P_{xx}\), is approximated by \(2L + 1\) weighted points given by \([12]\)

\[
\begin{align*}
\chi_0 & = \bar{x} \\
\chi_i & = \bar{x} + (\sqrt{(L + \lambda)}P_{xx})_i, \quad i = 1, ..., L \\
\chi_{i+L} & = \bar{x} - (\sqrt{(L + \lambda)}P_{xx})_i, \quad i = L + 1, ..., 2L \\
W^{(m)}_0 & = \lambda/(L + \lambda) \\
W^{(c)}_0 & = \lambda/(L + \lambda) + (1 - \alpha^2 + \beta) \\
W^{(m)}_i & = W^{(c)}_i = 1/2(L + \lambda) \quad i = 1, ..., 2L \\
\end{align*} \tag{3.4}
\]

where \(\lambda = \alpha^2(L + \kappa) - L\) is a scaling parameter. \(\alpha\) determines the spread of the sigma points around \(\bar{x}\) and is usually set to a small positive value (between \(1 \times 10^{-4}\) and 1). \(\kappa\) is a secondary scaling parameter which is usually set to 0, and \(\beta\) is used to incorporate prior knowledge of the distribution of \(x\) (for Gaussian distributions, \(\beta = 2\) is optimal). \((\sqrt{(L + \lambda)}P_{xx})_i\) is the \(i\)th row of the matrix square root \([9]\).

In order to provide an unbiased estimate the weights must satisfy \([9]\)

\[
\sum_{i=0}^{p} W^{(m)}_i = 1 \tag{3.6}
\]
3. Unscented Kalman Filter

The sigma vectors are propagated through the nonlinear function[9]

\[ Y_i = g(\chi_i) \quad i = 0, ..., 2L \] (3.7)

The mean and the covariance for \( y \) are approximated using a weighted sample mean and covariance of the posterior sigma points [9]

\[ \bar{y} \approx \sum_{i=0}^{2L} W^{(m)}_i Y_i \] (3.8)

\[ P_y \approx \sum_{i=0}^{2L} W^{(c)}_i (Y_i - \bar{y})(Y_i - \bar{y})^T \] (3.9)

3.3 Algorithm

The set of sigma points are created by applying equation (3.4) to the augmented system given by equation (1.18).

As one is in the presence of a Kalman filter-type approach, the algorithm can be subdivided in two sections, time update and measurement update.

1. Time Update

(a) The transformed set is given by instantiating each sigma-point through the process model

\[ \chi^x_{k|k-1} = F[\chi^x_{k-1}, \chi^v_{k-1}] \] (3.10)

(b) The predicted mean is computed as

\[ \hat{x}^-_k = \sum_{i=0}^{2L} W^{(m)}_i \chi^x_{i,k|k-1} \] (3.11)

(c) and the predicted covariance as

\[ P^-_k = \sum_{i=0}^{2L} W^{(c)}_i [\chi^x_{i,k|k-1} - \hat{x}^-_k][\chi^x_{i,k|k-1} - \hat{x}^-_k]^T \] (3.12)

(d) Each of the prediction points is instantiated through the observation model

\[ Y_{i,k|k-1} = H[\chi^x_{i,k|k-1}, \chi^n_{i,k|k-1}] \] (3.13)

(e) and the predicted observation is calculated by

\[ \hat{y}^-_k = \sum_{i=0}^{2L} W^{(m)}_i Y_{i,k|k-1} \] (3.14)

2. Measurement Update Equations
3.4 UKF preliminary results and discussion

(a) The innovation covariance is given by

\[ P_{y_k|y_k} = \sum_{i=0}^{2L} W_i^{(c)} [Y_{i,k|k-1} - \hat{y}_k] [Y_{i,k|k-1} - \hat{y}_k]^T \]  

(3.15)

(b) The cross covariance matrix is determined by

\[ P_{x_k|y_k} = \sum_{i=0}^{2L} W_i^{(c)} [\chi_{i,k|k-1} - \hat{x}_k] [Y_{i,k|k-1} - \hat{y}_k]^T \]  

(3.16)

(c) The Kalman gain can be computed as

\[ K = P_{x_k|y_k} P_{y_k|y_k}^{-1} \]  

(3.17)

(d) The \textit{a posteriori} estimation for the augmented state is given by

\[ \hat{x}_k = \hat{x}_k - K (y_k - \hat{y}_k) \]  

(3.18)

(e) and the \textit{a posteriori} estimate error covariance is

\[ P_k = P_k - K P_{y_k|y_k} K^T \]  

(3.19)

The UKF as valuable advantages in relation to EKF [9]

- the UKF is able to predict the state of the system more accurately than EKF in almost all applications;
- the UKF is much less difficult to implement, because it does not involve the computation of Jacobian matrices;
- the number of sigma points can be extended to yield a filter that matches moments up to the fourth order. This higher order extension effectively de-biases almost all common nonlinear coordinate transformations;
- the sigma points are chosen deterministically from the statistics of the transformation - second-order properties of the distribution can be propagated with only a small amount of statistical information; and
- the approximation itself can be interpreted more generally than as a probability distribution.

3.4 UKF preliminary results and discussion

As done in relation to EKF, the first attempt to test UKF implementation was to estimate the same generated synthetic data. All the conditions previously described for integration were kept.

For the UKF application, the initial conditions chosen for states and parameters were the same as in EKF, \( \hat{x}_0 = [50, 0.3, 0.85, 30, 0.01, 0.03] \) and \( \hat{p}_0 = 1.1 p_{nv} \). The observation model considered was the same. The two obtained systems are represented on Figure 3.1.
3. Unscented Kalman Filter

![Simulation and Estimated System](image)

**Figure 3.1:** Simulated system with synthetic data (on the left) and the respective estimated system with UKF (on the right).

The estimates obtained with UKF are compared with the integrated states and the nominal values of parameters in Figure 3.2(a) where it is possible to see that they are very close for observable variables (X1, X3, X45, and X7). The non directly measurable states present the same behavior but with an offset between the expected value and the estimated value (X2 and X6).

Looking at parameter estimates in figures 3.2(b), 3.2(c) and 3.2(d) it is possible to see that their behavior is a bit strange. It can be due to the fact that noise is included in the augmented state.

These results indicates that UKF can be a good method to deal with estimation in biochemical models, because observable states are very accurately estimated, but in this concrete case, probably UKF will have a worst performance than EKF, because the errors in estimative of hidden states and parameters with synthetic data are higher and some additional constrains must be imposed.
3.4 UKF preliminary results and discussion

(a) States estimates.

(b) Parameters $k$, $\alpha$, $\beta_1$, $h_{11}$, $h_{25}$, $\beta_2$ and $h_{22}$ estimates.

(c) Estimative of parameters $h_{2ATP}$, $\beta_3$, $h_{33}$, $h_{3P_i}$, $h_{3NAD}$, $\beta_5$, $h_{525}$, $\beta_5$ and $h_{513}$ estimates.

(d) Estimative of parameters $h_{515}$, $h_{51P_i}$, $\beta_6$, $h_{616}$, $h_{613}$, $h_{61NAD}$, $\beta_9$, $h_{626}$ and $k_{45}$ estimates.

Figure 3.2: Comparison between the nominal values (in blue) and the respective estimated values (in green) with UKF for synthetic data.
3. Unscented Kalman Filter

The quadratic-square error was computed according to (2.24) to quantify the estimation error and the results compiled in Table 3.1.

<table>
<thead>
<tr>
<th>State</th>
<th>ε_{syntheticdata}</th>
<th>Parameter</th>
<th>ε_{syntheticdata}</th>
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<td>$k$</td>
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</table>

In fact, the MSE errors computed are smaller than the ones computed for EKF for all the observable states. The maximum MSE occurs again to $X_1$, but now the value is around 1%, about six times smaller than the one obtained for the EKF estimate. However, the hidden states are much more difficult to estimate. It was necessary to impose an extra bound condition to guarantee filter convergence. If any state has a value higher than 100mM, their current time step value is replaced for the previous one. It is because of this condition that state $X_6$ presents a more or less constant value around 100mM. If this condition is not imposed, the filter diverges. $X_2$ is estimated with a reasonable accuracy, and the state behavior is kept along UKF process. The estimates for parameters are slight better than with EKF in the way that a higher number of parameters converge to their nominal value but this convergence is slower than with EKF, even for parameters that converge to a constant value distinct from their respective nominal value. Some parameters, like $\beta_{52}$, are still changing their value at the end of the simulation. Because of these reasons, a large error obtained for parameter estimation with UKF are expected.
4 Structural Analysis

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4. Structural Analysis

When a model is developed, not all the variables involved are equally relevant in system dynamics description. To understand which variables are more important to explain the system behavior is addressed along this chapter, along with a complementary stability study.

4.1 Sensitivity Studies

Before parameter estimation is crucial to have an idea about which variables are more influential in the system, because the difficulty of this task may be directly related with this aspect. If a parameter is important in the system, any variation of its value will be reflected in the whole system’s dynamics. In fact, the assumption above is not so simple as it seems to be because when one is dealing with a large set of parameters they can mask theirs influences behind others parameters, partially due to the existence of correlation between them. Even so, a parameter that by itself has not a strong influence in the system will be much more difficult to estimate (with the methods considered) than one for which small perturbations in its numerical value are reflected in large output changes.

Starting from the nominal values presented in Table 1.2 (on page 5), defined in this section as the true parameters values, the true simulation of the system was performed. After the true simulation, the system was simulated again, changing one parameter each turn, by a factor of ±10%, ±20% and ±30% in relation to its nominal value, keeping all the others with their respective nominal value. The simulation results consists of graphical representations, which results are compiled on Appendix 9.

4.1.1 Qualitative Analysis

The graphical results were carefully studied in a qualitatively way. That results are summarized on Tables 4.1 to 4.6 in order to facilitate their interpretation. The criteria adopted along tables construction to classify the relevance of the parameter to the system were the following: if the output system simulated with nominal values is distinguishable from the one simulated with a different parameter, a plus signal is put in the corresponding table entry; a +/− signal is put when there are variations but these variations are not large or when only during a time interval the state representations are different; an entry with a − signal is when the state remains more or less the same, with no large variations and the two representations are almost overlapped. If the state dynamics is completely lost, a * is put on the respective table entry.

When parameters are changed +10% in relation to their nominal values the state that is more influenced is $X_{45}$ and the least influenced is $X_{1}$. This is expected because $X_{1}$ depends on only three parameters (that are no longer used along the other states description) and itself; $X_{1}$ does not suffer influence from other parameters even if they change the restants states. $X_{45}$ is one of the states described by a higher number of parameters, so it is normal that its behaviour is
4.1 Sensitivity Studies

Table 4.1: Qualitative study when parameters change +10% in relation to their nominal values

<table>
<thead>
<tr>
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<th>State</th>
</tr>
</thead>
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</tr>
<tr>
<td>$h_{515}$</td>
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</tr>
<tr>
<td>$h_{51P_i}$</td>
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</tr>
<tr>
<td>$h_{51P_i}$</td>
<td>−</td>
</tr>
<tr>
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<td>−</td>
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<td>$h_{613}$</td>
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<td>$h_{61NAD}$</td>
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<td>$h_{616}$</td>
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<td>$h_{61}$</td>
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<td>$h_{2ATP}$</td>
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<td>$h_{33}$</td>
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<td>$h_{51P_i}$</td>
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<td>$h_{61NAD}$</td>
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</tr>
<tr>
<td>$h_{61}$</td>
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</tr>
</tbody>
</table>

Table 4.1: Qualitative study when parameters change +10% in relation to their nominal values

easier affected by small changes. The parameters that have the smallest influences are $\beta_{52}$, $h_{525}$, $h_{515}$, $\beta_{61}$, $h_{616}$, $h_{61NAD}$ and $k_{45}$. On the other side, $\beta$ is the parameter that conditiones all states dynamics, so it is expected that its estimation will not be hard. $h_{11}$, $h_{22}$, $h_{2ATP}$, $\beta_3$, $h_{33}$, $\beta_{51}$, $h_{513}$ and $h_{3P_i}$, are also very relevant, influencing all states but $X_1$, so their estimation is also expected to be easy.

According with what was expected, higher changes in parameters correspond to higher changes in system output, what can be seen comparing Tables 4.1 and 4.2. The parameters considered least relevant when a deviation of +20% is performed are the same as indicated for Table 4.1. The parameter $h_{3P_i}$ assumes now a relevant role on system dynamics, comparable to the more influent parameters refered for the +10% change.

When a change of +30% is performed only three parameters does not really affect the system behavior: $\beta_{52}$, $h_{525}$, and $h_{61NAD}$. All the other influence more than one state (see Table 4.3). The state more influenced is $X_6$, which is justified by the same reason presented above for $X_{45}$.

It is curious to see that when parameters change +10% or -10% the results are not the same (see Tables 4.1 and 4.4). This is an indication that system is nonsymmetric. The state more influenced is again $X_{45}$. The parameters that have small influences are the same that were indicated for when a +10% change is performed, plus $h_{613}$, that in this case only influence $X_7$. $\beta$, $h_{12}$, $h_{2ATP}$, $\beta_3$, $h_{33}$, $\beta_{51}$ and $h_{513}$ are the parameters that lead to higher changes in states dynamics for this variation of parameters (see Table 4.4).
4. Structural Analysis

Table 4.2: Qualitative study when parameters change +20% in relation to their nominal values

<table>
<thead>
<tr>
<th>Parameters</th>
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<th></th>
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</tr>
</thead>
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<td>+/−</td>
</tr>
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<tr>
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<td>+/−</td>
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<tr>
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<td>+/−</td>
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</table>

As can be seen in Table 4.5 when a change of -20% is performed the parameters with small contributions to system output variations are the same indicated for Table 4.4. A higher number of parameters is responsible for large output changes, what is in accordance with the discussion above. $X_{45}$ is the state more altered, being affected by twenty of the twenty-seven parameters.

When a deviation of -30% of parameter values in relation to their nominal values is performed only three parameters do not have large influences in system dynamics, $\beta_{52}$, $h_{525}$ and $h_{61NAD}$ (see Table 4.6). These three parameters are not expected to be able to estimate because they do not influence the system for any variation. A -30% change is more influential on system output than a +30% change: fifteen parameters influence at least five of the the six states. The parameters that are in $X_1$ definition ($k$, $\alpha$ and $\beta$) influence the six states.
### 4.1 Sensitivity Studies

#### Table 4.3: Qualitative study when parameters change +30% in relation to their nominal values

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#### Table 4.4: Qualitative study when parameters change -10% in relation to their nominal values

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Table 4.5: Qualitative study when parameters change -20% in relation to their nominal values

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Table 4.6: Qualitative study when parameters change -30% in relation to their nominal values

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</table>
4.1.2 Quantitative Analyses

In quantitative terms, a Sensitivity Matrix was computed for each % of variation of parameters, according to (4.1), where \( N \) is the number of time instants considered.

\[
S_{(\text{parameter, state})} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_{i, \text{nominal value}} - x_{i, \text{different parameter}})^2}
\] (4.1)

Based on these matrices it is possible to find which parameter is more influent on each state, looking for the maximum of each row. The results are summarized on Tables 4.7 to 4.12.

Table 4.7: Quantitative study when parameters change -30% in relation to their nominal value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>State 1</th>
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<th>State 3</th>
<th>State 45</th>
<th>State 6</th>
<th>State 7</th>
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<td>2.56</td>
<td>8.22</td>
<td>0.52</td>
<td>10.52</td>
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<tr>
<td>( \alpha )</td>
<td>0.85</td>
<td>0.50</td>
<td>1.95</td>
<td>5.12</td>
<td>0.42</td>
<td>7.39</td>
</tr>
<tr>
<td>( \beta )</td>
<td>3.63</td>
<td>10.18</td>
<td>8.48</td>
<td>21.40</td>
<td>1.87</td>
<td>35.53</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>0.63</td>
<td>2.58</td>
<td>12.73</td>
<td>0.42</td>
<td>12.75</td>
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</tr>
<tr>
<td>( h_{11} )</td>
<td>1.43</td>
<td>5.70</td>
<td>27.54</td>
<td>0.94</td>
<td>26.12</td>
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<tr>
<td>( h_{12} )</td>
<td>0.74</td>
<td>5.24</td>
<td>29.66</td>
<td>0.79</td>
<td>27.39</td>
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<tr>
<td>( h_{25} )</td>
<td>0.52</td>
<td>0.97</td>
<td>4.25</td>
<td>0.24</td>
<td>4.92</td>
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<td>0.28</td>
<td>2.44</td>
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<td>0.35</td>
<td>12.10</td>
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<tr>
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<td>4.60</td>
<td>21.33</td>
<td>0.73</td>
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<tr>
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<td>4.51</td>
<td>20.62</td>
<td>0.66</td>
<td>21.52</td>
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<tr>
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<td>7.02</td>
<td>48.99</td>
<td>1.42</td>
<td>32.17</td>
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<tr>
<td>( h_{33} )</td>
<td>0.88</td>
<td>6.65</td>
<td>49.19</td>
<td>1.99</td>
<td>35.95</td>
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<tr>
<td>( h_{3P_1} )</td>
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<td>3.72</td>
<td>33.18</td>
<td>0.65</td>
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<tr>
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<td>11.94</td>
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<tr>
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<tr>
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<td>0.00021</td>
<td>0.0038</td>
<td>0.42</td>
<td>0.00053</td>
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<td>2.73</td>
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To facilitate the analysis of the tables the maximum of each column, that is the parameter which has large influence in that state, was colored. The maximum differences occur for state \( X_{45} \), what implies the composed variable PEP+PGA is the one more sensible to small perturbations in parameters. One can think that it could be directly related with the elevated number of parameters used to describe \( X_{45} \); however, \( X_6 \) needs also a comparable number of parameters and does not show comparable variations.

The quantitative and qualitative analysis show that the parameters that have more influence in \( X_{45} \) are associated with large variations at qualitative level in all the states but \( X_1 \). In relation to \( X_6 \), it seems that a perturbation in one parameter is supported by the others, even when the parameter in question is one with influence in the whole system, suggesting that the mask effect, previously described, is really doing its role, what directly implies large difficulties to estimate the parameters.
4. Structural Analysis

Table 4.8: Quantitative study when parameters change -20% in relation to their nominal value

<table>
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<tr>
<th>Parameter</th>
<th>$X_1$</th>
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<td>4.62</td>
<td>5.99</td>
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<td>7.89</td>
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<td>2.40</td>
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</tbody>
</table>

Table 4.9: Quantitative study when parameters change -10% in relation to their nominal value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
<th>$X_4$</th>
<th>$X_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0.41</td>
<td>0.12</td>
<td>0.74</td>
<td>2.25</td>
<td>0.15</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.25</td>
<td>0.11</td>
<td>0.57</td>
<td>1.42</td>
<td>0.12</td>
</tr>
<tr>
<td>$\beta$</td>
<td>1.14</td>
<td>1.25</td>
<td>2.99</td>
<td>7.10</td>
<td>0.67</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>0.21</td>
<td>0.82</td>
<td>4.21</td>
<td>0.13</td>
<td>4.16</td>
</tr>
<tr>
<td>$h_{11}$</td>
<td>0.64</td>
<td>2.28</td>
<td>11.67</td>
<td>0.38</td>
<td>10.89</td>
</tr>
<tr>
<td>$h_{12}$</td>
<td>0.46</td>
<td>1.27</td>
<td>6.81</td>
<td>0.27</td>
<td>6.25</td>
</tr>
<tr>
<td>$h_{25}$</td>
<td>0.19</td>
<td>0.35</td>
<td>1.59</td>
<td>0.087</td>
<td>1.78</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>0.080</td>
<td>0.76</td>
<td>3.76</td>
<td>0.11</td>
<td>3.86</td>
</tr>
<tr>
<td>$h_{22}$</td>
<td>0.29</td>
<td>1.55</td>
<td>7.61</td>
<td>0.23</td>
<td>7.73</td>
</tr>
<tr>
<td>$h_{2ATP}$</td>
<td>0.13</td>
<td>1.67</td>
<td>8.07</td>
<td>0.23</td>
<td>8.25</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.19</td>
<td>2.14</td>
<td>24.55</td>
<td>0.42</td>
<td>10.01</td>
</tr>
<tr>
<td>$h_{33}$</td>
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<td>7.07</td>
<td>48.31</td>
<td>1.43</td>
<td>32.54</td>
</tr>
<tr>
<td>$h_{3P_i}$</td>
<td>0.085</td>
<td>1.23</td>
<td>12.06</td>
<td>0.20</td>
<td>5.24</td>
</tr>
<tr>
<td>$h_{3NAD}$</td>
<td>0.026</td>
<td>0.26</td>
<td>3.91</td>
<td>0.054</td>
<td>1.23</td>
</tr>
<tr>
<td>$\beta_5$</td>
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<td>0.020</td>
<td>0.65</td>
<td>0.0026</td>
<td>0.094</td>
</tr>
<tr>
<td>$h_{25}$</td>
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<td>0.0013</td>
<td>0.14</td>
<td>0.00018</td>
<td>0.0063</td>
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<td>31.21</td>
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<td>9.59</td>
</tr>
<tr>
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<td>79.86</td>
<td>0.29</td>
<td>19.05</td>
</tr>
<tr>
<td>$h_{5P_i}$</td>
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<td>0.055</td>
<td>1.31</td>
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<td>0.20</td>
</tr>
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<td>1.14</td>
<td>11.35</td>
<td>0.12</td>
<td>4.93</td>
</tr>
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<td>5.82</td>
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</tr>
<tr>
<td>$h_{616}$</td>
<td>0.055</td>
<td>7.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$h_{613}$</td>
<td>0.13</td>
<td>18.68</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$h_{61NAD}$</td>
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</tr>
<tr>
<td>$\beta_{52}$</td>
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<td>$h_{526}$</td>
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</tr>
<tr>
<td>$k_{45}$</td>
<td>0.056</td>
<td>0.15</td>
<td>0.33</td>
<td>0.031</td>
<td>0.83</td>
</tr>
</tbody>
</table>
## 4.1 Sensitivity Studies

### Table 4.10: Quantitative study when parameters change +10% in relation to their nominal value

<table>
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<tr>
<th>Parameter</th>
<th>State</th>
<th>( X_1 )</th>
<th>( X_2 )</th>
<th>( X_3 )</th>
<th>( X_{45} )</th>
<th>( X_6 )</th>
<th>( X_7 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k )</td>
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<td>0.37</td>
<td>0.088</td>
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<td>1.91</td>
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<td>1.84</td>
</tr>
<tr>
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<td></td>
<td>1.02</td>
<td>0.31</td>
<td>2.53</td>
<td>5.04</td>
<td>0.55</td>
<td>8.64</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td></td>
<td>0.21</td>
<td>0.80</td>
<td>4.19</td>
<td>0.13</td>
<td>4.10</td>
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</tr>
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<td></td>
<td>0.90</td>
<td>2.89</td>
<td>15.86</td>
<td>0.46</td>
<td>14.57</td>
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</tr>
<tr>
<td>( h_{12} )</td>
<td></td>
<td>0.78</td>
<td>0.85</td>
<td>3.74</td>
<td>0.31</td>
<td>3.25</td>
<td></td>
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<tr>
<td>( h_{25} )</td>
<td></td>
<td>0.21</td>
<td>0.38</td>
<td>1.79</td>
<td>0.094</td>
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<tr>
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<td>0.071</td>
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<td>3.66</td>
<td>0.010</td>
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<td>1.55</td>
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<td>0.22</td>
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<td>0.12</td>
<td>1.84</td>
<td>9.50</td>
<td>0.25</td>
<td>9.51</td>
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</tr>
<tr>
<td>( \beta_3 )</td>
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<td>0.15</td>
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<td>25.81</td>
<td>0.31</td>
<td>6.75</td>
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<td>0.829</td>
<td>8.676</td>
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<tr>
<td>( h_{3P_i} )</td>
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<tr>
<td>( h_{3NAD} )</td>
<td></td>
<td>0.026</td>
<td>0.27</td>
<td>3.82</td>
<td>0.056</td>
<td>1.29</td>
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</tr>
<tr>
<td>( \beta_{22} )</td>
<td></td>
<td>0.0011</td>
<td>0.020</td>
<td>0.64</td>
<td>0.0026</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>( h_{25} )</td>
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<td>7.18e-005</td>
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<td>0.15</td>
<td>0.00019</td>
<td>0.0064</td>
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<td>2.65</td>
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<td>0.25</td>
<td>10.75</td>
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<td>7.40</td>
<td>47.89</td>
<td>0.88</td>
<td>29.62</td>
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</tr>
<tr>
<td>( h_{515} )</td>
<td></td>
<td>0.0029</td>
<td>0.052</td>
<td>1.28</td>
<td>0.00080</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
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<td>5.73</td>
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<tr>
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<td></td>
<td>0.062</td>
<td>8.83</td>
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</tr>
<tr>
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<tr>
<td>( h_{61NAD} )</td>
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<td>0.0033</td>
<td>0.47</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>( \beta_{62} )</td>
<td></td>
<td>0.24</td>
<td>2.62</td>
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<tr>
<td>( k_{45} )</td>
<td></td>
<td>0.051</td>
<td>0.14</td>
<td>0.31</td>
<td>0.029</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.11: Quantitative study when parameters change +20% in relation to their nominal value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>State</th>
<th>( X_1 )</th>
<th>( X_2 )</th>
<th>( X_3 )</th>
<th>( X_{45} )</th>
<th>( X_6 )</th>
<th>( X_7 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k )</td>
<td></td>
<td>0.71</td>
<td>0.15</td>
<td>1.23</td>
<td>3.57</td>
<td>0.25</td>
<td>4.84</td>
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<tr>
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<td>0.43</td>
<td>0.14</td>
<td>0.96</td>
<td>2.27</td>
<td>0.20</td>
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<td>1.58</td>
<td>8.37</td>
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<td>103.58</td>
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<td>4.10</td>
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<td>0.19</td>
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<td>47.89</td>
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<td>29.62</td>
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<tr>
<td>( h_{3P_i} )</td>
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<tr>
<td>( h_{51NAD} )</td>
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<td>0.47</td>
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<tr>
<td>( \beta_{52} )</td>
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</tr>
<tr>
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<tr>
<td>( k_{45} )</td>
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<td>0.14</td>
<td>0.31</td>
<td>0.029</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

37
parameters only associated to $X_6$. The task to estimate these parameters are still harder if one remembers that $X_6$ is not directly measurable and the only way to correct its value is by correct $X_7$, because $f_7$ depends on $X_6$ but if $X_6$ does not change, $f_7$ also does not have large changes and it is quiet impossible to estimate the parameters.

$\beta$ is the parameter that is more influent in $X_1$ for all the percentage variations of its value. Maybe due to the dependency of $f_2$ on $X_1$, this parameter is also responsible for large changes in $X_2$ (Tables 4.7 to 4.9); on the other three situations (Tables 4.10 to 4.12) parameter $h_{11}$ is the one responsible for large $X_2$ dynamics changes. Both parameters are among the more influent parameters discussed along qualitative analysis. $X_3$ is more influenced by parameters $h_{513}$ (Tables 4.7, 4.8 and 4.10), $h_{33}$ (Table 4.9) and $h_{11}$ (Tables 4.11 and 4.12). $X_{45}$ is more influenced by $h_{513}$ when the variations are negative and $h_{33}$ when variations are positive. $X_6$ is conditioned by $h_{626}$ (Tables 4.7 and 4.8), $h_{33}$ (Table 4.9), $h_{513}$ (Table 4.10) and $h_{11}$ (Tables 4.11 and 4.12). $X_7$ variation depends on $h_{626}$ (Table 4.7), $h_{33}$ (Table 4.8), $h_{513}$ (Tables 4.9 and 4.10) and $h_{11}$ (Tables 4.11 and 4.12). It can be concluded that $h_{11}$ is the parameter in relation to which more system output changes occur; this parameter should be easy to estimate, if this study holds.
4.2 Stability and Finite-Time Escape

When one is working with differential equations a very important issue is concerned with their stability. In some equations a phenomenon known as Finite-Time Escape can occur. It means that the solution can go to infinity in finite time and the system diverges. If it happens during a estimation procedure with Kalman filter based approaches, the filter will also diverge.

With the aim of limit parameter value regions, to overcome these aspect, a qualitative study was performed. It is known that if the derivative does not grow faster than a linear function, then the solution does not go to infinity in finite time.

The system under study is in the form

$$\dot{x} = f(x, \theta)$$  \hspace{1cm} (4.2)

Explicitly, $x$ represent the states and $\theta$ is the array of parameters. Having a biological support, one knows that rate constants ($\alpha_i$ and $\beta_j$ parameters) are limited to the range [0,10] and the exponents($h_{mn}$) are in the range [-4,4]. $k_{45}$ is a ratio in the range of [1,3]. The simulations performed are presented in the following figures. The time instant considered was $t = 10\text{min}$.

4.2.1 GLU flux - $f_1$ stability study

$f_1$ depends on state $X_1$ and on parameters $k$, $\alpha$ and $\beta$. The qualitative study of $f_1$ stability is presented on Figure 4.1.

Looking to $f_1$ stability study figures, one can see that the function is linear for $X_1$, $k$ and $\alpha$. However, when $\beta$ goes to the upper limit, $f_1$ quickly goes to large negative values; the behavior observed corresponds to a scale problem, and not a some kind of singularity in the system.

4.2.2 G6P flux - $f_2$ stability study

$f_2$ depends on states $X_1$, $X_2$ and $X_{45}$ and on parameters $\beta_1$, $h_{11}$, $h_{12}$, $h_{25}$, $\beta_2$, $h_{22}$, $h_{2ATP}$ and $k_{45}$. The qualitative study of $f_2$ stability in relation to states variation is in Figure 4.2.

Looking at the Figure 4.2 it is possible to see that while state $X_1$ changes, $f_2$ changes linearly; when state $X_4$ changes, $f_2$ changes quickly than linear but does not go to infinity, so in relation to these two states $f_2$ is considered stable. However, when $X_2$ approaches zero, $f_2$ goes to infinity what is due to the fact that $h_{12}$ has negative nominal value. One should be careful with it during filtering calculations because $X_2$ is one of the hidden states and a large error prediction can compute a priori estimate for $X_2$ around zero, what may leads to system divergence.

The results for $f_2$ stability in relation to parameters variations are presented on Figure 4.3. Based on these results it does not seem that problems can arise when parameters change along the predefined interval; just some scale problems can arise, as it was comment about $f_1$ behavior.
4. Structural Analysis

\[ f_1 \]

\[ f_1 \] depends on states \( X_1 \) and \( X_3 \) and on parameters \( \beta_2, h_{22}, h_{2ATP}, \beta_3, h_{33}, h_{3P}, \) and \( h_{3NAD} \).

On Figure 4.4 is shown the behavior of \( f_3 \) in function of \( X_2 \) and \( X_3 \). \( f_3 \) behaves linearly along \( X_2 \) and \( X_3 \) variations, suggesting that the function is stable in relation to both states.

The variations of \( f_3 \) in relation to the mentioned parameters are in Figure 4.5. The evolution of \( f_3 \) with parameters does not seem to present instability problems. Some scale problems can arise when \( h_{22}, h_{2ATP}, h_{33}, h_{3P}, e h_{3NAD} \) becomes higher, but this is also function of the states because no singularities are associated directly to parameters. This can only be a problem if the
4.2 Stability and Finite-Time Escape

![Graphs showing variation of $f_2$ in function of $\beta_1$, $h_{11}$, $h_{25}$, $\beta_2$, $h_{22}$, $h_{2ATP}$, and $k_{45}$](image_url)

**Figure 4.3:** $f_2$ stability study in relation to parameters dependence.

![Graphs showing variation of $f_3$ in function of $X_2$ and $X_3$](image_url)

**Figure 4.4:** $f_3$ stability study in relation to states dependence.
state also goes to infinity what is not true. In relation to $\beta_3$, $f_3$ behaves like a constant.

4.2.4 PEP and PGA flux - $f_{45}$ stability study

$f_4$ depends on states $X_1$, $X_2$, $X_3$ and $X_{45}$ and on parameters $\beta_1$, $h_{11}$, $h_{12}$, $h_{25}$, $\beta_3$, $h_{31}$, $h_{3P1}$, $h_{3NAD}$, $h_{525}$, $h_{513}$, $h_{515}$, $h_{51P1}$, and $k_{45}$.

$f_{45}$ dependence on states can be seen on Figure 4.6. The behavior of $f_{45}$ in function of $X_2$ is similar to the one of $f_2$ in function of $X_2$. This is again because of the negative nominal value of $h_{11}$. In relation to the other states there are no relevant considerations to make.

About parameters, the behavior of $f_{45}$ in function of each parameter is in Figures 4.7 and 4.8. In relation to $\beta_3$, $f_{45}$ behaves like a constant, as well as in relation to $h_{525}$, $h_{525}$, $\beta_{51}$ and $h_{513}$. Scale problems arise when $f_{45}$ is represented in function of $h_{11}$, $h_{12}$, $h_{25}$, $h_{33}$, $h_{3P1}$, $h_{3NAD}$, $h_{515}$, $h_{51P1}$.
4.2 Stability and Finite-Time Escape

4.2.5 PYR flux - $f_6$ stability study

$f_6$ depends on states $X_1$, $X_2$, $X_3$, $X_45$ and $X_6$ and on parameters $\beta_1$, $h_{11}$, $h_{12}$, $h_{25}$, $\beta_{51}$, $h_{533}$, $h_{515}$, $h_{51P}$, $\beta_{61}$, $h_{616}$, $h_{613}$, $h_{61NAD}$, $\beta_{62}$, $h_{626}$ and $k_{45}$.

The results for $f_6$ stability are on Figure 4.9 for states and on Figures 4.11 and 4.11 for parameters. Analyzing these figures one can see that the behaviors presented are similar to the ones that are being discussed. Scale problems arise when $X_2$ approaches zero; in relation to $X_1$, $X_3$ and $X_6$ $f_6$ behaves linearly and in function of $X_{45}$ grows faster than a linear function but just for values of $X_{45}$ smaller than 10mM; after that $f_6$ grows slower.

About parameters, scale problems arise when $h_{11}$, $h_{12}$, $h_{25}$, $h_{513}$ and $h_{51P}$ approaches the upper limit defined for parameter range and when $k_{45}$ goes to the lower limit predefined. $f_6$ behaves linearly in function of $\beta_1$ and more or less like a constant for $\beta_{61}$, $h_{616}$, $h_{613}$, $\beta_{62}$ and $h_{626}$.

4.2.6 LAC flux - $f_7$ stability study

$f_7$ depends on states $X_3$ and $X_6$ and on parameters $\beta_{61}$, $h_{616}$, $h_{613}$ and $h_{61NAD}$.

The qualitative study of $f_7$ stability is presented on Figure 4.12. $f_7$ behaves linearly in relation to $X_3$ and $X_6$. Some bad scale problems can arise for parameters, but it is not probable due to

Figure 4.6: $f_{45}$ stability study in relation to states dependence.

and $k_{45}$. $f_{45}$ behaves linearly in function of $\beta_1$. 
4. Structural Analysis

Figure 4.7: $f_{45}$ stability study in relation to parameters dependence.

As conclusion, the study system is well-behave if parameters were kept in their biological range, that is the range of interest for this work.
4.2 Stability and Finite-Time Escape

**Figure 4.8:** $f_{45}$ stability study in relation to parameters dependence (continuation).

**Figure 4.9:** $f_{6}$ stability study in relation to states dependence.
Figure 4.10: $f_6$ stability study in relation to parameters dependence.
4.2 Stability and Finite-Time Escape

Figure 4.11: $f_6$ stability study in relation to parameters dependence (continuation).

Figure 4.12: $f_7$ stability study.
5 Results

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5. Results

5.1 Model Integration

The system is presented as a set of ODE’s in the form

\[ \dot{x} = f(x) \]  \hspace{1cm} (5.1)

Since no explicit analytical solution exists, some numeric integration method should be used. The Kalman-based filters need to know the model of the system with a high level of accuracy and for this reason the system was integrated using the Euler method.

Briefly, the Euler method is a first-order numerical procedure for solving ODE’s. The aim is to approximate the solution of the initial value problem

\[ y'(t) = f(t, y(t)) \]
\[ y(t_0) = y_0 \]  \hspace{1cm} (5.2)

using the first two terms of the Taylor expansion of \( y \). Basically it corresponds to the linear approximation around the point \((t_0, y(t_0))\). Defining \( \Delta \) as the integration step of the method from one instant \( t_n \) to the next one \( t_{n+1} \), the solution \( y_{n+1} \) is explicitly given by

\[ y_{n+1} = y_n + \Delta f(t_n, y_n) \]  \hspace{1cm} (5.3)

As mentioned, the method is explicit, what is a fundamental advantage in the posterior connection with the Kalman-based filters. In this particular case, the integration of the augmented state is done by

\[ X_{k+1} = \begin{bmatrix} x_{k+1} \\ \theta_{k+1} \end{bmatrix} = \begin{bmatrix} x_k + \Delta f(x_k, \theta) + w_k-1 \\ \theta_k \end{bmatrix} \]  \hspace{1cm} (5.4)

and the integration of only states

\[ x_{k+1} = x_k + \Delta f(x_k, \theta) + w_k \]  \hspace{1cm} (5.5)

Here, \( f \) represents the model in (1.3).

As the Euler method is the most basic method for numerical integration, some inconsistencies may occur along the calculations. As one is dealing with power-laws with fractional exponents, it should be kept in mind that mathematically it is possible to obtain complex solutions. However, the states have a biological meaning, so they must be real and, as they are concentrations, nonnegative. During integration, when one state presents a negative value it must be corrected, because the biological significant is lost, what is equivalent to say that the model is no longer useful. It is also important to notice that due to the fact that states are powers of non integer exponents, if one state is negative, its derivative will be complex, what, once again, does not have correspondence with biological phenomena. To overcome these limitations, a safe-guard must be imposed, avoiding the occurrence of negative states. The approach chose was to condition the global dynamic behavior of the system, preserving the local dynamics when one is in the
presence of numerical inaccuracies. In detail it means that at a generic instant $k$, $x_k$ is a vector of positive states; when the integration procedure is applied, if $x_{k+1}$ has one negative entry (one state is negative), that state is back to the value it had in $x_k$.

Coarsely, it corresponds to disdain the influence of first order derivatives in that time step. If one is considering very small time steps, this is not a big problem, but is a good solution. Many times, when a negative state appears it is due to a very brusque transition, attributed to some numerical errors.

During EKF estimation procedures it was verified that keep with the same numerical value only the state that is negative (letting all the rest system evolve) performed worst, so the strategy adopted was to arrest all the augmented state ($\tilde{X}_{k+1} = \tilde{X}_k$).

### 5.2 Construction of Splines

The major intention of this work is to estimate the experimental NMR data available and not to estimate the synthetic data that comes from the model (1.3) integration. The time series available have been acquired with a 2.2 minutes time-step between each measure and because of this reason there is no information between each measurement. In order to infer some information during each gap one can use splines, a very-well known method that interpolates a set of points with a 3rd order precision. The construction of splines might very useful in the correction (or measurement update) block of the Kalman filter; if one does not use it, corrections could only be made each time a measure is available.

Nevertheless, some considerations about splines construction must be done. GLU spline was constructed in the time interval $t \in [0, 16.5] \text{ min}$. One extra value for glucose concentration was added to glucose data (when $t = 16.5 \text{ min}$, $[GLU] = 0.01 \text{ mM}$) to give more information to spline function, having the goal of obtain an interpolation function that mimetizes better the experimental decayment observed. Outside the interval considered, spline was extrapolated with a constant function. FBP spline was done in time interval $t \in [0, 18.7] \text{ min}$. Two extra points of $[FBP]$ were added, the first was when $t = 0 \text{ min}$, $[FBP] = 0.01 \text{ mM}$ and the second was when $t = 18.7 \text{ min}$, $[FBP] = 0.01 \text{ mM}$. Outside the time interval used, the spline was extrapolated by a constant function. LAC spline was construct along all time interval used in the simulations. Because one does not have the basal concentration of lactate (there is no record of $[LAC]$ when $t = 0 \text{ min}$), the value of $0.01 \text{ mM}$ was imposed. The reason why one does not have access to this information was discussed in the NMR spectroscopy section (see Chapter 1); it is simply because the normal carbon present in cells is not magnetic active - only the isotope $^{13}\text{C}$, with which the glucose given in pulse was labeled, is magnetic active and one can only have a measure after one complete glycolysis. The PEP and PGA spline is the one that deserves more considerations. The model (1.3) considers a variable $X_{45}$ that is the sum of these two metabolites. To use the data
5. Results

available coherently, PEP and PGA experimental data should be added. Another important point is the fact known from literature about the basal levels (before glucose pulse) of these metabolites are not zero [4]; the same can be inferred from the two-glucose-pulse experiments, during which it was observed that the decayment of PEP and PGA is quickly and approximately quadratic. This suggests that spline extrapolation in this time interval should be done quadratically. As summary, the PEP and PGA spline was done in the interval \( t \geq 7.7 \text{min} \). Two extra points were added in the begin, to overcome the blindness of extrapolation; for \( t = 0 \text{min} \) \([\text{PEP} + \text{PGA}] = 40 \text{mM}\) and for \( t = 1.1 \text{min} \) \([\text{PEP} + \text{PGA}] = 20 \text{mM}\). The splines obtained were presented in Figure [5.1] they are overlapped with experimental data and model equations integrated.

![Figure 5.1: Representation of splines (broken lines), model equations integrated (full line) and experimental data points. Splines follow all data points. Model equations are closer to GLU and FBP data points. LAC is overestimated by the model, as well as PEP and PGA sum.](image)

5.2.1 Estimation of spline data with EKF

To proceed to the estimation of the spline data the first step is to obtain the system to estimate. State \( X_1 \) is obtained evaluating GLU spline in each time step; the same is done for \( X_3, X_{45} \) and \( X_7 \), by evaluation of the respective spline. \( X_2 \) and \( X_6 \) correspond to non directly measurable states so their time evolution is not needed to the filter, but just to have an idea how the system should look like (for comparison purposes), \( f_2 \) and \( f_6 \) were integrated by (5.5).

In relation to the filter, the initial conditions were the same used for synthetic data estimation (\( \hat{x}_0 = [50, 0.3, 0.85, 30, 0.01, 0.03] \) and \( \hat{p}_0 = 1.1 p_{\text{ms}} \)). Along EKF procedures, \( W, H, V \) and \( A \) matrices were computed according to equations (2.8), (2.9), (2.10) and (2.7), respectively. The \( H \) matrix is coincident with the matrix used for estimation of synthetic data (2.23). \( Q \) and \( R \) were defined as in (1.12). The \textit{a priori} state \( \hat{x}^-_{k} \) and covariance \( P^-_{k} \) estimative were computed applying (2.20). After a measurement \( z_k \) (2.4), the estimative are updated by (2.21), what the gives the \( a \)
posteriori estimative for state $\hat{x}_k$ and covariance error $P_k$. Before the filter goes to the next time step, the estimated system is checked with to intuit to find any negative state; if it happens, then all the augmented state is retained, that is, the current estimative $\hat{x}_k$ is replaced by the previous $\hat{x}_k = \hat{x}_{k-1}$, as it was described on the previous section. The simulated and estimated systems are presented on Figures 5.2(a) and 5.2(b) respectively.

![Figure 5.2: Simulated system with spline data (on the left) and the respective estimated system with EKF (on the right). The points on the left plot correspond to the experimental data points and the lines are the spline functions. While the interpolated system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.](image)

As can be seen both systems look very similar, what is confirmed in the Figure 5.3(a) where each reconstructed state is directly compared with its estimative.

States $X_2$ and $X_6$ are hidden states - the blue line in Figure 5.3 is purely indicative and is obtained integrating the respective state equation of the model (1.3) (on page 4), the filter does not know these data. State estimation are very good, specially all directly measurable states whose estimative is very accurately. For hidden states, state dynamics is accompanied, but there are large errors. $X_2$ estimation presents a the more or less constant offset after the state achieve steady-state dynamics, as can be seen on Figure 5.3(a).

Parameter estimation is not so good as state estimation, but an interesting observation is that parameters quickly converge to constant values that for the majority of parameters are different from nominal values. The best estimative are for parameters $h_{25}$, $\beta_2$, $\beta_3$, $h_{3P_i}$ and $\beta_{52}$. It is important to mentioned that an identifiability problem could be in the root of this result, because the set of parameters with nominal values and the estimated set of parameters both are able to reproduce the system dynamics. Connecting this result with the structural analysis, $\beta_3$ and $h_{3P_i}$ are two parameters that by itself have a large influence in the system, so it is expected a result like the one observed. By its turn, $\beta_{52}$ is one example of a parameter that by itself have a small influence in the system behavior but its estimation is well performed by EKF, indicating that the system behavior is a result of synergy, where each part cannot be study in separated from the others.
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In relation to the filter, the initial conditions were the same used for synthetic data estimation.

Results

Comparison between the nominal values (in blue) and the respective estimated values (in green) are presented on Figures 5.4(a) and 5.4(b).

5.2.2 Estimation of spline data with UKF

Figure 5.3: Comparison between the nominal values (in blue) and the respective estimated values (in green) with EKF for spline-based reconstructed data.

(c) Parameters $h_{2ATP}$, $\beta_3$, $h_{33}$, $h_{3PI}$, $h_{3NAD}$, $\beta_2$, $h_{25}$, $\beta_1$ and $h_{511}$ estimates.

(d) Parameters $h_{515}$, $h_{51PI}$, $\beta_{61}$, $h_{616}$, $h_{613}$, $h_{61NAD}$, $\beta_{62}$, $h_{626}$ and $k_{45}$ estimates.

A more detailed comparison about state estimation is presented on Figure 5.5(a), where it is
5.3 Experimental Data Measurements

Figure 5.4: Simulated system with spline data (on the left) and the respective estimated system with UKF (on the right). The points on the left plot correspond to the experimental data points and the lines are the spline functions. While the interpolated system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.

It is possible to see that non directly measurable states estimates are worst than with EKF. Once again, UKF does not have access to the blue line data represented along with $X_2$ and $X_6$ estimates. In relation to measurable states, they are accurately estimated.

About parameters, whose estimates are represented on Figures 5.5(b) to 5.5(d) most of them only converge to a constant value after the system has achieved steady-state. Parameters $k$, $h_{11}$, $h_{25}$, $h_{22}$, $h_{3P}$, $h_{51P}$, and $\beta_{62}$ converge to their nominal value; $h_{11}$, $h_{3P}$, and $h_{51P}$, are among the parameters that should be easy to estimate, based on structural analysis (see Chapter 4). A curious observation is that parameters $\alpha$ and $\beta$ seem do not converge to constant values during simulation time.

5.2.3 Comparison between EKF and UKF in the estimation of spline data

To have a quantitative criteria to compare both EKF and UKF estimate, the respective quadratic-square errors were computed according to (2.24) and the results compiled in Table 5.1.

The observable states are better estimated with UKF. The maximum error for $X_1$ is less than 6%, about five times smaller than the one obtained with EKF. In relation to hidden states, $X_2$ is estimated with more or less the same accuracy with both methods (the error is about 64% with EKF and 58% with UKF). $X_6$ is better estimated with EKF. In relation to parameters, all of them are better estimated with EKF. The large deviations obtained with UKF are due to difficulties of parameter estimation convergence, what presents large oscillations during first minutes of simulation.

5.3 Experimental Data Measurements

One can even go in a harder task - what happens if no spline data were used to measurement update in filters, but only the data points from experimental time series?
To use data spline to correct predictions is not the ideal solution because one cannot be sure that extrapolation is being done in the right conditions.

A more realistic approach is to separate prediction and measurement update cycles inside the filter. Doing so, one can compute Kalman filter only with the prediction step and just when a measure is available introduce the measurement correction. This leads to another problem that is the fact that observation matrix $H$ (equation (2.9)) cannot be constant. $H$ has to be dynamically

Figure 5.5: Comparison between the nominal value (in blue) and the respective estimated values (in green) with UKF for spline-based reconstructed data.
generated, that is, as not all metabolites have coincident time series, in some instants one can have measures of four metabolites, three metabolites or only two metabolites. Because of it, matrix $H$ needs to be computed each time instant. But this is only one trouble! In a system that is in $\mathbb{R}^{33}$, to have only two states with measurements could not be enough to assure the EKF convergence - the filter has to be adapted again! The divergence problem occurs associated with bad conditions of covariance matrix. To overcome these aspect one has to use the structure `try...catch`, available in MatLab®. Imagining that one has a measurement, if it makes the filter diverges, what one does is to ignore the measure, and assumes that the updated estimate is the predicted one.

5.3.1 Estimation of experimental data with EKF

This subsection is concerned with, perhaps, the best results of the whole work.

To proceed to the estimation with EKF the initial conditions were once again set to $\hat{x}_0 = [50, 0.3, 0.85, 30, 0.01, 0.03]$ and $\hat{P}_0 = 1.1P$. The noise variance was kept the same as previous. The big difference here is on measurement update. As one is only using experimental data points, without splines interpolation or another method to reconstruct the whole time series for each metabolite, matrix $H$ cannot be a constant because the time series have different lengths. Because of this, $H$ has to be dynamically generated at each time step, as it was said before. Another important point is that prediction and measurement have to be made independent, because in each time step of the filter cycle one has a prediction, but only when there is an experimental
5. Results

measurement at the same time instant the correction of the prediction is done. In a total of 62701 predictions for each metabolite, only 7 are corrected for GLU, 29 for LAC, 26 for PEP+PGA and 8 for FBP - a total of 70 corrections out of 62701×6 predictions. The result is amazing! X1 is very well described, the experimental data is accompanied by the estimated system in a very soft way. X3 is also very well estimated, despite some oscillations on the initial phase. X45 is one of the most interesting variables, the first observation occurs only about 10 minutes of simulation, so until there system is evolving freely; when a measurement correction is done, and after the third observation, the estimated system converges for the experimental data points and keeps that trend until the end. X7 presents large oscillations during the first minutes, but more or less after 10 minutes experimental data are very well covered by the model.

The estmative of parameters are also very close to nominal values, specially for k, α, h_{3NAD}, β_{52}, h_{525}, h_{515}, β_{61} and h_{61NAD}. Making a parallelism between these results and results of structural analysis, it seems strange that β_{52}, h_{525}, h_{515}, β_{61} and h_{61NAD} are very well estimated by EKF, because they do not have a strong influence by itself in system dynamics; h_{525} is the parameter that during structural analysis shown the smallest influence to system dynamics, any state is changed when this parameter changes but in this situation EKF is able to estimate it, what once again reinforces the idea that only a study where all the parts are taken in account together is completely valid.

Figure 5.6: Experimental data points (on the left) and EKF estimated system (on the right). The points on the left plot correspond to the experimental data points. While the experimental system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.
5.3 Experimental Data Measurements

(a) States estimates.

(b) Parameters $k$, $\alpha$, $\beta_1$, $h_{11}$, $h_{12}$, $h_{25}$, $\beta_2$ and $h_{22}$ estimates.

(c) Estimative of parameters $h_{2ATP}$, $\beta_3$, $h_{33}$, $h_{3P_i}$, $h_{3NAD}$, $\beta_2$, $h_{525}$, $\beta_1$ and $h_{513}$ estimates.

(d) Estimative of parameters $h_{515}$, $h_{51P_i}$, $\beta_6$, $h_{616}$, $h_{613}$, $h_{61NAD}$, $\beta_2$, $h_{626}$ and $k_4$ estimates.

Figure 5.7: Comparison between the nominal value (in blue) and the respective estimated values (in green) with EKF for experimental data.
5. Results

In order to have a quantitative criteria to discusses EKF estimates when only the experimental points are available to correct the predictions done by the filter, the respective MSE was computed by (2.24) and the results compiled on table 5.2.

| Table 5.2: MSE (%) for estimation of experimental data with EKF |
|-----------------|-----------------|-----------------|-----------------|
| State           | $\varepsilon_{\text{experimental data}}$ | Parameter       | $\varepsilon_{\text{EKF}}$ |
| $X_1$           | 49.02           | $k$             | 35.79           |
| $X_2$           | 46.27           | $\alpha$       | 43.45           |
| $X_3$           | 24.09           | $\beta$        | 10.29           |
| $X_{45}$        | 6.73            | $\beta_1$      | 10.11           |
| $X_6$           | 318.86          | $h_{11}$       | 10.85           |
| $X_7$           | 3.62            | $h_{12}$       | 9.54            |
|                 |                 | $h_{25}$       | 12.49           |
|                 |                 | $\beta_2$      | 12.83           |
|                 |                 | $h_{22}$       | 10.53           |
|                 |                 | $h_{2ATP}$     | 10.54           |
|                 |                 | $\beta_3$      | 12.91           |
|                 |                 | $h_{33}$       | 10.77           |
|                 |                 | $h_{3P_i}$     | 14.01           |
|                 |                 | $h_{3NAD}$     | 14.61           |
|                 |                 | $\beta_{52}$   | 18.78           |
|                 |                 | $h_{525}$      | 23.35           |
|                 |                 | $\beta_{51}$   | 11.00           |
|                 |                 | $h_{513}$      | 11.14           |
|                 |                 | $h_{515}$      | 47.10           |
|                 |                 | $h_{51P_i}$    | 15.10           |
|                 |                 | $\beta_{61}$   | 54.35           |
|                 |                 | $h_{610}$      | 11.31           |
|                 |                 | $h_{613}$      | 10.82           |
|                 |                 | $h_{61NAD}$    | 27.26           |
|                 |                 | $\beta_{62}$   | 10.46           |
|                 |                 | $h_{626}$      | 10.58           |
|                 |                 | $k_{45}$       | 10.39           |

The MSE’s for both states and parameters estimates are comprised between 7% and 54%, except for $X_6$ estimate, to which the MSE is about 318%. For $t$ higher than 20min, $X_6$ is approximately 0mM and the estimate, that is also near from 0mM, presents an offset to which is attributed higher importance because the expected value is very very small.

The MSE for $X_1$ and $X_2$ estimates are comparable, what means that EKF has a very good performance along the estimation. $X_{45}$ and $X_7$ are accurately estimated, with MSE around 7% and 4% respectively.

Relatively to parameters, MSE’s are comparable with the ones obtained for spline estimation 5.1(a). It is important to reinforce the identifiability problem, because, once again, despite the final numerical value, all parameters quickly converge to a constant value that is kept in the majority of time simulation.
Conclusions and Future Work
6. Conclusions and Future Work

This thesis was developed on the interface between biochemistry and control theory. It was really motivator to see how two different areas, that come from completely distinct backgrounds are so complementary.

Kalman-based filtering techniques proved to be adequate methods to estimate states and parameters in these kind of systems. The EKF has shown a good performance during estimation, for states and parameters. Even the non directly measured states are estimated accurately. The principal limitation of the EKF (linearization of nonlinear systems at the first step of the filter) seems not to compromise the results for this system. EKF is very stable and able to keep the system in its natural range only with few measurement corrections, as described along Chapter 5.

UKF implementation was a harder task, not because of the filter itself, which is much more easy than EKF (UKF implementation does not need to compute the Jacobian matrices), but because of the system that is far from trivial. The fact that the system has to be positive had numerous implications on UKF algorithm. The construction of sigma-points can lead to nonpositive points, that are iterated through the model according to (5.4). Because of it, complex states can appear. To avoid it only the real part of derivatives should be considered in the subsequent calculations. However, as discussed before, it can lead to numerical errors than may influence all the system dynamics and also the covariance error matrix (3.19). In the UKF, a posteriori covariance error matrix must be positive defined, because the algorithm implies the determination of the matrix square root, what may be done by Cholesky factorization, that is only defined for matrices that hold this condition. There is no intensive discussion on literature about the parameters of the UKF (α, β and κ) and it was verified that these parameters are determinant on filter performance. Their adjustment was done based on try-error attempts and the values chosen were the ones tested that best fits the model in study. Despite of it, a more systematic study should be done, in order to try to figure out what are the exact relevance of parameters on filter performance.

In the first section of Chapter 5 some considerations about integration method were done, namely one related with the guarantee of positive states. The strategies used do not have an exact mathematical background, being just conditions that allow to approximate the result to what is intended. This suggests that some kind of new theory for positive systems should be developed, to avoid either complex either non positive states that do not have biological meaning.

During estimation procedure one problem that was needed to deal with was the few number of experimental points. The ideal solution would be to have much more experimental points in order to use only these data, to avoid synthetic data and splines or another type of interpolation. The results shown that the studies of metabolic networks modeling with Kalman filter are going on the right direction, suggesting that filter techniques could be appropriated methods to deal with these kind of systems biology problem. As future work proposals there is one task for biochemistries that is to have a higher number of measurements in the same experimental conditions, maybe alternating the experience times. It means, the data with one is working starts at $t = 1.1\text{min}$ and
after goes on with a step of $2.2\text{min}$; if another experience, with the same start conditions, begins at $t = 2\text{min}$, for example, and goes on with the same time step, these two experimental data could be compiled and used to have a more realistic idea of the system. With this approach one would have more real data points in the gaps that one has now and these results would be crucial along filtering procedures. Another important point would be to limit concentrations of metabolites, even those that are non directly measurable. To know what is the minimal concentration that is able to be detected by NMR experiments for each metabolite is an extremely valuable information that can be incorporated to guide the estimation.

The estimation of experimental data points, having only the few experimental measurements, as done with the EKF (on Section 5.3.1) was also tried with UKF but the UKF diverges during the first instants of the simulation, before the first measurement correction to be available (at $t = 1.1\text{min}$). To avoid the UKF divergence some additional constrains must be used and because some of them do not have neither biological neither mathematical support the results were not included on the thesis. Once again, to know the detection limit for each metabolite with a high level of accuracy will have a crucial importance and might be used to impede filter divergence. A more intensive study should be performed in order to understand what can be done to improve the estimation with UKF of the few points of the experimental time series. Two recent publication that applier Kalman-based filter techniques to biological systems present two distinct results. Quach et al. [15] discuss very well the UKF and intensively advise its use. However, Sun et al. [16] indicate that UKF has a worst performance than EKF. It suggests that there is a lot of work to do in order to understand the suitability of this techniques in function of the mathematical models.

An important point that was not discussed is the coherence between the detection limits and the model predicted concentrations. The detection limit is about 3mM but particularly for $X_2$, that is one of the not directly measurable variables, the model attributes a concentration higher than the limit during the first minutes, even for time instants where other specimens have experimental measurements.

From the modeling point of view, the results with Kalman filters were promising so the natural next step is to try other filters, like particle filters (for example Unscented Particle Filter). To test the implemented filters in others biochemical systems is also a future work proposal, being the Appendix A a first draft in that direction.

However, the study of the system with EKF and UKF is not a closed chapter. One task that should also be done is to determine the convergence regions for the filters, that is, how far can one starts from real system values and guarantee filter convergence.

An idea that has been present since the work began was to construct a toolbox that facilitates the application of the implemented filters to other systems, different from the system studied. This toolbox would also give the possibility to estimate only a subset of the complete set of parameters, for any system and would be prepared to make the estimations with both EKF and UKF and to
6. Conclusions and Future Work

compare the results. To do it for the UKF is relatively easy because the filter implementation is not difficult. However, for the EKF is mandatory to compute the Jacobian matrices. It implies that symbolic calculations must be performed what in MatLab® means an enormous augment of computation time. This problem as to be carefully analyzed and the solution may be related with some kind of interface between two different programing languages, like Mathematica® that is very powerful with symbolic calculations.

As summary, the realization of this thesis was interesting and motivating. This study shown that the interaction between different disciplines is a fundamental point to science development. The main goals of this work were achieved and a lot of new knowledge was acquired. This thesis is a contribution to Systems Biology field, establishing a connection between traditional control techniques, from Electronic engineering, and biology. These techniques proved to be versatile and showed that they might be applied to a panoply of biochemical systems.
Bibliography


Bibliography


Mathematical Model of Glycolysis in Red Blood Cells
A. Mathematical Model of Glycolysis in Red Blood Cells

The main intention of this appendix is to describe the mathematical model of glycolysis in red blood cells, based on the work done by Holzhütter et al [17]. The appendix includes a brief introduction about red blood cells and their role in the regulation of microcirculation. After that the mathematical model is analyzed; the algorithm to deal with the model is explained in detail, as well as the UKF implementation to estimate one state and two parameters of this model. Results of estimation and conclusions are also presented.

A.1 Motivation

Red blood cells, or erythrocytes, are small, flexible, biconcave discs [18]. Their shape provides a large surface area for the diffusion of molecules gas, in comparison with a sphere or a cube [19]. Mature erythrocytes do not have a nucleus, mitochondria or other internal organelles [18] and because of that they cannot divide or carry on extensive metabolic activities [19]. Basically, they consist of a selectively permeable plasma membrane, cytosol and hemoglobin. Hemoglobin is the most abundant protein and also the pigment that gives whole blood its color [19].

Erythrocytes are responsible for one of the most important tasks in the body: the supply of oxygen to all cells. However, it has become increasingly clear that erythrocytes does not only carry and deliver oxygen, they also have an important participation in the regulation of its own distribution within microcirculation [18].

The current understanding about the fundamental physiological process of oxygen delivery is based on a century model proposed by Erlang and Krogh [20], which points out that perfused capillary density must be actively regulated in response to changing metabolic activity to ensure adequate tissue oxygenation. Many attempts have been done in order to propose a reasonable explanation for these mechanisms [18], but until now none fully explains the experimental observations [18].

A more recent study, by Stein and Ellsworth [21] suggested that in severe hypoxia, oxygen content (the extent of binding of oxygen to hemoglobin within the erythrocyte) is more important than oxygen tension (the extent of diffusive transfer of oxygen from the erythrocyte to the tissue). If it holds, then erythrocyte must have a crucial role in the regulation of oxygen delivery [18]. Another important point is the fact that erythrocytes release small amounts of ATP after an exposure to reduced oxygen content [22]. The figure A.1 represents esquematically the cascade of events initiated by the entrance of erythrocytes into a tissue region in which oxygen demand exceeds oxygen supply.

The energy metabolism in erythrocytes is also related with hemolysis. For example, pyruvate-kinase-deficient red blood cells exhibit a shortening of their life span in circulation [17], consequence of a disorder of the energy metabolism [17]. All these aspects are really interesting and because of them, red blood cells are a very stimulating case-study.
When oxygen supply does not meet oxygen demand, tissue oxygen tension \( P_{O_2} \) decreases. This decrease in tissue \( P_{O_2} \) causes the hemoglobin oxygen content of the erythrocytes that perfuse the tissue region to decrease proportionally. This decrease in oxygen content initiates a series of events resulting in the release of ATP from the erythrocyte. The ATP then diffuses to the endothelium (Endo) where it binds to purinergic (P2y) receptors resulting in the production of vasoactive mediators, either within the endothelium or the smooth muscle (SMC), which initiate vasodilation. This vasodilation is conducted (dashed arrow) in a retrograde fashion increasing flow and thus oxygen supply to the tissue region in need. In [18].

The mathematical model of glycolysis in human erythrocytes studied is based on reaction scheme shown in figure A.2. The calculations in [17] were performed for steady-state conditions. Because of that, the following flux reaction have to hold:

\[
\begin{align*}
    v_{HK} &= v_{PFK} \\
    v_{PK} &= 2v_{HK} \\
    v_{P_2GM} &= v_{P_2Gase} \\
    v_{ATPase} &= v_{PK} - v_{P_2GM}
\end{align*}
\]
A. Mathematical Model of Glycolysis in Red Blood Cells

For metabolites \((X)\) which may exist in a free \((X)\) or a magnesium complexed \((Mg \cdot X)\) form, \([X] = [\tilde{X}] + [Mg \cdot X]\), according to the equilibrium relation [17]

\[
\frac{[\tilde{X}][Mg]}{Mg \cdot X} = K_{Mg \cdot X}
\]

(A.2)

it follows that

\[
[\tilde{X}] = \frac{K_{Mg \cdot X}}{K_{Mg \cdot X} + [Mg]}[X]
\]

(A.3)

and

\[
[Mg \cdot X] = \frac{[Mg]}{K_{Mg \cdot X} + [Mg]}[X]
\]

(A.4)

where \(K_{Mg \cdot X}\) denotes the magnesium-complex binding constant and \([Mg]\) refers to the concentration of free magnesium [17].

The rate equations used in the model are presented in the following three subsections and the parameters values are collected in a table after equations [17].

A.2.1 Non-equilibrium enzymes

1. Hexokinase

\[
v_{HK} = v_{max}^{HK} \frac{[Mg \cdot ATP]}{K_{Mg \cdot ATP}} \left(1 + \frac{[Mg \cdot ATP]}{K_{Mg \cdot ATP}}\right) \left(1 + \frac{[Mg]}{K_{Mg}}\right) \left(1 + \frac{[G6P]}{K_{G6P}} + 1.55\right) \left(1 + \frac{[Mg]}{K_{Mg}} + \frac{[2,3P2G]}{K_{2,3P2G}}\right)
\]

(A.5)

2. Phosphofructokinase

\[
v_{PFK} = v_{max}^{PFK} \frac{[F6P][Mg \cdot ATP]}{([F6P] + K_{F6P}^{PFK})([Mg \cdot ATP] + K_{Mg \cdot ATP}^{PFK})(1 + L_{PFK}^{PFK} \left(\frac{[ATP]}{K_{ATP}^{PFK}}\right)^4)}
\]

(A.6)

3. 2,3 - Bisphosphoglycerate mutase

\[
v_{P2GM} = \frac{v_{max}^{P2GM} [1,3P2G]}{1 + \frac{[2,3P2G]}{K_{2,3P2G}^{P2GM}}}
\]

(A.7)

4. 2,3 - Bisphosphoglycerate phosphatase

\[
v_{P2Gase} = \frac{v_{max}^{P2Gase} [2,3P2G]}{K_{2,3P2G}^{P2Gase} + [2,3P2G]}
\]

(A.8)

5. Pyruvate kinase

\[
v_{PK} = \frac{v_{max}^{PK}}{(1 + \frac{K_{ADP}^{PK}}{[Mg \cdot ADP]})(1 + \frac{K_{ATP}^{PK}}{[Mg \cdot ATP]})(1 + \frac{[ATP]}{K_{ATP}^{PK}})^4(1 + \frac{[ADP]}{K_{ADP}^{PK}})^4)\frac{[Mg]}{K_{Mg}^{PK}}\frac{[2,3P2G]}{K_{2,3P2G}^{PK}}}
\]

(A.9)
A.2.2 Equilibrium enzymes

1. Adenylate kinase

\[ q_{AK} = \frac{[Mg \cdot ATP][Mg \cdot AMP]}{[Mg \cdot ADP]^2} \]  

(A.11)

2. Phosphoglucoisomerase

\[ q_{PGI} = \frac{[F6P]}{[G6P]} \]  

(A.12)

3. Aldolase

\[ q_{Ald} = \frac{[GAP][DHAP]}{[F2P]} \]  

(A.13)

4. Triose phosphate isomerase

\[ q_{TPI} = \frac{[DHAP]}{[GAP]} \]  

(A.14)

5. Glyceraldehydephosphate dehydrogenase

\[ q_{GAPD} = \frac{[1,3P2G][NADH]}{[P2][GAP][NAD]} \]  

(A.15)

6. Phosphoglycerate kinase

\[ q_{PGK} = \frac{[Mg \cdot ATP][3PG]}{[Mg \cdot ADP][1,3P2G]} \]  

(A.16)

\[ ^1 k_{ATPase} \text{ is a variable parameter} \]
A. Mathematical Model of Glycolysis in Red Blood Cells

7. Phosphoglyceromutase

\[ q_{\text{PGM}} = \frac{[3\text{PG}]}{[2\text{PG}]} \]  

(A.17)

8. Enolase

\[ q_{\text{Enol}} = \frac{[\text{PEP}]}{[2\text{PG}]} \]  

(A.18)

<table>
<thead>
<tr>
<th>Equilibrium constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( q_{\text{AK}} )</td>
<td>0.5</td>
</tr>
<tr>
<td>( q_{\text{PGI}} )</td>
<td>0.42</td>
</tr>
<tr>
<td>( q_{\text{Ald}} )</td>
<td>81</td>
</tr>
<tr>
<td>( q_{\text{TPI}} )</td>
<td>17.5</td>
</tr>
<tr>
<td>( q_{\text{GAPD}} )</td>
<td>0.18</td>
</tr>
<tr>
<td>( q_{\text{PGK}} )</td>
<td>1800</td>
</tr>
<tr>
<td>( q_{\text{PGM}} )</td>
<td>6.8</td>
</tr>
<tr>
<td>( q_{\text{Enol}} )</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table A.2: Values of the equilibrium constants. In [17].

A.2.3 Conservation quantities

1. Sum of adenine nucleotides ([\( A_0 \)] is a variable parameter of the model)

\[ [A_0] = [\text{AMP}] + [\text{ADP}] + [\text{ATP}] \]  

(A.19)

2. Total magnesium ([\( M_{g_0} = 2.8mM \)]

\[ [M_{g_0}] = [Mg] + [Mg \cdot \text{AMP}] + [Mg \cdot \text{ADP}] + [Mg \cdot \text{ATP}] + [Mg \cdot P_2G] \]  

(A.20)

3. Redox potential \( q_H = \frac{1}{1000} \)

\[ q_H = \frac{[\text{NADH}]}{[\text{NAD}]} \]  

(A.21)

4. Inorganic phosphate

\[ [P_i] = 0.94mM \]  

(A.22)

A.3 Algorithm

The algorithm to simulate the system steady-state used was similar to the one proposed in [17]. First of all, a value of [\( A_0 \)] is defined; after that [\( \text{ATP} \)] and [\( Mg \)] are chosen respecting the
conditions \( 0 \leq [ATP] \leq [A_0] \) and \( 0 \leq [Mg] \leq [Mg_0] \), respectively. The first two variables computed are \([ADP]\) and \([AMP]\), combining equations (A.11) and (A.19). With these two quantities is possible to find \([Mg \cdot ATP]\), \([Mg \cdot ADP]\) and \([Mg \cdot AMP]\) by equation (A.4). \([Mg \cdot P_2G]\) is computed with (A.20) and used in equation (A.4) to calculate \([2, 3P_2G]\). With this quantity is possible to determine \([1, 3P_2G]\) by the third flux relation (A.1). At this point is possible to compute \(v_{P_2GM}\) and \(v_{P_2Gase}\), respectively by equations (A.7) and (A.8). With (A.16) is possible to calculate \([3PG]\) and use it to find \([2PG]\) by equation eq:qpgm. \([2PG]\), by its turn, is used in (A.18) to determine \([PEP]\). \([GAP]\) is computed by (A.15) and introduced in (A.14) to determine \([DHAP]\); these two metabolites are used in (A.13) to calculate \([FP_2]\). \([ATP_{free}]\) is find due to its equilibrium with \([ATP]\) by equation (A.3). \(v_{FK}\) is computed by (A.9). Combining equation (A.12) with the first two flux relations (A.1) it is possible to calculate \([G6P], [F6P]\) and \([K^{HKG}_6P]\). \(v_{HK}\) and \(v_{PFK}\) are computed by their own equations, (A.5) and (A.6), respectively. Finally, \(v_{ATPase}\) and \(k_{ATPase}\) are computed by the last flux relation in (A.1) and by (A.10), respectively. This algorithm was implemented in MatLab®.

### A.4 UKF estimation of the red blood cells model

The aim is to illustrate how Kalman filter can be used to estimate this kind of system. A simplified version of UKF was implemented. The model consists of one state \((k_{ATPase})\) and two parameters \(([Mg] \text{ and } [ATP])\); the augmented state for joint estimation is \(x \in \mathbb{R}^3\).

\[
x = \begin{bmatrix} k_{ATPase} \\ [Mg] \\ [ATP] \end{bmatrix}
\]  

(A.23)

Only the state is observable, so \(z \in \mathbb{R}^1\).

As one is in steady state no time variation is introduced, so the models for the system (1.10) and observations (1.11) are very simple

\[
x_k = F(x_{k-1}) + w_{k-1}
\]

\[
z_k = H x_k + v_k
\]  

(A.24)

\(w\) and \(v\) are the process and measurement noise (respectively) in each step of simulation (1.12). The H matrix was defined as

\[
H = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix}
\]  

(A.25)

The process model F is

\[
F_{k-1} = \begin{bmatrix} f(x^{(2)}_{k-1}, x^{(3)}_{k-1}) \\ x^{(2)}_{k-1} \\ x^{(3)}_{k-1} \end{bmatrix}
\]  

(A.26)

where \(f\) is the function that implements the algorithm described in the previous section to simulate the system steady-state.
To begin the computation one has to define \([A_0]\) and choose reasonable values for \([ATP]\) and \([Mg]\). \(k_{ATPase}\) is calculated in the way described above. The state is these three quantities plus the noise contribution. For each step in the simulation the state is generated in this way, using the previous values for \([ATP]\) and \([Mg]\) to determine \(k_{ATPase}\).

In relation to UKF, the state considered was not augmented with the noise contributions, because the noise is additive. Sigma-points are calculated using equation (3.4). During time update process, each sigma-point is instantiating through the process model according to equation (3.10); the predicted mean and covariance are computed, respectively, by equations (3.11) and (3.12). In time update measurements, the predicted points are instantiated through observation model, according with equation (3.13) and the predicted observation is calculated by equation (3.14). In measurement update the innovation and cross covariance matrices are computed, respectively by equations (3.15) and (3.16). Kalman gain are computed as in equation (3.17). The \textit{a posteriori} estimative for the augmented state and for error covariance are computed as in equations (3.18) and (3.19), respectively.

The results are presented in figure A.3, where it is possible to see that the state \(k_{ATPase}\) is very well estimated; parameters estimative do not converge exactly to the true value but they keep in the same range of values.

![Figure A.3: Estimation of state and parameter with UKF for red blood cells model. In blue are the real values of state and parameters and in green the estimative.](image)

This example is just an illustration about what can be done with these kind of filter techniques. A more careful study must be done on this model, having the present work as the start point, because there is a lot of information to explore in this field.
Complementary Sensitivity Study

This appendix presents the graphical results that were the basis for discussion along the first section of Chapter 4.
B. Complementary Sensitivity Study

Figure B.1: Parameter $k$ changes -30% relatively to its nominal value

Figure B.2: Parameter $\alpha$ changes -30% relatively to its nominal value

Figure B.3: Parameter $\beta$ changes -30% relatively to its nominal value

Figure B.4: Parameter $\beta_1$ changes -30% relatively to its nominal value

Figure B.5: Parameter $h_{11}$ changes -30% relatively to its nominal value

Figure B.6: Parameter $h_{12}$ changes -30% relatively to its nominal value
Figure B.7: Parameter $h_{25}$ changes -30% relatively to its nominal value

Figure B.8: Parameter $h_{2ATP}$ changes -30% relatively to its nominal value

Figure B.9: Parameter $h_{2\beta}$ changes -30% relatively to its nominal value

Figure B.10: Parameter $h_{2ATP}$ changes -30% relatively to its nominal value

Figure B.11: Parameter $\beta_2$ changes -30% relatively to its nominal value

Figure B.12: Parameter $h_{3\beta}$ changes -30% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.13: Parameter $h_{3p1}$ changes -30% relatively to its nominal value

Figure B.16: Parameter $h_{25}$ changes -30% relatively to its nominal value

Figure B.14: Parameter $h_{3NAD}$ changes -30% relatively to its nominal value

Figure B.17: Parameter $\beta_{31}$ changes -30% relatively to its nominal value

Figure B.15: Parameter $\beta_{32}$ changes -30% relatively to its nominal value

Figure B.18: Parameter $h_{313}$ changes -30% relatively to its nominal value
Figure B.19: Parameter $h_{315}$ changes -30% relatively to its nominal value

Figure B.20: Parameter $h_{313}$ changes -30% relatively to its nominal value

Figure B.21: Parameter $\beta_{31}$ changes -30% relatively to its nominal value

Figure B.22: Parameter $h_{616}$ changes -30% relatively to its nominal value

Figure B.23: Parameter $h_{613}$ changes -30% relatively to its nominal value

Figure B.24: Parameter $h_{61,NAD}$ changes -30% relatively to its nominal value
**B. Complementary Sensitivity Study**

Figure B.25: Parameter $\beta_{h2}$ changes -30% relatively to its nominal value

Figure B.26: Parameter $h_{22}$ changes -30% relatively to its nominal value

Figure B.27: Parameter $k_{12}$ changes -30% relatively to its nominal value

Figure B.28: Parameter $k$ changes -20% relatively to its nominal value

Figure B.29: Parameter $\alpha$ changes -20% relatively to its nominal value

Figure B.30: Parameter $\beta$ changes -20% relatively to its nominal value
Figure B.31: Parameter $\beta_1$ changes -20% relatively to its nominal value

Figure B.32: Parameter $h_{11}$ changes -20% relatively to its nominal value

Figure B.33: Parameter $h_{12}$ changes -20% relatively to its nominal value

Figure B.34: Parameter $h_{25}$ changes -20% relatively to its nominal value

Figure B.35: Parameter $\beta_2$ changes -20% relatively to its nominal value

Figure B.36: Parameter $h_{22}$ changes -20% relatively to its nominal value
Figure B.37: Parameter $h_{2ATP}$ changes -20% relatively to its nominal value

Figure B.38: Parameter $\beta_3$ changes -20% relatively to its nominal value

Figure B.39: Parameter $h_{3ADP}$ changes -20% relatively to its nominal value

Figure B.40: Parameter $h_{3P_i}$ changes -20% relatively to its nominal value

Figure B.41: Parameter $h_{3NAD}$ changes -20% relatively to its nominal value

Figure B.42: Parameter $\beta_{32}$ changes -20% relatively to its nominal value
Figure B.43: Parameter $h_{525}$ changes -20% relatively to its nominal value

Figure B.44: Parameter $\beta_{51}$ changes -20% relatively to its nominal value

Figure B.45: Parameter $h_{513}$ changes -20% relatively to its nominal value

Figure B.46: Parameter $h_{515}$ changes -20% relatively to its nominal value

Figure B.47: Parameter $h_{51,13}$ changes -20% relatively to its nominal value

Figure B.48: Parameter $\beta_{51}$ changes -20% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.49: Parameter $h_{116}$ changes -20% relatively to its nominal value

Figure B.50: Parameter $h_{113}$ changes -20% relatively to its nominal value

Figure B.51: Parameter $h_{1NAD}$ changes -20% relatively to its nominal value

Figure B.52: Parameter $\beta_{12}$ changes -20% relatively to its nominal value

Figure B.53: Parameter $h_{26}$ changes -20% relatively to its nominal value

Figure B.54: Parameter $k_{12}$ changes -20% relatively to its nominal value
Figure B.55: Parameter $k$ changes -10\% relatively to its nominal value

Figure B.56: Parameter $\alpha$ changes -10\% relatively to its nominal value

Figure B.57: Parameter $\beta$ changes -10\% relatively to its nominal value

Figure B.58: Parameter $\beta_1$ changes -10\% relatively to its nominal value

Figure B.59: Parameter $h_{11}$ changes -10\% relatively to its nominal value

Figure B.60: Parameter $h_{12}$ changes -10\% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.61: Parameter $h_{25}$ changes -10% relatively to its nominal value

Figure B.62: Parameter $\beta_3$ changes -10% relatively to its nominal value

Figure B.63: Parameter $h_{2ATP}$ changes -10% relatively to its nominal value

Figure B.64: Parameter $h_{2ATP}$ changes -10% relatively to its nominal value

Figure B.65: Parameter $\beta_3$ changes -10% relatively to its nominal value

Figure B.66: Parameter $h_{25}$ changes -10% relatively to its nominal value
Figure B.67: Parameter \( h_{3P1} \) changes -10% relatively to its nominal value

Figure B.70: Parameter \( h_{326} \) changes -10% relatively to its nominal value

Figure B.68: Parameter \( h_{3NAD} \) changes -10% relatively to its nominal value

Figure B.71: Parameter \( \beta_{1} \) changes -10% relatively to its nominal value

Figure B.69: Parameter \( \beta_{2} \) changes -10% relatively to its nominal value

Figure B.72: Parameter \( h_{313} \) changes -10% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.73: Parameter $h_{515}$ changes -10% relatively to its nominal value

Figure B.74: Parameter $h_{515P}$ changes -10% relatively to its nominal value

Figure B.75: Parameter $h_{513}$ changes -10% relatively to its nominal value

Figure B.76: Parameter $h_{516}$ changes -10% relatively to its nominal value

Figure B.77: Parameter $h_{513}$ changes -10% relatively to its nominal value

Figure B.78: Parameter $h_{51NAD}$ changes -10% relatively to its nominal value
Figure B.79: Parameter $\beta_{26}$ changes -10% relatively to its nominal value

Figure B.80: Parameter $k_{26}$ changes -10% relatively to its nominal value

Figure B.81: Parameter $k_{45}$ changes -10% relatively to its nominal value

Figure B.82: Parameter $k$ changes +10% relatively to its nominal value

Figure B.83: Parameter $\alpha$ changes +10% relatively to its nominal value

Figure B.84: Parameter $\beta$ changes +10% relatively to its nominal value
B. Complementary Sensitivity Study

**Figure B.85:** Parameter $\beta_1$ changes +10% relatively to its nominal value

**Figure B.86:** Parameter $h_{11}$ changes +10% relatively to its nominal value

**Figure B.87:** Parameter $h_{12}$ changes +10% relatively to its nominal value

**Figure B.88:** Parameter $h_{25}$ changes +10% relatively to its nominal value

**Figure B.89:** Parameter $\beta_3$ changes +10% relatively to its nominal value

**Figure B.90:** Parameter $h_{22}$ changes +10% relatively to its nominal value
Figure B.91: Parameter $h_{2ATP}$ changes +10% relatively to its nominal value

Figure B.92: Parameter $\beta_1$ changes +10% relatively to its nominal value

Figure B.93: Parameter $h_{33}$ changes +10% relatively to its nominal value

Figure B.94: Parameter $h_{3F}$ changes +10% relatively to its nominal value

Figure B.95: Parameter $h_{3NAD}$ changes +10% relatively to its nominal value

Figure B.96: Parameter $\beta_{32}$ changes +10% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.97: Parameter $h_{25}$ changes +10% relatively to its nominal value

Figure B.98: Parameter $\beta_{21}$ changes +10% relatively to its nominal value

Figure B.99: Parameter $h_{213}$ changes +10% relatively to its nominal value

Figure B.100: Parameter $h_{515}$ changes +10% relatively to its nominal value

Figure B.101: Parameter $h_{51,P_1}$ changes +10% relatively to its nominal value

Figure B.102: Parameter $\beta_{61}$ changes +10% relatively to its nominal value
Figure B.103: Parameter $h_{616}$ changes +10% relatively to its nominal value

Figure B.104: Parameter $h_{613}$ changes +10% relatively to its nominal value

Figure B.105: Parameter $h_{61NAD}$ changes +10% relatively to its nominal value

Figure B.106: Parameter $\beta_{62}$ changes +10% relatively to its nominal value

Figure B.107: Parameter $h_{626}$ changes +10% relatively to its nominal value

Figure B.108: Parameter $k_{45}$ changes +10% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.109: Parameter $k$ changes +20% relatively to its nominal value

Figure B.110: Parameter $\alpha$ changes +20% relatively to its nominal value

Figure B.111: Parameter $\beta$ changes +20% relatively to its nominal value

Figure B.112: Parameter $\beta_1$ changes +20% relatively to its nominal value

Figure B.113: Parameter $h_{11}$ changes +20% relatively to its nominal value

Figure B.114: Parameter $h_{12}$ changes +20% relatively to its nominal value
Figure B.115: Parameter $h_{25}$ changes +20% relatively to its nominal value

Figure B.116: Parameter $\beta_2$ changes +20% relatively to its nominal value

Figure B.117: Parameter $h_{32}$ changes +20% relatively to its nominal value

Figure B.118: Parameter $h_{2ATP}$ changes +20% relatively to its nominal value

Figure B.119: Parameter $\beta_3$ changes +20% relatively to its nominal value

Figure B.120: Parameter $h_{33}$ changes +20% relatively to its nominal value
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Figure B.121: Parameter $h_{3P}$ changes +20% relatively to its nominal value

Figure B.122: Parameter $h_{SNAD}$ changes +20% relatively to its nominal value

Figure B.123: Parameter $\beta_2$ changes +20% relatively to its nominal value

Figure B.124: Parameter $h_{525}$ changes +20% relatively to its nominal value

Figure B.125: Parameter $\beta_{51}$ changes +20% relatively to its nominal value

Figure B.126: Parameter $h_{513}$ changes +20% relatively to its nominal value
Figure B.127: Parameter *h*₁₁₅ changes +20% relatively to its nominal value

Figure B.128: Parameter *h*₁₁₆ changes +20% relatively to its nominal value

Figure B.129: Parameter *β*₁₁ changes +20% relatively to its nominal value

Figure B.130: Parameter *h*₁₁₇ changes +20% relatively to its nominal value

Figure B.131: Parameter *h*₁₁₃ changes +20% relatively to its nominal value

Figure B.132: Parameter *h*₁₁₈ changes +20% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.133: Parameter $\beta_{62}$ changes +20% relatively to its nominal value

Figure B.136: Parameter $k$ changes +30% relatively to its nominal value

Figure B.134: Parameter $h_{626}$ changes +20% relatively to its nominal value

Figure B.137: Parameter $\alpha$ changes +30% relatively to its nominal value

Figure B.135: Parameter $k_{45}$ changes +20% relatively to its nominal value

Figure B.138: Parameter $\beta$ changes +30% relatively to its nominal value
Figure B.139: Parameter $\beta_1$ changes +30% relatively to its nominal value

Figure B.140: Parameter $h_{11}$ changes +30% relatively to its nominal value

Figure B.141: Parameter $h_{12}$ changes +30% relatively to its nominal value

Figure B.142: Parameter $h_{25}$ changes +30% relatively to its nominal value

Figure B.143: Parameter $\beta_2$ changes +30% relatively to its nominal value

Figure B.144: Parameter $h_{22}$ changes +30% relatively to its nominal value
B. Complementary Sensitivity Study

Figure B.145: Parameter $h_{\text{ATP}}$ changes +30% relatively to its nominal value

Figure B.146: Parameter $\beta_3$ changes +30% relatively to its nominal value

Figure B.147: Parameter $h_{\text{ADP}}$ changes +30% relatively to its nominal value

Figure B.148: Parameter $h_{\text{PI}}$ changes +30% relatively to its nominal value

Figure B.149: Parameter $h_{\text{NAD}}$ changes +30% relatively to its nominal value

Figure B.150: Parameter $\beta_2$ changes +30% relatively to its nominal value
Figure B.151: Parameter $h_{25}$ changes +30% relatively to its nominal value

Figure B.152: Parameter $\beta_{31}$ changes +30% relatively to its nominal value

Figure B.153: Parameter $h_{113}$ changes +30% relatively to its nominal value

Figure B.154: Parameter $h_{515}$ changes +30% relatively to its nominal value

Figure B.155: Parameter $h_{51,P1}$ changes +30%

Figure B.156: Parameter $\beta_{51}$ changes +30% relatively to its nominal value
B. Complementary Sensitivity Study

**Figure B.157**: Parameter $h_{616}$ changes +30% relatively to its nominal value

**Figure B.158**: Parameter $h_{613}$ changes +30% relatively to its nominal value

**Figure B.159**: Parameter $h_{61,NAD}$ changes +30% relatively to its nominal value

**Figure B.160**: Parameter $h_{62}$ changes +30% relatively to its nominal value

**Figure B.161**: Parameter $h_{626}$ changes +30% relatively to its nominal value

**Figure B.162**: Parameter $k_{45}$ changes +30% relatively to its nominal value