Simultaneous Estimation of States and Parameters in Metabolic Networks

from Filtering Techniques to Systems Biology

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Abstract

This work addresses the problem of state and parameter estimation in metabolic network models, using 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. The task of parameter estimation revealed to be a harder one, justified by the fact that certain parameters are constants whose individual variations do not strongly condition the system evolution. It is important to mentioned that certain parameters variation can be compensated by others maintaining the systems dynamics, although with a different numerical set of parameters, thereby suggesting that this model has an identifiability problem. Based on structural analysis, where the system sensitivity was studied in relation to each parameter, one can have an idea about which parameters would be more difficult to estimate. The techniques used revealed to be adequate to the problem in question and the results are promising to an investigation that goes in this direction.

Keywords: *Nonlinear state-space models, Extended Kalman filter, Unscented Kalman filter, parameter estimation, structural analysis, metabolic networks.*

1 INTRODUCTION

1.1 Motivation

Systems biology is an emerging area that intends to integrate several different sciences in order to achieve a better understanding of a biological system. 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 [1]. Metabolic control studies, specially dynamic modelling of metabolic pathways, is one of the main topics in Systems Biology. Both complementary areas of mathematical modelling and experimental biology 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.

1.2 The Problem

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 [2]. It is the most primitive pathway and exists in all actual living organisms, suggesting that it is a vital process in cells [2]. *Lactococcus lactis* is a gram-(+) bacterium, member of the lactic acid bacteria [3].

The glycolysis network can be reduced to a simple version and graphically translated into a flux diagram, without loss of crucial information, as in the Figure 1.

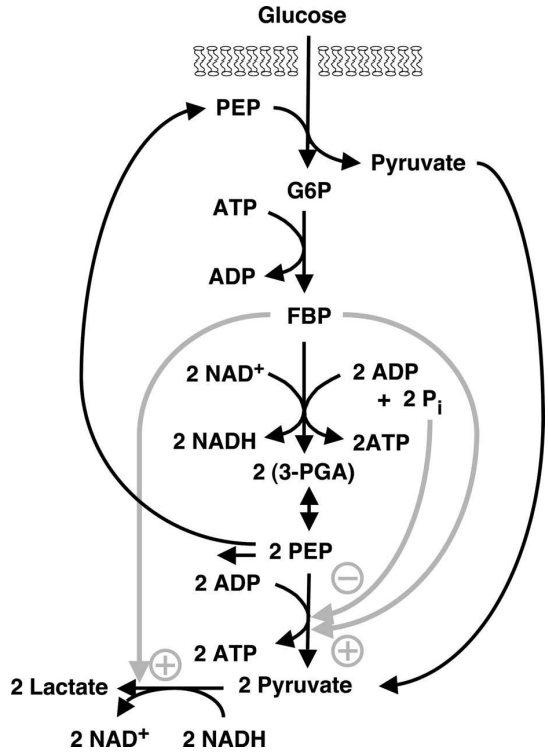


Figure 1: Metabolic pathway of glycolysis in *L. lactis*. Each vertix represents a metabolite and each edge corresponds to either a flux of mass or activation/inhibitory signals. In grey are activation and inhibitory signals. In [4].

Vinga *et al* [5] proposed a new model, based on the work developed by Voit *et al* [4], that intends to describe the metabolic pathway of glycolysis in *Lactococcus lactis*. It considers six equations characterized by a set of twenty-seven parameters. This model, described in equation (1)

below, is the starting point of the present work.

$$\begin{aligned}
f_1 &= -k(1+\alpha t^{\beta})X_1 \\
f_2 &= \beta_1 X_1^{h_{11}} X_2^{h_{12}} X_5^{h_{25}} - \beta_2 X_2^{h_{22}} ATP^{h_{2ATP}} \\
f_3 &= \beta_2 X_2^{h_{22}} ATP^{h_{2ATP}} - \beta_3 X_3^{h_{33}} Pi^{h_{3Pi}} NAD^{h_{3NAD}} \\
f_{45} &= 2\beta_3 X_3^{h_{33}} Pi^{h_{3Pi}} NAD^{h_{3NAD}} - \beta_1 X_1^{h_{11}} X_2^{h_{12}} X_5^{h_{25}} \\
&\quad -\beta_{51} X_3^{h_{513}} X_5^{h_{515}} Pi^{h_{51Pi}} - \beta_{52} X_5^{h_{525}} \\
f_6 &= \beta_1 X_1^{h_{11}} X_2^{h_{12}} X_5^{h_{25}} + \beta_{51} X_3^{h_{513}} X_5^{h_{515}} Pi^{h_{51Pi}} \\
&\quad -\beta_{61} X_6^{h_{616}} X_3^{h_{613}} NAD^{h_{61NAD}} - \beta_{62} X_6^{h_{626}} \\
f_7 &= \beta_{61} X_6^{h_{616}} X_3^{h_{613}} NAD^{h_{61NAD}} \qquad (1)
\end{aligned}$$

Here, the metabolite concentrations are represented by a state variable. Each f_i $(i = 1...7)$ functions corresponds to the respective $\dot{X}_i$; in other words, f_i corresponds to the time derivative (flux of mass) of the respective metabolite concentration:

$$f_i \equiv \dot{X}_i \qquad (2)$$

The integrated model is represented on Figure 2.

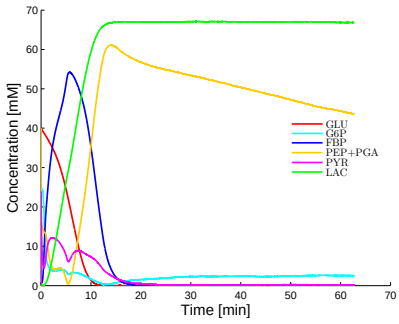


Figure 2: Graphical representation of the system after the integration of the model (1).

The specimens included in the model and their respective state variable are summarized in Table 1.

Table 1: Correspondence between metabolites and state variables

Metabolite	State variable
Glucose (GLU)	X_1
Glucose-6-Phosphate (G6P)	X_2
Fructose 1,6-Biphosphate (FBP)	X_3
3-Phosphoglycerate (PGA)	X_4
Phosphoenolpyruvate (PEP)	X_5
Pyruvate (PYR)	X_6
Lactate (LAC)	X_7

The other metabolites needed in the model, 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 modelling. 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 [5].

The model parameters obtained by Vinga *et al* [5] are summarized in Table 2; these values are considered the nominal values of parameters.

Table 2: Nominal values for the parameters. The experience behind is a glucose pulse of 40mM under aerobic conditions. For further information see [5].

Parameters					
k	0.0530251	h_{2ATP}	1.51599	h_{515}	0.0382342
α	0.0419958	β_3	0.338423	h_{51Pi}	0.211149
β	2.68092	h_{33}	1.09298	β_{61}	0.0324743
β_1	7.20321	h_{3Pi}	0.258372	h_{616}	0.675486
h_{11}	0.997546	h_{3NAD}	-0.0966562	h_{613}	1.03221
h_{12}	-1.48643	β_{52}	0.134164	h_{61NAD}	-0.0519436
h_{25}	0.38576	h_{525}	0.0940446	β_{62}	1.74742
β_2	0.345889	β_{51}	0.862421	h_{626}	1.40312
h_{22}	1.54399	h_{513}	0.7663	k_{45}	2.04035

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

1.3 Models for Metabolic Networks

Information on biological processes is becoming available in the form of metabolic and genetic time series, but to quantitatively characterize these processes is still a challenge [3]. 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 analysis, which is applied to variables in logarithmic space [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:

$$V_i = \gamma_i \prod_{j=1}^{n+m} X_j^{f_{ij}} \qquad (3)$$

where γ_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 [4].

The most useful alternative representations offered by BST are the Generalized Mass Action (GMA) and the S-Systems representations [4]. In the particular case of the model (1) the formalism used was GMA. **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 [4]; the generic GMA structure is

$$\dot{X}_i = \sum_{p=1}^{P_i} (\pm\gamma_{ip} \prod_{j=1}^{n} X_j^{f_{ipj}}), i = 1, ..., n \qquad (4)$$

The advantages and disadvantages of these formalism along with several examples of application are explored elsewhere (see for instance [1]).

1.4 Filtering and Nonlinear Estimation

Filtering can be defined as the problem of recursively estimating the states of a system from a set of observations available on-line [6]. Kalman Filter (KF) variants [7] are the most widely used approximation techniques for tracking and estimation due to its simplicity, optimality, tractability and robustness. KF algorithm is a very general treatment from the "state" point of view to the discrete-data linear filtering problem [7]. 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 [7]. Some fundamental assumptions are made in filter design:

1. the system should be linear or, at least, linearizable in a neighbourhood 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; and
3. the system's noise and the measurement's noise are not correlated.

The KF addresses the general problem of trying to estimate the state $x \in \Re^n$ of a discrete-time controlled system described by a linear stochastic difference equation [8]. 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.

The task of parameter estimation is crucial for modelling and basically corresponds to find the set of parameters able to minimize the predefined cost function [9]. The KF 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. An hypothesis to do it is to concatenate into a single joint state vector the states (x) and the parameters (θ) [9]:

$$X = \begin{bmatrix} x \\ \theta \end{bmatrix} \tag{5}$$

In this case, the process model reads as

$$X_k = F(X_{k-1}) = \begin{bmatrix} f(x_{k-1}, \theta_{k-1}) \\ \theta_{k-1} \end{bmatrix} \tag{6}$$

For this approach, known as **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 θ [9].

1.5 Original Contributions

This work explores the case study presented on [5], using KF based approaches (EKF and UKF) in a context different from the one they are commonly used for. 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 paper. The study shows that the use of filter techniques is adequate to metabolic networks modelling studies.

1.6 Paper Structure

The structure of the present paper is as follows. After Section 1, that motivates the problem to be solved and summarizes the overall contributions, Section 2 reviews the relationship between KF and EKF for nonlinear systems. Section 3 presents the limitations of EKF and motivates the UKF. Section 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 Section 5. Global discussion about the work and summary of principal results are in Section 6, as well as future work proposals.

2 EXTENDED KALMAN FILTER

The KF assumes systems linearity but its major application is on nonlinear systems analysis [10], 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 [8].

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 [9]. 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 [11].

3 UNSCENTED KALMAN FILTER

Although the EKF keeps the elegant and computationally efficient recursive update form of the KF, there are three major drawbacks that limit its use [11]:

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.

One of the most fundamental tasks in filtering and estimation is to calculate the statistics of a random variable which has undergone a transformation [12]. 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 [11].

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

$$y = g(x) \tag{7}$$

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 statistics from that distribution [11]. 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 [11].

The Unscented Transform (UT) is a method for calculating the statistics of a random variable which undergoes a nonlinear transformation [10]. A set of points - sigma points - are chosen so that their sample mean and sample covariance are $\bar{x}$ and P_{xx}, respectively [9]. 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 [10].

4 STRUCTURAL ANALYSIS

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

4.1 Sensitivity Studies

Before any parameter estimation procedure is crucial to have an idea about which variables are more influent in the system, because the difficult 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 2 (on page ii), 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 $\pm 10\%$, $\pm 20\%$ and $\pm 30\%$ in relation to its nominal value, keeping all the others with their respective nominal value.

4.1.1 Qualitative Analysis

The results were carefully studied in a qualitatively way. 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 remaining states. X_{45} is one of the states described by a higher number of parameters, so it is normal that its behavoir is easier affected by small changes.

The parameters that have the smallest influences are β_{52}, h_{525}, h_{515}, β_{61}, h_{616}, h_{61NAD} and k_{45}. On the other side, β is the parameter that conditions all states dynamics, so it is expected that its estimation will not be hard. h_{11}, h_{22}, h_{2ATP}, β_3, h_{33}, β_{51}, h_{513} and h_{51Pi} are also very relevant, influencing all states but X_1, so their estimation is also expected to be easy.

According with what was expected, the system output has large changes when the higher variation on parameters are performed. The parameters considered least relevant when a deviation of +20% is performed are the same as indicated above for a +10% deviation. The parameter h_{3Pi} 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 behaviour: β_{52}, h_{525} and h_{61NAD}. All the other influence more than one state. 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 what indicates that system is nonsymmetric. The state more influenced when the deviation in parameters is -10% 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. β, h_{12}, h_{2ATP}, β_3, h_{33}, β_{51} and h_{513} are the parameters that lead to higher changes in states dynamics for this variation of parameters.

When a change of -20% is performed the parameters with small contributions to system output variations are the same indicated for a deviation of -10%. 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, β_{52}, h_{525} and h_{61NAD}. 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 influent on system output than a +30% change: fifhteen parameters influence at least five of the the six states. The parameters that are in X_1 definition (k, α and β) influence the six states.

4.1.2 Quantitative Analysis

In quantitative terms, a Sensitivity Matrix was computed for each % of variation of parameters, according to (8), where N is the number of time instants considered.

$$S_{(parameter,state)} = \sqrt{1/N \sum_{i=1}^{N} (x_i^{\mathrm{nv}} - x_i^{\mathrm{dp}})^2} \qquad (8)$$

Here, *nv* and *dp* stand for nominal value and different parameter, respectively.

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 maximal difference occurs for state X_{45}, what implies that 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 is role, what directly implies large difficulties to estimate the parameters only associated to X_6. The task of estimate these parameters is still hard if one remembers that X_6 is not directly measurable and the only way to correct its value is by correcting 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 quite impossible to estimate the parameters. 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 phenomena 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 KF 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

$$\dot{x} = f(x, \theta) \qquad (9)$$

Explicitly, x represent the states and θ is the array of parameters. Having a biological support, one knows that rate constants (α_i and β_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 to make the study was $t = 10min$. Despite some scale problems, that can arise for example when state X_2 approaches zero (because parameter h_{12} has negative nominal value), the observed behaviour for the system indicates that the system is well-behave if parameters were kept in their biological range, that is the range of interest for this work.

5 RESULTS

5.1 Model Integration

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

$$\dot{x} = f(x) \qquad (10)$$

Since no explicit analytical solution exists, some numerical 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. This method is explicit, which 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

$$X_{k+1} = \begin{bmatrix} x_{k+1} \\ \theta_{k+1} \end{bmatrix} = \begin{bmatrix} x_k + \Delta f(x_k, \theta) + w_{k-1} \\ \theta_k \end{bmatrix} \qquad (11)$$

Here, f represents the model in (1) and w the noise present along the process.

The Euler method is the most basic method for numerical integration and 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.

5.2 Construction of Splines

The 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) 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 be 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.

The splines were constructed along all the time interval considered for the directly measurable variables, GLU, FBP, PEP/PGA and LAC. The obtained functions are presented in Figure 3; they are overlapped with experimental data and model equations integrated.

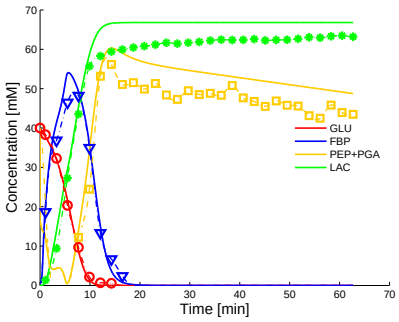


Figure 3: Representation of splines (broken lines), model equations integrated (full line) and experimental data points. Splines follow all data points. Model equations are closer to GLU and FBP data points. LAC is overestimated by the model, as well as PEP and PGA sum.

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 (11). In relation to the filter, the initial conditions were $\hat{x}_0 = [50, 0.3, 0.85, 30, 0.01, 0.03]$ and $\hat{p}_0 = 1.1p_{nv}$. Following EKF procedures, W, H, V and A matrices were computed as well as the *a priori* state $\hat{x}_k^-$ and covariance P_k^- estimative. After a measurement z_k, the estimative are updated, 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 4(a) and 4(b), respectively.

As can be seen both systems look very similar, what is confirmed in the Figure 5(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 is purely indicative and is obtained integrating the respective state equation of the model (1) (on page ii), the filter does not know these data. State estimation are

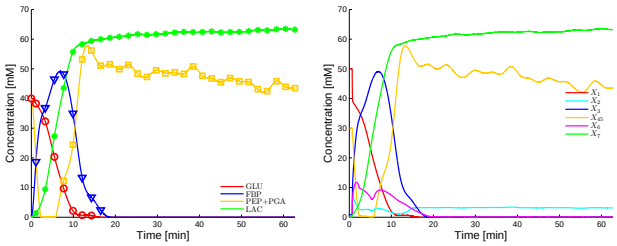
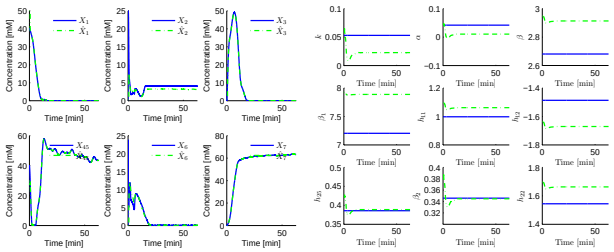
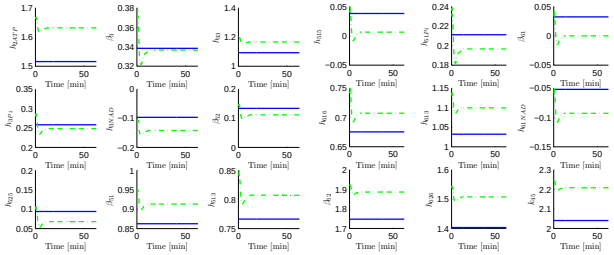


Figure 4: Simulated system with spline data (on the left) and the respective estimated system with EKF (on the right). The points on the left plot correspond to the experimental data points and the lines are the spline functions. While the interpolated system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.



(a) States estimates.

(b) Parameters k, α, β, β_1, h_{11}, h_{12}, h_{25}, β_2 and h_{22} estimates.



(c) Parameters h_{2ATP}, β_3, h_{33}, h_{3Pi}, h_{3NAD}, β_{52}, h_{525}, β_{51} and h_{513} estimates.

(d) Parameters h_{515}, h_{51Pi}, β_{61}, h_{616}, h_{613}, h_{61NAD}, β_{62}, h_{626} and k_{45} estimates.

Figure 5: Comparison between the nominal values (in blue) and the respective estimated values (in green) with EKF for spline-based reconstructed data.

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(a).

Parameter estimation is not as accurate 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}, β_2, β_3, h_{3Pi} and β_{52}. It is important to mentioned that an identifiability problem could be the cause of this result, because the set of parameters with nominal values and the estimated set of parameters are both able to reproduce the system dynamics. Connecting this result with the structural analysis,

β_3 and h_{3Pi} are two parameters that by itself have a large influence in the system, so a result like the one observed is expected. β_{52} is one example of a parameter that by itself have a small influence in the system behaviour but its estimation is well performed by EKF, indicating that the system behaviour is a result of synergy, where each part cannot be study in separated from the others.

5.2.2 Estimation of spline data with UKF

For UKF, the spline-based reconstructed system was done in the same way that for EKF. In relation to the filter, the initial conditions were the same as used for EKF estimation. The spline-based reconstructed system and the UKF estimated system are presented on Figures 6(a) and 6(b).

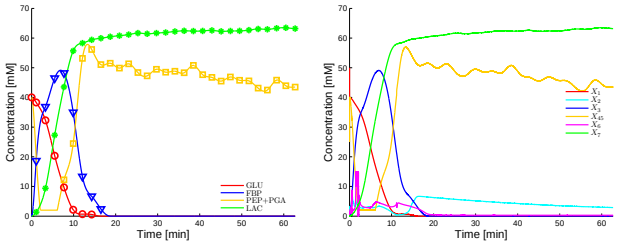
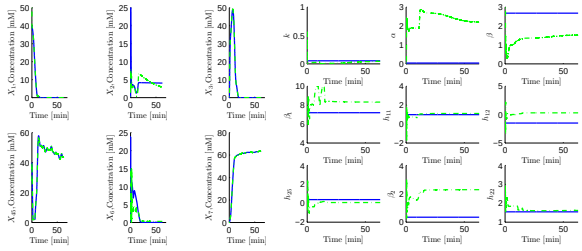
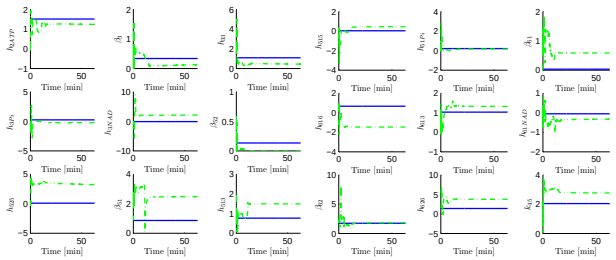


Figure 6: Simulated system with spline data (on the left) and the respective estimated system with UKF (on the right). The points on the left plot correspond to the experimental data points and the lines are the spline functions. While the interpolated system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.



(a) States estimates.

(b) Parameters k, α, β, β_1, h_{11}, h_{12}, h_{25}, β_2 and h_{22} estimates.



(c) Parameters h_{2ATP}, β_3, h_{33}, h_{3Pi}, h_{3NAD}, β_{52}, h_{525}, β_{51} and h_{513} estimates.

(d) Parameters h_{515}, h_{51Pi}, β_{61}, h_{616}, h_{613}, h_{61NAD}, β_{62}, h_{626} and k_{45} estimates.

Figure 7: Comparison between the nominal value (in blue) and the respective estimated values (in green) with UKF for spline-based reconstructed data.

A more detailed comparison about state estimation is presented on Figure 7(a), where it is possible to see that non directly measurable states estimates are worst than those obtained with EKF. Once again, UKF does not use the blue line data represented along with X_2 and X_6 estimates for measurement corrections purposes. In relation to measurable states, they are accurately estimated.

About parameters, whose estimates are represented on Figures 7(b) to 7(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_{3Pi}, h_{51Pi} and β_{62} converge to their nominal value; h_{11}, h_{3Pi} and h_{51Pi} are among the parameters that should be easy to estimate, based on structural analysis. A curious observation is that parameters α and β seem not to 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 EKF and UKF estimatives, the respective quadratic-square errors were computed according to (12)

$$\varepsilon_{EKF}^{syntheticdata} = \frac{\sqrt{\sum (X - \hat{X})^2}}{\sum X} \tag{12}$$

where X are the expected values for the augmented state and $\hat{X}$ the respective estimates. The results are compiled in Table 3.

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 the first minutes of simulation.

5.3 Experimental Data Measurements

One can even go in a harder task - what happens if no spline data is used to measurement update in filters, but only the data points from experimental time series? Using 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 KF 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 cannot be constant. H has to be dynamically 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

Table 3: MSE (%) for estimation of spline data.

(a) Estimation with EKF.

State	$\varepsilon^{\text{splinedata}}_{\text{EKF}}$
X_1	30.00
X_2	64.01
X_3	6.18
X_{45}	1.81
X_6	65.27
X_7	0.18
k	58.57
α	76.53
β	8.67
β_1	9.51
h_{11}	6.46
h_{12}	12.41
h_{25}	1.85
β_2	1.91
h_{22}	7.70
h_{2ATP}	7.66
β_3	2.00
h_{33}	6.76
h_{3Pi}	4.57
h_{3NAD}	47.38
β_{52}	17.28
h_{525}	28.78
β_{51}	5.91
h_{513}	5.41
h_{515}	85.02
h_{51Pi}	7.55
β_{61}	101.83
h_{616}	4.82
h_{613}	6.58
h_{61NAD}	79.66
β_{62}	7.97
h_{626}	7.47
k_{45}	8.26

(b) Estimation with UKF.

State	$\varepsilon^{\text{splinedata}}_{\text{UKF}}$
X_1	5.47
X_2	58.12
X_3	0.50
X_{45}	0.71
X_6	116.96
X_7	0.024
k	59.27
α	5583.19
β	50.64
β_1	18.88
h_{11}	20.04
h_{12}	116.46
h_{25}	103.23
β_2	527.02
h_{22}	11.85
h_{2ATP}	20.27
β_3	64.83
h_{33}	58.28
h_{3Pi}	215.37
h_{3NAD}	2435.43
β_{52}	99.16
h_{525}	3502.72
β_{51}	194.94
h_{513}	87.00
h_{515}	1234.41
h_{51Pi}	107.04
β_{61}	2017.88
h_{616}	309.23
h_{613}	31.91
h_{61NAD}	642.20
β_{62}	29.68
h_{626}	167.34
k_{45}	39.28

of it, matrix H needs to be computed at each time instant. But this is only one of the problems. In a system in $\Re^{33}$, having 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

To proceed to the estimation with EKF the initial conditions were the same as for spline data estimation. The major 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. Another important point is that prediction and measurement have to be made independently, because in each time step of the filter cycle one has a prediction, but only when there is an experimental 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. Since X_1 is very well described, the experimental data is accompanied by the estimated system in a very smooth way. X_3 is also very well estimated, despite some oscillations on the initial phase. X_{45} is one of the most interesting variable, 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. X_7 presents large oscillations during the first minutes, but more or less after 10 minutes experimental data are very well covered by the model.

The estimative 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 when all the parts are taken in to account together, the study is completely valid.

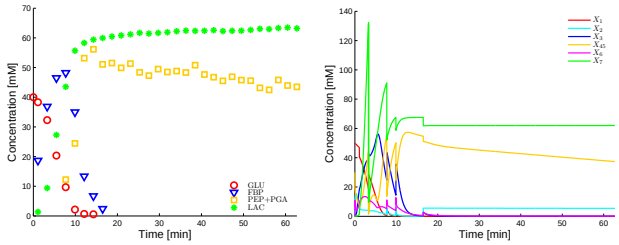
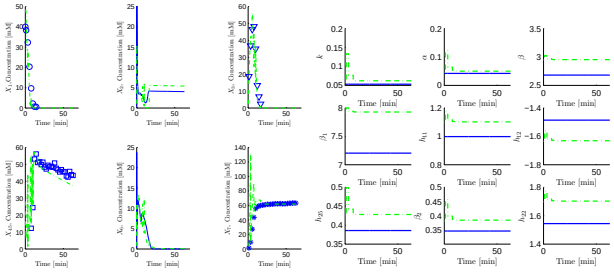


Figure 8: Experimental data points (on the left) and EKF estimated system (on the right). The points on the left plot correspond to the experimental data points. While the experimental system has only four variables (corresponding to the observable states), the estimated system has six variables because all the model is estimated.

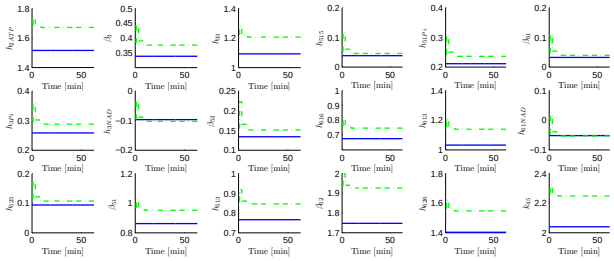
In order to have a quantitative criteria to compare EKF estimates when only the experimental points are available for the filter corrects the predicted state and covariance, the respective MSE was computed by (12) and the results compiled on Table 4.

The MSE 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 atributed higher importance because the expected value is very small. The MSE for X_1 and X_2 estimates are comparable, what means that EKF has a very good performance



(a) States estimates.

(b) Parameters k, α, β, β_1, h_{11}, h_{12}, h_{25}, β_2 and h_{22} estimates.



(c) Estimative of parameters h_{2ATP}, β_3, h_{33}, h_{3Pi}, h_{3NAD}, β_{52}, h_{525}, β_{51} and h_{513} estimates.

(d) Estimative of parameters h_{515}, h_{51Pi}, β_{61}, h_{616}, h_{613}, h_{61NAD}, β_{62}, h_{626} and k_{45} estimates.

Figure 9: Comparison between the nominal value (in blue) and the respective estimated values (in green) with EKF for experimental data.

Table 4: MSE (%) for estimation of experimental data with EKF

State	$\varepsilon_{\mathbf{EKF}}^{\mathbf{experimentaldata}}$	Parameter	$\varepsilon_{\mathbf{EKF}}^{\mathbf{experimentaldata}}$
X_1	49.02	k	35.79
X_2	46.27	α	43.45
X_3	24.09	β	10.29
X_{45}	6.73	β_1	10.11
X_6	318.86	h_{11}	10.85
X_7	3.62	h_{12}	9.54
		h_{25}	12.49
		β_2	12.83
		h_{22}	10.53
		h_{2ATP}	10.54
		β_3	12.91
		h_{33}	10.77
		h_{3Pi}	14.01
		h_{3NAD}	14.61
		β_{52}	18.78
		h_{525}	23.35
		β_{51}	11.00
		h_{513}	11.14
		h_{515}	47.10
		h_{51Pi}	15.10
		β_{61}	54.35
		h_{616}	11.31
		h_{613}	10.82
		h_{61NAD}	27.26
		β_{62}	10.46
		h_{626}	10.58
		k_45	10.39

along with the estimation. X_{45} and X_7 are accurately estimated, with MSE around 7% and 4% respectively. Relatively to parameters, MSE are comparable with the ones obtained with the spline data estimation (Table 3(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.

6 CONCLUSIONS AND FUTURE WORK

This work was developed on the interface between biochemistry and control theory. Kalman-based filtering techniques proved to be adequate methods to estimate states and parameters in these type of systems.

The EKF has shown a good performance during estimation, for states and parameters. Even the non directly measured states were 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.

UKF implementation was a harder task, not because of the filter itself, which is easier 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. Also, 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.

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 $t = 1.1min$ and after goes on with a step of $2.2min$; if another experience, with the same start conditions, begins at $t = 2min$, 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.

From the modelling point of view, the results with EKF and UKF 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. 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.

This study showed 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 paper 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.

Acknowledgements

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