Examensarbete utfört i Reglerteknik vid Tekniska högskolan i Linköping av

Tibor Maksai

LiTH-ISY-EX--08/3789--SE

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Modeling Anaerobic Muscle Metabolism
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**Abstract**

Is it possible for a minimal model of anaerobic muscle contraction to describe measured data? There have been many models trying to describe separate parts of the human body with various results. In this thesis a model has been created to describe all the essential biochemical reactions of anaerobic muscle metabolism during contraction but with as few states and parameters as possible. A toolbox in Matlab was used for simulation and also for parameter estimation. The best model eventually got validated to see statistically how well it can describe the measured data. During the simulations an unnecessary assumption got revealed which helped us to understand the system better. The vision of a whole-body model may not be so far into the future as many think and the first step is to understand smaller biochemical systems like muscle contraction.
Abstract

Is it possible for a minimal model of anaerobic muscle contraction to describe measured data? There have been many models trying to describe separate parts of the human body with various results. In this thesis a model has been created to describe all the essential biochemical reactions of anaerobic muscle metabolism during contraction but with as few states and parameters as possible. A toolbox in Matlab was used for simulation and also for parameter estimation. The best model eventually got validated to see statistically how well it can describe the measured data. During the simulations an unnecessary assumption got revealed which helped us to understand the system better. The vision of a whole-body model may not be so far into the future as many think and the first step is to understand smaller biochemical systems like muscle contraction.
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Chapter 1

Introduction

In this chapter a short introduction will be followed by the thesis disposition. It also contains the thesis objectives and the problem definition.

1.1 Introduction

Each year since 1977 there is a competition called “The world’s strongest Man” where athletes are testing the limits of human strength and endurance. They are pulling lorries and airplanes, lifting stones that weight over 100 kg and other physically demanding events. Of course they are blessed with good genes and they train more in a month than an average person does in a year, but in fact their muscles work the same way as ours. Food we eat turns into energy in our cells by several complicated reactions. Biochemistry describes these chemical processes that take place in our cells and organs and has been studied intensely the past 100 years. The latest technology has opened up new ways of approaching biochemistry, it is easier measuring substances and with help from powerful computers it is possible to create small models of isolated biochemical subsystems. But even though we have made remarkable progress in technology and also biochemistry it is still very hard to predict measurements on the human body due to its complexity and we still do not have all the required information.

1.2 Thesis objectives

The investigation of biological behaviour and its transformation to mathematical language is called biosimulation. Even though scientists know a lot about the human body they have problems creating working biochemical models of it. Instead the goal is to model smaller parts: the function of the kidneys, the liver, the human metabolism etc. Melissa J. Lambeth and Martin J. Kushmerick tried to

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simulate muscle metabolism quite successfully [16]. But their paper also raised a lot of new questions. How well can a model describe muscle metabolism?

It is of great value to be able to create a fully functional model of muscle metabolism. It will help understanding the system but it is also relevant from a medical point of view. Millions of dollars could be spared if we could predict how the substances in a new drug effects our body without testing it in labs or on human test persons.

1.3 Problem definition

My thesis has its origin in a paper by Lambeth and Kushmerick and describes a computational model for glycogenolysis in skeletal muscle [16]. Based on this paper, scientists from the university of Copenhagen (Department of medical biochemistry and genetics)\(^3\) tried to make a smaller, less complicated model, describing anaerobic muscle metabolism. The question asked in this thesis is whether this model can describe the measured data they collected.

1.4 Thesis disposition

The first chapter is the introduction chapter where it is explained what this thesis is investigating, the problem definition, what the objectives are, and why it is relevant to make this research at all.

The second chapter will contain the biochemical background that is needed for the reader but also measurement data, elementary statistics, basics for model validation and a short introduction about optimization. These are all relevant fields of science which should be well procured in order to understand the thesis.

The third chapter is about modeling. Here, our model is simulated and modified in order to achieve our objectives. Assumptions and changes in the model are explained and motivated.

The fourth chapter contains results. All the results from the modeling process are collected and presented in an eloquent way. The work is divided into phases for better understanding, different models are presented to illustrate the progress of the thesis.

The fifth and last chapter is for discussion and for making conclusions.

\(^3\)http://www.mfi.ku.dk/nmr/
Chapter 2

Background

This chapter contains background information to facilitate understanding of the assumptions and theories used in the thesis. Some biochemical terms are being explained, which will help the understanding of the model structure and the relevance of some biological formulas used. The measurement data we received from Copenhagen is presented, and it is explained how it was collected using NMR technology. Different optimization methods are explained because they will be used during the system identification part of the thesis. The last sections will explain elementary hypothesis testing, and give some background on modeling.

2.1 Biochemical terms

This section explains some elementary biochemical terms and reactions. These will prove to be quite important to understand in the following sections. For more information about biochemistry and anatomy of the human body, see [11].

2.1.1 Glycolysis

Glycogen is a polymer of glucose \((C_6H_{12}O_6)\), and as other polymers they are connected by covalent chemical bonds which form a long chain of residues. Glucose is stored as glycogen predominantly in liver and muscle cells. The catabolism of glycogen is called glycogenolysis and occurs when the muscles or cells need energy. This process uses glycogen phosphorylase (an enzyme) to break down glycogen into glucose-1-phosphate (G1P).

\[
\text{Glycogen(N residues) + P} \rightarrow \text{Glycogen(N-1 residues) + Glucose-1-phosphate}
\]

Phosphoglucomutase is an enzyme that produces glucose-6-phosphate (G6P) from glucose-1-phosphate by transferring a phosphoryl group within the molecule.
Figure 2.1. Phosphoglucomutase catalyzes the reversible reaction: G1P $\rightleftharpoons$ G6P.

This occurs at the first step in figure 2.2. Glycolysis is when G6P is oxidized to two molecules of pyruvic acid and generates high-energy ATP.

Figure 2.2. Glycolysis; all the steps from glucose to pyruvic acid. At step 1 we have G1P from glucose which has been generated by the glycogenolysis.
2.1.2 NAD\(^+\) and NADH

Nicotinamide adenine dinucleotide (NAD\(^+\)) is an important substance in muscle metabolism because without it, the enzymes can not start the reactions. These substances are called cofactors. NADH is the reduced form of (NAD\(^+\)) and is being produced during glycolysis.

2.1.3 3 important nucleotides

A nucleotide is a chemical substance consisting of different chemically bonded chemical elements, with some additional phosphate groups. They play an important role in muscle metabolism and in energy production in general in our cells. Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are 3 important nucleotides during muscle metabolism. Energy is released when ATP is dephosphorylized into ADP, and this released energy can activate other chemical reactions. This decomposition of ATP is catalyzed by enzymes called ATPases and works as an energy releasing process in all forms of living cells.

2.2 Creating a minimal model

This section will show how the minimal model is created; which reactions are important, how they effect the model and also how we determine ordinary differential equations for the model.

An anaerobic model is desired since it decreases the input of energy sources to the cells which in turn makes the model a lot easier to describe and also to overview. With no oxygen or new blood supply, the ATP production is limited to glycolysis and to the creatine kinase. This is quite easily obtained on a test person by stopping the blood from reaching the muscles by blocking major blood vessels. To be able to create a minimal model of anaerobic muscle metabolism we need to decide which major parts of the reactions we should consider important. The following equations are reaction kinetics and they describe the rate of the chemical processes.

1. Glycogen Phosphorylase: \(P \rightarrow G6P\)

\[
v_{GP} = V_1 \cdot \frac{P - \frac{PME}{K_{eq}}}{K_P + P} \cdot \frac{AMP_{g1AMP} \cdot (Ca^{2+})_{g1Ca}}{K_{AMP} + AMP_{g1AMP} \cdot K_{Ca} + (Ca^{2+})_{g1Ca}}
\]

This reaction describes the glycogenolysis, how G6P is being produced out of glucose. Actually several reactions are baked together in this equation and some are neglected to make the model easier. \(Ca^{2+}\) works as a catalyst that starts the reaction when the muscle contracts. G6P is approximated with PME (phosphomonoester) because it is PME the scientists can measure, and it is believed that G6P is the dominating molecule. PME is one of the measured signals and more information can be found in section 2.4 (Measurement data).
2. Lumped Glycolysis:

\[ \text{G6P} + 2\text{NAD}^+ + 2\text{P} + 3\text{ADP} \rightarrow \text{PyR} + 3\text{ATP} + 2\text{NADH} + \text{H} \]

\[ v_{\text{Glyc}} = V2 \cdot \frac{\text{ADP}}{K_{2_{\text{ADP}}}} \cdot \frac{\text{PME}}{K_{2_{\text{HP}}}} \cdot \frac{\text{NAD}}{K_{2_{\text{NAD}}}} \cdot \frac{\text{P}}{K_{2_{\text{P}}}} \]

This equation describes the glycolysis, an irreversible reaction which releases two molecules of pyruvic acid and also ATP when G6P is oxidized. G6P has again been approximated with PME, compare with the previous reaction.

3. Lactate Dehydrogenase: Pyr + NADH + H \equiv \text{Lac} + \text{NAD}

\[ v_{\text{LDH}} = V3 \cdot \frac{\text{Pyr} \cdot \text{NADH} - \frac{\text{NAD-Lac}}{K_{3_{\text{eq}}}}}{K_{3_{\text{Lac}}} \cdot K_{3_{\text{NAD}}} \cdot (1 + K_{3_{\text{NAD}}} \cdot \text{Lac} + v_{\text{LDHterms}})} \]

\[ v_{\text{LDHterms}} = \frac{\text{Pyr}}{K_{3_{\text{Pyr}}}} + \frac{\text{NADH}}{K_{3_{\text{NADH}}}} + \frac{\text{Pyr} \cdot \text{NADH}}{K_{3_{\text{Pyr}}} \cdot K_{3_{\text{NADH}}}} + \text{NADterms} \]

\[ \text{NADterms} = K_{3_{\text{Lac}}} \cdot \text{NAD} + \text{Lac} \cdot \text{NAD} \]

Lactate dehydrogenase (LDH) is also important in our model because it balances \( \text{NAD}^+ \) and NADH. It is difficult to describe it by mathematical equations but this reduced version seems efficient for our minimal model.

4. ATPase : ATP \rightarrow \text{ADP} + \text{P} + \text{H}

\[ v_{\text{ATPase}} = v_{\text{rest}} + V4 \cdot \frac{\text{ATP}}{K_{4_{\text{ATP}}}} \cdot \frac{(\text{Ca}^{2+})^{g_{4_{\text{Ca}}}}}{K_{4_{\text{Ca}}} + (\text{Ca}^{2+})^{g_{4_{\text{Ca}}}}} \]

The energy release that is needed in the cells is supplied through ATPase, where the ATPase-enzymes decomposes ATP into ADP. This reaction is catalyzed by Ca\(^{2+}\) just like glycogenolysis and occurs when the muscle contracts. \( v_{\text{rest}} \) is the initial value for \( v_{\text{ATPase}} \) when Ca\(^{2+}\) is zero.

5. Creatine Kinase (assumed fast) : H + PCr + ADP \rightleftharpoons \text{ATP} + \text{Cr}

\[ v_{\text{CK}} = V5 \cdot (\text{PCr} \cdot \text{ADP} \cdot \text{H} - \frac{\text{ATP} \cdot \text{Cr}}{K_{5_{\text{eq}}}}) \]

Creatine kinase (CK) also known as phosphocreatine kinase or creatine phosphokinase (CPK) is also an important reaction because it consumes ATP and generates ADP by converting creatine to phosphocreatine. This reaction is also reversible and can thereby work as an important energy reservoir when ATP is needed to be regenerated. The energy necessary for muscle contraction is provided by ATP. Because the muscle stores a limited amount of ATP, the \( v_{\text{CK}} \) mechanism must be in place to synthesize new ATP fast to enable continued muscle activity. Mass-action kinetics gives the reaction rate for \( v_{\text{CK}} \).
6. Adenylate Kinase: \( ATP + AMP \rightleftharpoons 2ADP \)

This is assumed to be fast and can be replaced with the steady-state relation:

\[
AMP = \frac{(ADP)^2}{ATP \cdot K_{eq}}
\]

These 3 nucleotides balance each other in our cells with this reaction, also called myokinase.

### 2.2.1 ODEs for the model

We have selected the reactions that are necessary to model the contraction. Now we need to know how they behave, meaning we would like to predict their derivatives. Since we know how the reactions interact with each other, we can find differential equations for the system’s input values. The input values are in this case the molecules and substances which build up our model, for example; P, ATP, ADP etc. They will from now on be called the system signals.

\[
\begin{align*}
\frac{d}{dt}(G) &= v_{GP} \\
\frac{d}{dt}(G6P) &= v_{GP} - v_{Glyc} \\
\frac{d}{dt}(P) &= -v_{GP} - 2v_{Glyc} + v_{ATPase} \\
\frac{d}{dt}(ATP) &= 3v_{Glyc} - v_{ATPase} + v_{CK} \\
\frac{d}{dt}(ADP) &= -3v_{Glyc} + v_{ATPase} - v_{CK} \\
\frac{d}{dt}(PYR) &= 2v_{Glyc} - v_{LDH} \\
\frac{d}{dt}(NADH) &= 2v_{Glyc} - v_{LDH} \\
\frac{d}{dt}(NAD) &= -2v_{Glyc} + v_{LDH} \\
\frac{d}{dt}(Lac) &= v_{LDH} \\
\frac{d}{dt}(PCr) &= -v_{CK} \\
\frac{d}{dt}(Cr) &= v_{CK} \\
\frac{d}{dt}(H) &= \frac{v_{Glyc} - v_{LDH} + v_{ATPase} - v_{CK}}{S_{buf}}
\end{align*}
\]
2.3 Nuclear Magnetic Resonance

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. Nuclear magnetic resonance spectroscopy is the use of the NMR (Nuclear Magnetic Resonance) phenomenon to study physical, chemical, and biological properties of matter. By placing a particle with a net spin in a magnetic field of strength $B$, it will absorb a photon of frequency $\nu$. It is known that the frequency $\nu$ depends on the gyromagnetic ratio, $\gamma$ of the particle.

$$\nu = \gamma \cdot B$$

The scientist at the university of Copenhagen have access to their own NMR device, helping them with the data measurements. Since the measured data comes from a testperson it is relieving to know that NMR is a safe way to analyze samples non-destructively. For more information about NMR, see [9].

2.4 Measurement data

The measured data received from the university of Copenhagen (Department of medical biochemistry and genetics) contained 9 independent time series measurements of ATP, P, PCr, pH and PME. Unfortunately it was not possible to measure G6P directly, but since it is the main molecule in PME (phosphomonoester group) it could be estimated. A test person’s blood supply to the arm was restrained by blocking the major blood vessels, so anaerobic conditions could occur during contraction. Before the actual contraction the test person rested 5 minutes to make sure no oxygen remained and during this time we could measure the initial values in the cell by NMR. After the resting time the test person pulled a handle for 60 seconds and the he rested for another 60 seconds before turning on the blood supply. These 2 phases are the contraction and the relaxation phase of the time series and the substances were measured with NMR all the time. The resting phase was 5 min long but it is sufficient with the last 2 min before the contraction because it does not contain any valuable information besides ensuring that no oxygen is present during contraction. Following table will show how many samples there are during the different phases of each experiment.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of samples</th>
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<tbody>
<tr>
<td>Resting</td>
<td>24</td>
</tr>
<tr>
<td>Contraction</td>
<td>13</td>
</tr>
<tr>
<td>Recovery</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
</tbody>
</table>

Table 2.1. Number of samples during the different phases of the experiment.
2.4 Measurement data

2.4.1 Measured signal - PME

Phosphomonoester (PME) is a measurement which includes several molecules containing a phosphate group, and glucose 6-phosphate is one of the molecules. Glycolysis occurs when glucose 6-phosphate is oxidized to two molecules of pyruvic acid. G6P (glucose 6-phosphate) is glucose sugar phosphorylated on carbon 6 but it cannot be measured directly by NMR since it cannot distinguish it from other phosphorylated sugars. G6P is the main molecule in phosphomonoester (PME) but NMR measures all phosphorylated sugars. The measured PME signal is quite noisy but the signal characteristics can be distinguished.

2.4.2 Measured signal - P

Phosphorus is found as a free phosphate ion in most biological systems and is called inorganic phosphate. High energy phosphate, in the form of ADP, ATP and AMP, is carrying the energy within our bodies. The measured signal gets more noisy during the contraction and the relaxation part.
2.4.3 Measured signal - PCr

Phosphocreatine or creatine phosphate is a phosphorylated creatine molecule which generates ATP from ADP. Observing the measurement data, it is possible that the initial values differ for every experiment and that it might be wrong taking the average value for all 9 experiments.
2.4.4 Measured signal - ATP

The energy necessary for muscle contraction is provided by ATP. ATP is unfortunately very hard to measure. The noise makes it very hard to use the actual information, but by taking an average value during the 3 phases of the experiment we can still use it in our parameter prediction.

2.4.5 Measured signal - pH

---

**Figure 2.6.** 9 measured ATP series.

**Figure 2.7.** 9 measured pH series.
NMR measures the concentration of $H^+$ and from that we can calculate the pH-value with the formula:

$$\text{pH} = -\log_{10}[H^+]$$  \hspace{1cm} (2.13)

### 2.5 Optimization

A model will be created and that model should match the collected measured data, or at least be close to it. The parameters in the model will be optimized so the model can simulate the data. The optimization will try to minimize a calculated cost function and thereby find the parameters that will generate a good, or even maybe the best possible model. The cost function usually have the following equation:

$$\text{Cost} = \sum_{i=1}^{N} (X_M - X_D)^2$$  \hspace{1cm} (2.14)

$N$ = Number of time points  
$X_M - X_D$ = Difference between model and measured data

Minimizing the difference between the model and measured data will generate a good set of parameter values. And since the cost function squares the difference, it does not matter if the difference is negative or positive.

If there is information about the input and output data of a process, it is called supervised learning, meaning that for each input the measured process output is known. When it comes to optimizing parameters in a model it is the case of supervised learning, which can be divided into 3 major types: the linear optimization, the nonlinear local and the nonlinear global optimization [14, page 25]. The distinction between local and global optimization techniques is only necessary in nonlinear optimization since linear problems always have a unique optimum. When solving nonlinear local optimization problems the most common and effective way is to start from an initial point and then search in directions based on neighborhood information such as first and second order derivatives. This method leads to a local optimum relatively close to the starting point and it is most likely not the global one. A special case occurs for convex optimization problems, namely that if a local minimum exists, then it is the global minimum. Some global methods can converge to the global optimum but that is not likely to happen within finite time. That is why it is not expected to find the global optimum but instead a good local one, especially for larger problems. Multi-starting a nonlinear local optimization technique with different parameter values is the simplest way searching for a good local optimum. The best of these local optima can be picked as the final result. Most nonlinear global optimization methods contain some stochastic elements and this is because we would like to escape from local optima. When the iteration is finally finished, we will choose the best overall solution and not the best solution in the final iteration.
Therefore, it is wise to store the best solution over all iterations. The global methods's convergence to any optimum is very slow since they are examining the whole parameter space, but this procedure can be fastened up by applying a local optimization method when approaching an optimum. In general, global methods are good at finding regions while local methods are good at finding points [14, page 114].

Two different optimization methods will be presented that will be used in the thesis. Both of them are implemented in the System Biology Toolbox, which is used for simulation and optimization.

### 2.5.1 Nelder-Mead simplex method

The Nelder-Mead method, also called *downhill simplex* method, is a nonlinear optimization algorithm based on the concept of direct search algorithms. It is not a global method, and like other direct search algorithms, no derivatives are required. Instead these methods use the evaluation of loss function values only. The goal of the Nelder-Mead simplex method is to find the N-dimensional parameter vector that minimizes the loss function. The basic idea of simplex search is to calculate the loss function at each vertex, which has a form of a polyhedron composed of N+1 points in N dimensions. The worst point, the vertex that generates the largest loss function, is being reflected to the centroid and is thereafter being deleted to generate a new simplex. By iterating this algorithm we will eventually roll downhill until a local optimum is reached. One loss function evaluation is enough for each new iteration but it is important to avoid that the algorithm gets stuck by cycling between two or more identical simplexes. This problem can be avoided in several ways. For example, when cycling is detected we can choose the second worst vertex instead of the worst for reflection, or we can reduce the size of the simplex. The Nelder-Mead simplex method is an extended version of a basic simplex search where the following problems are eliminated [14, page 87]:

1. All parameters have to be scaled by the same factor if the simplex size is reduced. This is a problem because often it is advantageous to choose different scale factors for the parameters depending on how they affect the loss function.

2. The size of the simplex can only be reduced and therefore no acceleration of the convergence speed is possible. This is a problem because it is not always beneficial to reduce the size of the simplex. The Nelder-Mead simplex algorithm enables stretching and shrinking of the simplex in all directions, leading to a convergence speed-up.

3. Contracting the simplex requires the recomputation of all vertices.

To further speed up the convergence it is advisable to start the search with a large simplex, and to shrink it when cycling is detected.
2.5.2 Simulated annealing method

Simulated annealing (SA) is an efficient way to solve the nonlinear global optimization problems of minimizing multivariate functions. The inspiration behind SA comes from metallurgy where a controlled heating and cooling process reduces the material’s defects. First the heat makes the atoms more flexible, while the slow cooling allows them to find configurations with lower internal energy than before. In the optimization algorithm the particles represent the parameters in the search space and the potential energy represents the loss function. It means that the virtual heating enables us to leave local minima and to find better solutions. The general form of a simulated annealing is presented in the following list [14, page 116]:

1. Choose an initial temperature $T_0$.
2. Choose an initial parameter vector $\theta_0$.
3. Evaluate the loss function value for the initial parameters $I(\theta_0)$.
4. Iterate for $k = 1, 2, \ldots$.
5. Generate a new point in search space $\theta_{\text{new}}$, which has the deviation $\Delta \theta_k = \theta_{\text{new}} - \theta_{k-1}$ from the old point with the generation probability density function $g(\Delta \theta_k, T_k)$.
6. Evaluate the loss function value for the new parameters $I(\theta_{\text{new}})$.
7. Accept this new point with a acceptance probability $h(\Delta I_k, T_k)$ where $\Delta I_k = I(\theta_{\text{new}}) - I(\theta_{k-1})$. Meaning, either set $\theta_k = \theta_{\text{new}}$ or keep the old point, $\theta_k = \theta_{k-1}$.
8. Decrease the temperature according to the annealing schedule $T_k$.
9. Test for the termination criterion and either go to Step 4 or stop.

All nonlinear problems are different and because of that it is very difficult to choose the initial temperature $T_0$. It is a tradeoff between globality and locality of the search, and usually a trial-and-error approach is required. If $T_0$ is too high the convergence will be very slow and if it is too small the algorithm will concentrate on the neighborhood around the initial point, making it impossible to find remote optima. At Step 5 a new point in search space is generated by a probability density function (pdf) and it has 2 properties:

1. Smaller parameter changes have higher probability than higher ones.
2. Large parameter changes are more likely for higher temperatures than for lower ones.

It means that from the start the algorithm examines a very wide parameter space and as time progresses (and temperature decreases) it focuses more and more on regions. Gaussian distribution is the traditional choice of the probability
density function \( g(\Delta \theta_k, T_k) \). When a new point is generated, it is time to evaluate its loss function value. Simulated annealing accepts this point with a probability of \( h \) (Step 7). This probability function enables us to escape from local minima, and has two main properties:

1. Acceptance is more likely for better points than for worse.
2. Acceptance of worse points has a higher probability for larger temperatures than for smaller ones.

The acceptance probability in standard simulated annealing is chosen as [14, page 117]:

\[
h(\Delta I_k, T_k) = \frac{1}{1 + \exp(\frac{\Delta I_k}{T_k})}
\]

\( \Delta I_k \) = Difference in loss function value between the new and the old parameters. It can be shown that the algorithm is statistically guaranteed to find the global optimum, if the pdf \( g(\Delta \theta_k, T_k) \) is a Gaussian distribution and if the following annealing schedule is applied [14, page 118]:

\[
T_k = \frac{T_0 \ln k}{k}
\]

This annealing schedule is known as Bolzmann annealing (BA). The algorithm guarantees that it will not get stuck in a local optimum and that it thereby will find the global optimum in \textit{infinite} time. This method is of course very slow and it is possible to speed up the simulated annealing by choosing the Fast Annealing (FA) method. In FA the Gaussian pdf is replaced by a Cauchy distribution and because this distribution has a “fatter” tail the search will get more global and also faster. Fast annealing statistically finds the global optimum, with probability 1, for the following annealing schedule [14, page 118]:

\[
T_k = \frac{T_0}{k}
\]

The problem with FA is that the Cauchy distribution leads to a higher probability of rejected points (see step 7 in the simulated annealing list) but the faster annealing schedule compensates this effect. It means that we have a trade-off between time and effectiveness. Unfortunately this trade-off is quite common when it comes to mathematical optimization.
2.6 Testing and validating the model

In this section we will present how it is possible to validate an estimated/identified model. Different methods of validation will be presented and essential tools such as skewness and kurtosis will be discussed. We would like to apply a statistical test for the validation and therefore a $\chi^2$ test is explained in more detail.

2.6.1 Identification cycle

The road to a validated model is often quite long with numerous experiments behind the work. Gathering information and deciding how the model should be constructed in order to cover the vital parts are the first step towards a model. The following picture describes the major steps in the fine art of modeling.

![Identification cycle diagram](image)

*Figure 2.8. Identification cycle. Rectangles: the computers’ main responsibility. Ovals: the user’s main responsibility [7, page 521].*
The choice of model structure in this thesis may be questionable since the model have been minimized and some minor parts have been left out. If we find any errors in the model structure during modeling, we are free to change it as long as they agree with the biochemical structure. The validation part will also be important but there are 3 major questions we should consider [7, page 424]:

1. Does the model agree sufficiently well with the observed data?
2. Is the model good enough for my purpose?
3. Does the model describe the “true system”?

During the thesis focus will be on the first question since we are not creating the model for any specific purpose and we do not know what the “true system” looks like. Instead we are interested in how well the model can describe the measured data we received.

If there are a lot of samples available it is appropriate to divide them into two parts; the training set, which is used for model estimation, and the validation set, which is used for validating the estimated model. The method above is called cross-validation and is frequently used with large sets of tests because overfitting can be prevented. The likelihood of overfitting depends mostly on the number of parameters and the data, but the model error compared to the expected level of noise in the data is also important.

### 2.6.2 Statistical test and validation

A statistical test of a hypothesis consists of 5 parts [15, page 308]:

1. The null hypothesis, denoted by $H_0$.
2. The alternative hypothesis, denoted by $H_a$.
3. The test statistic.
4. The rejection region.
5. The conclusion.

Mostly it is the alternative hypothesis we would like to show is true and we use the null hypothesis to prove it. The null hypothesis is a contradiction to the alternative hypothesis, and we make the assumption that if $H_0$ is false we can accept $H_a$. Though it is $H_a$ we would like to increase our confidence in, $H_0$ is the hypothesis to be tested, so the procedure may be thought of as “acceptance by contradiction”. Hypothesis testing can either be two-tailed or one-tailed depending on how the test statistic is formulated. In the two-tailed hypothesis a value might either be above or below the acceptance region. For the one-tailed hypothesis there is only one critical value for comparison. The critical value which separates the acceptance region from the rejection region is a “decision
maker” which is called the test statistic. The values that the statistic set may assume is divided into 2 regions. The rejection region consists of those values which support the alternative hypothesis. And the acceptance region consists accordingly of all the other values where the null hypothesis can not be rejected. These two regions are separated by a critical value of the test statistic.

Confidence intervals are used to indicate the reliability of the estimated model. The most often used confidence level in modern applied practise is 95% [13]. It means that even if the null hypothesis \((H_0)\) is true we will reject it 5% of the time. The probability used in the criterion for rejection is called the significance level, denoted by \(\alpha\). Rejecting a null hypothesis when it is actually true is a Type I error, while a Type II error occurs when we can not reject the null hypothesis when it is false. The Type II error is represented by \(\beta\), and the power of a statistical test is defined as 1-\(\beta\).

<table>
<thead>
<tr>
<th></th>
<th>(H_0) is true</th>
<th>(H_0) is false</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_0) is rejected:</td>
<td>Type I error</td>
<td>No error</td>
</tr>
<tr>
<td>(H_0) is not rejected:</td>
<td>No error</td>
<td>Type II error</td>
</tr>
</tbody>
</table>

Table 2.2. The two types of errors in hypothesis testing [13, page 83].

2.6.3 Residual analysis

Since model validation is not an exact science, visual evaluation of a model can sometimes be sufficient. Residual analysis is an important part of a model validation, mostly because the difference between an estimated model and measured data can be so eloquently illustrated. The optimal case is when the difference is very small (insignificant) and when it is randomly distributed. This would agree with the assumption that the residuals are noise in the data. In our model 9 separate experiments were used and the evaluation of the residuals will be for each time point based on the calculated mean value for the experiments. Calculating the standard deviation, the skewness and the kurtosis of the data will prove to be helpful during the validation process.

Standard deviation

The standard deviation is a measure of the spread of values and is defined as the square root of the variance. It means it is the root mean square (RMS) deviation of values from their arithmetic means, and it is needed for the model validation. It is estimated by the expression:

\[
\hat{\sigma} = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2}
\]

(2.15)

\(N\) = Number of samples
\(\hat{x}\) = Calculated mean value
2.6 Testing and validating the model

In our case we have 9 separate experiments, and we will use all of them at each time point to calculate $\hat{\sigma}$.

**Skewness & kurtosis**

By calculating the skewness in the residuals for each signal, it is possible to measure the asymmetry of the collected data. A normal (Gaussian) distribution has zero skewness, and with this fact in hand, we would like the skewness of the residuals to be as small as possible. A negative skewness means that the data is spread out more to the left of the mean than to the right, and vice-versa, a positive skewness is a proof of that the data is spread more to the right. The skewness of a random variable $z$ is:

$$s = \frac{E(z - \bar{z})^3}{\sigma^3}$$  \hspace{1cm} (2.16)

$\bar{z}$ = Mean value of $z$

$\sigma$ = Standard deviation of $z$

$E(t)$ = The expected value of $t$

According to the central limit theorem, a large sum of independent and identically distributed random variables follows approximately a Gaussian distribution, if the sum of the variables has a finite variance. This approximation becomes more accurate as the sample size $N$ becomes larger. The expected value for a Gaussian distribution is zero but when replacing the expected value with a calculated one, it will most likely deviate from the mean. The following equations gives the estimate $\hat{s}$:

$$\hat{s} = \frac{1}{N} \sum_{i=1}^{N} (z_i - \hat{z})^3$$  \hspace{1cm} (2.17)

$$\hat{z} = \frac{1}{N} \sum_{i=1}^{N} z_i$$  \hspace{1cm} (2.18)

Under the hypothesis that $z$ are independent and identically distributed random variables (IID), giving $\hat{s}$ approximately a Gaussian distribution according to the central limit theorem (CLT), the standard deviation of $\hat{s}$ becomes [8, page 72]:

$$S_{\hat{s}} = \sqrt{\frac{6}{N}}$$

The null hypothesis is that the residuals are randomly distributed, meaning that $z_i - \hat{z}$ follow a Gaussian distribution. The rejection region is based on a two-sided hypothesis test where the null hypothesis is rejected when $|\hat{s}| > 2 \cdot S_{\hat{s}}$. When the null hypothesis is rejected, we can conclude that there is a skewness problem. The standard error is decreasing for larger $N$, meaning it is more likely that the null hypothesis is rejected for smaller deviations. We have 49 time points and that gives us that the skewness value should lie between -0.70 and 0.70 for us to accept it as noise.
While skewness measures the asymmetry, the kurtosis measures how outlier-prone a distribution is. The kurtosis of the normal distribution is 3, and distribution that are more outlier-prone than the normal distribution have kurtosis higher than 3. It also follows that distributions that are less outlier-prone have kurtosis less than 3. The basic equation for kurtosis gives a value of 3 when the distribution is normal but usually 3 is subtracted so that the expected value of k is zero. Hence, the kurtosis of a random variable $z$ is defined as:

$$k = \frac{E(z - \bar{z})^4}{\sigma^4} - 3$$  \hspace{1cm} (2.19)$$

$z$ = Mean value of $z$

$\sigma$ = Standard deviation of $z$

$E(t)$ = The expected value of $t$

It is now possible to use the same method as for skewness and calculate an estimate $\hat{k}$, with the following equations:

$$\hat{k} = \frac{1}{N} \sum_{i=1}^{N} (z_i - \hat{\bar{z}})^4 - 3$$  \hspace{1cm} (2.20)$$

$$\hat{\bar{z}} = \frac{1}{N} \sum_{i=1}^{N} z_i$$  \hspace{1cm} (2.21)$$

The conclusions about the rejection region and the null hypothesis we made for the skewness can be applied for the kurtosis. The mean value is zero but the distribution around it will generate a rejection area. The standard deviation under the hypothesis that $z$ is Gaussian distributed is approximately [8, page 72]

$$S_k = \sqrt{\frac{24}{N}}$$

If $|\hat{k}| > 2 \cdot S_k$ we can reject the null hypothesis and the we can conclude that there is a kurtosis problem. We have 49 time points which gives us the limitation for $k$:

$$2 \cdot \sqrt{\frac{24}{49}} \approx 1.40$$
2.6 Testing and validating the model

2.6.4 $\chi^2$ test

There are several ways of testing and validating a model statistically and the $\chi^2$-test is one of them. A $\chi^2$-test is any statistical hypothesis test in which the test statistic has a $\chi^2$ distribution under the assumption that the null hypothesis is true. Apparently it is requested to form a $\chi^2$ distribution for a validation.

We know that if $X_i$ are $k$ independent, normally distributed random variables with mean 0 and variance 1, then the random variable $Q$ is distributed according to the $\chi^2$ distribution.

\[
Q = \sum_{i=1}^{k} X_i^2 \iff Q \sim \chi_k^2
\]  

(2.22)

We assume that the residuals $\epsilon(t)$ are Gaussian white noise with mean value zero.

\[
\epsilon(t) = \hat{y}(t) - y(t)
\]  

(2.23)

Gaussian variables with mean value zero divided by their standard deviation have a $N(0,1)$ distribution and it is now obvious that we can use equation 2.22 to form a $\chi^2$ distribution. We are now able to create a $\chi^2$ test where the null hypothesis is that the model generating the residuals $\epsilon(t)$ is the true one. However, instead of seeking “acceptance by contradiction” (rejecting $H_0$), we would like to accept it. The critical value $T$ can be calculated by the following equation:

\[
T = \sum_{t=1}^{N} \left( \frac{\hat{y}(t) - y(t)}{\sigma(t)} \right)^2
\]  

(2.24)

$N = \text{Degree of freedom (Number of time points used in the experiments)}$

$\sigma(t) = \text{The standard deviation for each time point}$

If the calculated value is below the critical value $T$ we can not reject $H_0$, and we can assume that the model is acceptable with the assumption that the residuals $\epsilon(t)$ are Gaussian with mean value zero. The confidence level will be 95%, which have been discussed in previous sections.
Chapter 3

Modeling

The Systems Biology Toolbox (SBTB) offers modeling, simulation and parameter estimation and can easily be modified in Matlab when needed [17]. Besides the benefits already mentioned, there are others, in form of steady-state and stability analysis, parameter sensitivity analysis etc. This toolbox will be used throughout the thesis.

This chapter will contain the modifications made in the model and also how to improve the optimization phase. How the contraction was implemented and why the different changes in the model were crucial are explained and motivated.

3.1 Implementing the contraction

The contraction is implemented as an event in the model during simulation and parameter estimation.

--- Example 3.1: Contraction ---

\[
\text{contractionOn} = \text{ge}(\text{time}, 2), \text{Ca}, 1 \\
\text{contractionOff} = \text{ge}(\text{time}, 3), \text{Ca}, 0
\]

This implementation works as an on-off switch for the contraction during the experiment. It will only be activated during the contraction phase and will be set to zero during the resting and the recovery phase.

3.2 Modifying the original model

This section contains all information about the changes that were made to the original model. Even before the first simulation took place we knew what might be wrong. For example, the original model excluded the \( v_{GP} \) reaction in the expression for the derivative of H.
Actually the $v_{GP}$ reaction also had to be modified because it was only activated by Ca$^{2+}$, and during the other phases, when Ca$^{2+}$ was set to zero, it could not function. This mistake was corrected because it is known that glycogen phosphorylase occurs without being activated by Ca$^{2+}$. Measuring G6P involved also some controversies, because it is not possible to measure it directly due to its similarities with other particles. There were also some minor simplifications with the Ca-terms which led to a reduction in the numbers of parameters.

### 3.2.1 Simplifying the Ca-terms

During the simulations we will consider Ca$^{2+}$ as a switch turning on and off the reactions, meaning it will have either 0 or 1 as possible values. This assumption simplifies both the $v_{GP}$ and the vATPase expressions.

\[
\frac{(Ca^{2+})^g_{1Ca}}{K_{1Ca} + (Ca^{2+})^g_{1Ca}} \rightarrow Ca^{2+} \quad (3.1)
\]

\[
\frac{(Ca^{2+})^g_{4Ca}}{K_{4Ca} + (Ca^{2+})^g_{4Ca}} \rightarrow Ca^{2+} \quad (3.2)
\]

With this simplification we have reduced the number of parameters by 4. The new reactions have the form:

\[
v_{GP} = V_1 \cdot \frac{(P - HP)}{K_{1eq}(K_1P + P)} \cdot \frac{AMP^{g_{1AMP}}}{K_{1AMP} + AMP^{g_{1AMP}}} \cdot Ca^{2+}
\]

\[
v_{ATPase} = v_{rest} + V_4 \cdot \frac{ATP}{K_{4ATP} + ATP} \cdot Ca^{2+}
\]

### 3.2.2 Expanding the differential equation for H

The differential equation for H is quite simple and does not take the $v_{GP}$-reaction in consideration.

\[
\frac{d}{dt}(H) = \frac{v_{Glyc} - v_{LDH} + v_{ATPase} - v_{CK}}{S_{buf}} \quad (3.3)
\]

To create a more realistic model we would like to include the $v_{GP}$-reaction and also put on some weight on the different terms to illustrate how they affect the pH-value. It will be necessary to create 5 new terms which will be multiplied with the existing reactions. These new terms will be calculated according to their contribution to the pH-value.

\[
s_{GP} = \frac{-\alpha_{G6P}}{\alpha_{G6P} + 1} + \frac{\alpha_{Pi}}{\alpha_{Pi} + 1} \quad (3.4)
\]

\[
s_{Glyc} = \frac{-\alpha_{G6P}}{\alpha_{G6P} + 1} - \frac{2 \cdot \alpha_{Pi}}{\alpha_{Pi} + 1} - \frac{3 \cdot \alpha_{ADP}}{\alpha_{ADP} + 1} + \frac{2 \cdot \alpha_{Pyr}}{\alpha_{Pyr} + 1} + \frac{3 \cdot \alpha_{ATP}}{\alpha_{ATP} + 1} \quad (3.5)
\]

\[
s_{LDH} = \frac{-\alpha_{Lac}}{\alpha_{Lac} + 1} + \frac{\alpha_{Pyr}}{\alpha_{Pyr} + 1} + 1 \quad (3.6)
\]
3.2 Modifying the original model

\[
S_{\text{ATPase}} = \frac{-\alpha_{\text{ATP}}}{\alpha_{\text{ATP}} + 1} + \frac{\alpha_{\text{ADP}}}{\alpha_{\text{ADP}} + 1} + \frac{\alpha_{\text{Pi}}}{\alpha_{\text{Pi}} + 1} \quad (3.7)
\]

\[
S_{\text{CK}} = \frac{-\alpha_{\text{ATP}}}{\alpha_{\text{ATP}} + 1} + \frac{\alpha_{\text{ADP}}}{\alpha_{\text{ADP}} + 1} \frac{\alpha_{\text{PCr}}}{\alpha_{\text{PCr}} + 1} \quad (3.8)
\]

The \( \alpha \)-terms are related to \( H \) through following equations:

\[
\alpha_{\text{G6P}} = 10^{-\log_{10}(H) - pK_{\text{G6P}}} \quad (3.9)
\]

\[
\alpha_{\text{Pi}} = 10^{-\log_{10}(H) - pK_{\text{Pi}}} \quad (3.10)
\]

\[
\alpha_{\text{ADP}} = 10^{-\log_{10}(H) - pK_{\text{ADP}}} \quad (3.11)
\]

\[
\alpha_{\text{ATP}} = 10^{-\log_{10}(H) - pK_{\text{ATP}}} \quad (3.12)
\]

\[
\alpha_{\text{PYr}} = 10^{-\log_{10}(H) - pK_{\text{PYr}}} \quad (3.13)
\]

\[
\alpha_{\text{Lac}} = 10^{-\log_{10}(H) - pK_{\text{Lac}}} \quad (3.14)
\]

\[
\alpha_{\text{PCr}} = 10^{-\log_{10}(H) - pK_{\text{PCr}}} \quad (3.15)
\]

The pK-terms are new parameters and they need to be estimated. And since \( H \) is present in a logarithmic form in the \( \alpha \)-terms, we need to multiply the derivative of \( H \) with the inner derivative of the terms. It gives that the new expanded ODE for \( H \) has the form:

\[
\frac{d}{dt}(H) = \frac{-S_{\text{GP}} \cdot V_{\text{GP}} + S_{\text{Glyc}} \cdot V_{\text{Glyc}} - S_{\text{LDH}} \cdot V_{\text{LDH}} + S_{\text{ATPase}} \cdot V_{\text{ATPase}} - S_{\text{CK}} \cdot V_{\text{CK}}}{H \cdot S_{\text{buf}}} \quad (3.16)
\]

3.2.3 Measuring G6P

It is not possible to measure G6P directly due to its similarities to other particles. This has led to an assumption that G6P is approximately the PME value the NMR measurements gave. It is true that G6P is the major element in the measurements but since there are other elements present, scientists recommended that we modify the assumption to create a more realistic model. This can be achieved by introducing a parameter \( H_{\text{const}} \) which will be subtracted from the measured PME value:

\[
[G6P] = [\text{PME}] - H_{\text{const}} \quad (3.17)
\]
Where we previously used PME should now be replaced with PME-HP\textsubscript{const} to get a better estimation of G6P. HP\textsubscript{const} is estimated to be approximately 0.5 and will be optimized with the rest of the parameters. A natural question which arise with this modification is how to prevent HP\textsubscript{const} to be too small and maybe even transform G6P to an unrealistic negative value? But it is believed that if the model can simulate the measured signals in a correct way then the value for HP\textsubscript{const} should be quite close to its approximated value.

3.2.4 Expanding the v\textsubscript{GP}-reaction

The v\textsubscript{GP}-reaction was presented in section 2.2 and in that form the glycogen phosphorylase can not produce G6P during neither the resting nor the recovery phase. This statement is false because the v\textsubscript{GP}-reaction is active all the time, but its production is increased when Ca\textsuperscript{2+} is released during the contraction. To have a model that agrees with biochemical facts it is necessary to complete the reaction with another biochemical term. The new v\textsubscript{GP} has the following form:

\[
v_{GP} = V_1 \cdot \frac{Ca^{2+}(P - \frac{\text{PME-HP}_{\text{const}}}{K_{\text{1eq}}})}{K_{1P} + P} \cdot \frac{\text{AMP}_{\text{g1AMP}}}{K_{1\text{AMP}} + \text{AMP}_{\text{g1AMP}}} + \frac{V_1\text{bas}(P - \frac{\text{PME-HP}_{\text{const}}}{K_{\text{1eq}}})}{K_{1P} + P}
\]

Adding this new term to the reaction makes it acceptable from a biochemical point of view.

3.2.5 Re-writing some parameters as constants

We would like to have PME, P and ATP as constant as possible during the resting phase. It means that their derivatives should be zero or at least very small. Therefore, we choose to set the derivatives to zero at \(t = 0\) which gives the following 3 equations:

\[
0 = v_{GP}(0) - v_{\text{Glyc}}(0)
\]

\[
0 = -v_{GP}(0) - 2 \cdot v_{\text{Glyc}}(0) + v_{\text{ATPase}}(0)
\]

\[
0 = 3 \cdot v_{\text{Glyc}}(0) - v_{\text{ATPase}}(0) + v_{\text{CK}}(0)
\]

This system of equations can be solved and gives the following result:

\[
v_{GP}(0) = v_{\text{Glyc}}(0)
\] (3.18)

\[
v_{\text{ATPase}}(0) = 3 \cdot v_{GP}(0)
\] (3.19)

\[
0 = v_{\text{CK}}(0)
\] (3.20)
At \( t = 0 \) we have \( \text{Ca}^{2+} = 0 \), and by using equation (3.18) we can calculate \( V_{1_{\text{bas}}} \):

\[
V_{1_{\text{bas}}} = \frac{v_{\text{Glyc}}(0) \cdot (K_1 P + P(0))}{P(0) - \frac{P(0) - HP_{\text{const}}}{K_{\text{eq}}}}
\]

By using equation (3.19) we can calculate \( v_{\text{rest}} \):

\[
v_{\text{rest}} = 3 \cdot v_{\text{Glyc}}(0)
\]

By re-writing some of the expressions we have reduced 2 of the unknown parameters to constants and thereby simplified the optimization.

### 3.3 Assumptions about \( v_{\text{CK}} \)

A widely used assumption in biochemistry is to put \( v_{\text{CK}} = 0 \), but we will not use this assumption in the thesis due to the fact that if the assumption is true, \( v_{\text{CK}} \) will be zero by itself. The assumption has its origin in the ODE’s for the model. As described in section 2.2.1 (equation (2.10)), the derivative of PCr equals a negative \( v_{\text{CK}} \).

\[
\frac{d}{dt}(\text{PCr}) = -v_{\text{CK}} \tag{3.21}
\]

\( \text{PCr} \) is one of the measured signals and is quite stable during the experiment, except for the contraction part. It has been assumed that \( v_{\text{CK}} \) is at equilibrium all the time, but we would like to see what happens during the contraction phase when this assumption does not hold.

\[
v_{\text{CK}} = V_5 \cdot (\text{PCr} \cdot \text{ADP} \cdot H - \frac{\text{ATP} \cdot \text{Cr}}{K_{5_{\text{eq}}}}) \tag{3.22}
\]

This equation is used, but instead of assuming that it is zero during the anaerobic muscle contraction simulation, we let it be estimated by the signals. The removal of this assumption will prove to be quite interesting for the model behavior. More information can be found in chapter 4 where the different simulations will be evaluated.

### 3.4 Optimizing the optimization

There are different methods for manipulating the optimization in a desirable way. The measured signals can be scaled to the same magnitudes so they are treated equally during a simulation. Otherwise the larger signals will be dominating the smaller ones and the cost function will thereby be affected during an optimization. The measured error will not have the same proportion when the difference between the signals are high. The estimated parameters can also be weighted, and by this measure we can assure that they will be treated equally during an optimization. Instead of optimizing the parameters directly, it is beneficial to introduce another parameter with initial value 1 and to hold the
original parameters constant. This is a weighting method which guarantees that the optimization does not favor any parameter, regardless of the value.

3.4.1 Scaling the measured signals

Since the measured signals have different dynamic ranges the optimization does not give the best possible solution. For example, the pH-value is neglected compared to the other signal because its relative value is smaller. By scaling the signals we can assure that the optimization will treat the signals equally, or we can choose which signal or signals we want to prioritize. Since there is a significant difference in the maximum and minimum values, before and after the contraction, it is possible to choose a simple way of scaling the signals. By simply dividing all the measured points with the largest value we can create a transform that will make all graphs lie within 0 and 1, thereby making the optimization treat the measurements more equal.

\[ \text{signal}_{\text{scale}} = \frac{\text{signal}}{\text{signal}_{\text{max}}} \]  

(3.23)

The pH-value has very small relative variation and in that case it is relevant to pick another transform that increases the difference in the maximum and minimum values yet lying in the same scale as the other signals. This can be obtained by subtracting the smallest value from the every measured pH-value and dividing the result by the difference between the largest and smallest value:

\[ \text{pH}_{\text{scale}} = \frac{\text{pH} - \text{pH}_{\text{min}}}{\text{pH}_{\text{max}} - \text{pH}_{\text{min}}} \]  

(3.24)

3.4.2 Weighting the parameters

The parameters that are optimized can have enormous differences, ranging from $10^{-6}$ to $10^6$. The optimization algorithm will try to find the optimal values but the differences between the parameters cause a disadvantage for the larger parameters because the iteration step is the same for all parameters. This can be prevented by weighting the parameters that will be optimized. A very simple weighting method is sufficient in our case, just by replacing the parameters with scale-parameters that are optimized instead.

\[ \text{Parameter}_{\text{Weight}} = \text{Parameter} \cdot \text{Parameter}_{\text{Optimized}} \]  

(3.25)

Parameter$_{\text{Optimized}}$ is chosen to start with the value 1 for all the parameters in the optimization. It means that the starting values will remain the same but when the optimization starts they will be handled with the same weight regardless of their value. There are other benefits with this weighting method, for example, it is a lot easier to evaluate the result from the optimization. The optimized values will correspond to the direct change of the parameters. An optimization which gives the value 2 means that the initial parameter has been doubled, and a value of 0.5 means that it is only half its initial value.
3.4.3 Weighting and adjusting the cost function

Scaling measurement data and weighting parameters are not enough to obtain a satisfying model. After a first optimization we can see which areas in the model that might need more weighting, and that can be easily achieved by modifying the cost function. By putting a weight on each point in the measured time series it is possible to make a new optimization where we obtain new parameters and a better model is created.

Example 3.2: Code weighting in the cost function

```matlab
variableWeighting = ones(49, 5);
variableWeighting(1:20,1) = 2;
variableWeighting(25:30,1) = 2;
variableWeighting(2:30,2) = 3;
```

In this example 2 signals are weighted, and it is possible to decide at which time points the weighting will take place. This is very useful if some part in the model is more important to behave correctly then other parts. The contraction phase is the most crucial part in the model because radical changes occur during the 60 seconds it lasts. These changes can be hard to simulate and it is possible that we need to force the parameter estimation to give this area higher priority.
Chapter 4

Results

Results from the modeling process are presented in this chapter along with other relevant information, including tables, plots and evaluations. Model predictions at major steps, from the starting model to the final one, are plotted to illustrate the development. Statistical tests are performed for model validation.

The first and second sections contain information about the measurement data, their mean value and standard deviation. It is also explained how the standard deviation was filtered to eliminate noise.

The third section explains how an assumption, due to lack of simulations, was wrongly accepted which got revealed during the work with the modeling process. Fortunately a lucky discovery managed to solve a contradicting problem in regulating anaerobic muscle glycolysis.

The fourth section will contain information about the starting model. This model is the one the modeling process started with; it contains no modifications and works mostly as a starting point for creating a better model. The changes made to create a better model from the starting model were explained in chapter 3.

The fifth section illustrates how the changes made in the starting model affected the simulation. This improved model is called “the scaled and modified model” because the signals and the parameters have been scaled to gain a better optimization and all the necessary modifications have been made. There are four subsections evaluating the phases of the experiment separately, and finally the whole model is evaluated.

The sixth and final section contains the final model which is a further improvement of the scaled and modified model. It also contains the weighting used to obtain the final model, evaluation of the 3 phases of the experiment, and a whole model evaluation.
4.1 Standard deviation and mean value of measurement data

9 separate experiments were performed to give a good approximation of the calculated arithmetic mean value. At each time course and at each signal the sums of the data are divided by 9, which gives the mean value for the measured signals. This data has been used while optimizing the model.

![Graphs of PME, P, PCr, ATP, and pH over time](image)

**Figure 4.1.** Mean value for measurement data

The ATP signal is too noisy and it is impossible to get information how much it changes during the 3 phases of the experiment. This problem is handled by replacing the signal with a constant value during the optimizations. The constant is defined as the mean value of all ATP signals, approximately 5.47.
As the plots show, there are small differences between the 3 phases of the experiment. For PME and ATP, the standard deviation seems to be more or less constant during the phases but the other 3 signals have changes worth mentioning. For pH and P the standard deviation get a bit larger during the contraction and the recovery phase. PCr shows the opposite tendency, with lower standard deviation during the last 2 minutes. The ATP is very noisy and contains very little information while pH has really low values making it important to simulate in an accurate way, at least from a statistical point of view.
4.2 Filtering standard deviation

During the validation we use the model’s standard deviation, though it is important to note that these values are quite noisy. By filtering them we might obtain a more satisfying and realistic plot of the models standard deviation. We chose a Butterworth low pass filter of second order and made it go through the calculated values both back and forward. Since the signal PME was relatively constant we did not have to filter it, instead it was replaced with a constant based on the mean value. The cutoff frequency for the other signals were determined by visual inspection.

<table>
<thead>
<tr>
<th>Data</th>
<th>Wn</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>N/A</td>
</tr>
<tr>
<td>PCr</td>
<td>0.21</td>
</tr>
<tr>
<td>P</td>
<td>0.15</td>
</tr>
<tr>
<td>ATP</td>
<td>0.16</td>
</tr>
<tr>
<td>pH</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 4.1. Values for the cutoff frequency that gave the best visual approximation.

Figure 4.3. Comparisons between filtered and non-filtered standard deviation.
4.3 Wrong assumption revealed

There has been an assumption among biochemists, that creatine kinase is at equilibrium during the contraction which has led to several contradictions. This assumption is discussed in section 3.3 more deeply. The glycolytic flux is regulated by ADP, PME, NAD and P as has been described in chapter 2.

\[
\frac{v_{\text{Glyc}}}{v} = V_2 \cdot \frac{\text{ADP}}{K_{2\text{ADP}} + \text{ADP}} \cdot \frac{\text{PME}}{K_{2\text{HP}} + \text{PME}} \cdot \frac{\text{NAD}}{K_{2\text{NAD}} + \text{NAD}} \cdot \frac{P}{K_{2P} + P}
\]

During the contraction these regulators increase and since they are multiplied into the \(v_{\text{Glyc}}\)-term we have a rapid increase in the glycolytic flux as well. But when the flux stops and the recovery phase begins, the regulators remain at their high level but \(v_{\text{Glyc}}\) decreases drastically. This is a contradiction that has not been explained before. We believe that the contradiction is due to the false assumption that \(v_{\text{CK}} = 0\). Instead we let \(v_{\text{CK}}\) be determined by the signals and the estimated parameters.

\[
v_{\text{CK}} = V_5 \cdot (\text{PCr} \cdot \text{ADP} \cdot H - \frac{\text{ATP} \cdot \text{Cr}}{K_{5\text{eq}}})
\]

Next plot illustrates what happens when the assumption is revoked and the equation for \(v_{\text{CK}}\) is used.

![Figure 4.4](image-url)
It is very clear that $v_{\text{CK}}$ is not close to zero during the contraction as we assumed it would be. This also affects the ADP which increases a lot more and lifts $v_{\text{Glyc}}$ as needed during the contraction and then falls back during the recovery phase. By revoking the equilibrium for $v_{\text{CK}}$ we get a drastic change in ADP, enough to explain the behavior for the glycolytic flux during the experiment without any contradictions.

**Figure 4.5.** ADP level during the experiment.

**Figure 4.6.** Simulating the glycolysis.
4.4 Simulating the first model

Before any changes were made in the model, simulation was needed to see how well the initial minimalistic model behaved. The initial conditions were changed to suit the measured data better.

As the plots show, the simulation is not even close to the measurements. The resting phase is constant and can thereby be easily described just by modifying the initial conditions, but the contraction and the recovery phases are totally wrong. PME does not raise high enough though the recovery phase is relatively constant. P decreases after a couple of seconds into the contraction which is wrong, and it stays at a low level during the recovery phase. PCr is not decreasing fast enough during the contraction and the level it converges to during the recovery phase is not acceptable. ATP-level drops to zero shortly after the contraction takes place and is not capable of recovering to acceptable values throughout the remaining phase. After a Downhill-simplex optimization the model did not look much better, and three major reasons were noticed.

1. Some major errors were present in the model making the optimization unacceptable. The \( v_{\text{GP}} \)-reaction is totally Ca-dependant which makes the ATP-value decrease drastically when the contraction starts. The differential equation is too much simplified and does not consider changes
in the $v_{GP}$-reaction at all. By simulating the model these errors were revealed and could be addressed.

2. It is necessary to adjust some of the parameter values so the optimization method can find an adequate minimum at all. The Downhill-simplex method implemented in the toolbox can not find any minima unless it starts relatively close to one. The simulation helped us determinate how the parameter values interact. Simulated annealing method was used during these researches because the generated parameters could be used with Downhill-simplex. The simulated annealing method itself could not be used throughout the whole optimization because there were too many problems with it. Error messages frequently interrupted or stopped the optimization, but most of the times it made Matlab freeze totally. Still it helped us obtain parameters which the Downhill-simplex could finish an optimization with.

Since Downhill-simplex is a local optimization-method we need to start relatively close to the optimal value to obtain it. It is necessary to adjust some of the parameter values so the optimization method can find an adequate minimum. The simulation helped us determinate how the parameter values interact. Simulated annealing method was used during these researches because the simulations could finish.

3. The relative error in the model varies too much, making the pH-value less important than the plots with higher values. This can easily be modified by scaling the measured signals making all of them equally important in the cost-function.
4.5 Simulating a scaled and modified model

This is how the model describes measurement data after scaling the signals, scaling the parameters, making all the necessary modifications in the model. Downhill-simplex was used to optimize the parameters.

![Graphs of measured and simulated data](image)

**Figure 4.8.** Comparisons between measurement data and simulated model after scaling the signals, parameters and using Downhill-simplex as optimization-method.

The model describes the measured data very well, better than expected from start, but there are some minor errors in the model that we can not disregard. During the recovery phase, the measurement data is relatively constant but the simulation have difficulties stabilizing itself and have either a tendency of decreasing or increasing too much. Both P, PME and pH have some difficulties with stabilizing during the recovery phase, they decrease too much making us believe that the model prioritize the contraction phase more due to higher error-values during that phase. By weighting the measured data we might get model that is visually and also statistically better. By plotting the residuals it is possible to evaluate the model.
Figure 4.9. Residuals for the scaled and modified model.

The residual analysis will be divided into 3 parts; the resting phase, the contraction and the recovery phase.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>24</td>
</tr>
<tr>
<td>Contraction</td>
<td>13</td>
</tr>
<tr>
<td>Recovery</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>49</strong></td>
</tr>
</tbody>
</table>

Table 4.2. Degrees of freedom for the different phases of the model.

### 4.5.1 Resting phase

The resting phase lasts for two minutes, and by evaluating the residuals during the first 2 minutes we can see that they are quite constant and they seem to be randomly distributed around zero. We will plot histograms for all the signals during the resting phase and calculate their skewness and kurtosis values for evaluation.
There are 24 time points during the resting phase and it permits the values to deviate from a gaussian distribution even if they were randomly distributed. But by evaluating the histograms and the other tests we can clearly announce that the ATP signal is not randomly distributed, it fails both the skewness and the kurtosis tests. It is difficult to draw any conclusions about the other signals based on the tests and the histograms since they passed the tests and seem quite randomly distributed considering the few samples. It is however disturbing to notice that PME has many negative values while pH shows the opposite result. However, considering the few samples and by evaluating the skewness and kurtosis test it is possible to say that they might be randomly distributed except for the ATP signal.
Table 4.4. Validating the resting phase by comparing the calculated values with a test value $T$ given by $\chi^2$ distribution.

All the signals pass the $\chi^2$-test but since ATP is not randomly distributed the assumptions the test was based on are not fulfilled. Thereby the model’s resting phase can neither be rejected or accepted according to the null hypothesis. The signals show a very good fit but unfortunately it is not possible to evaluate the model during the resting phase with a $\chi^2$-test because of ATP.

### 4.5.2 Contraction phase

![Graphs of PME, P, PCr, ATP, pH over time](image)

**Figure 4.11.** Residuals for the scaled and modified model during the contraction.

The contraction phase lasts 60 seconds and takes place between the second and third minute of the experiment. By looking at the residuals during this phase, we
can observe that they are not randomly distributed and that they have either raising or declining patterns. The PME and the ATP signals can be regarded as exceptions since they seem quite randomly distributed. There is no use plotting histograms for this phase since we have very few samples and it would be impossible to evaluate the results. PME and ATP are 2 of the 5 signals that could be randomly distributed but we will calculate the mean, skewness and kurtosis for all signals to have as a comparison with future models.

<table>
<thead>
<tr>
<th>Data</th>
<th>Mean</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>0.015</td>
<td>0.115</td>
<td>-1.154</td>
</tr>
<tr>
<td>P</td>
<td>0.525</td>
<td>-0.094</td>
<td>-0.970</td>
</tr>
<tr>
<td>PCr</td>
<td>-0.397</td>
<td>0.153</td>
<td>-1.473</td>
</tr>
<tr>
<td>ATP</td>
<td>-0.015</td>
<td>-0.711</td>
<td>-0.152</td>
</tr>
<tr>
<td>pH</td>
<td>0.015</td>
<td>0.468</td>
<td>-1.247</td>
</tr>
</tbody>
</table>

Table 4.5. The mean, skewness and kurtosis for the scaled and modified model during the contraction.

The table shows that even though ATP seemed randomly distributed, it has a skewness problem and is not valid for a validation. This conclusion gives that the only signal for which a validation could be accepted is PME.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>1.45</td>
<td>22.36</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 4.6. Validating the PME signal during the contraction phase by comparing its calculated value with a test value $T$ given by $\chi^2$ distribution.

The PME signal passes the test with good margin, and fulfills the criterions for a validation. Unfortunately it is not possible to evaluate the other signals due to the non-randomly distributed residuals.

4.5.3 Recovery phase

The recovery phase lasts 60 seconds and is the last minute during the experiment. The contraction has been switched off and the signals should stabilize. By evaluating the residuals it is clear that most of them are not randomly distributed. Just as for the contraction phase, there are only 2 parameters that could be randomly distributed; PME and ATP. In order to be consequent, the mean, skewness, and kurtosis will be calculated for all the signals for comparison with future models.

Fortunately we can see that ATP passes both the skewness and the kurtosis test, which means it can be validated. Comparing this phase with the contraction, we can observe that the acceptance of ATP for validation is the only difference.

The validation for the two signals was successful. But it is impossible to evaluate the whole phase using the $\chi^2$ test due to non-randomly distributed residuals for the other signals.
Table 4.7. The mean, skewness and kurtosis for the scaled and modified model during the recovery phase.

<table>
<thead>
<tr>
<th>Data</th>
<th>Mean</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>-0.243</td>
<td>0.020</td>
<td>-1.357</td>
</tr>
<tr>
<td>P</td>
<td>-0.214</td>
<td>-1.239</td>
<td>0.853</td>
</tr>
<tr>
<td>PCr</td>
<td>-0.770</td>
<td>0.374</td>
<td>-1.218</td>
</tr>
<tr>
<td>ATP</td>
<td>-0.570</td>
<td>0.181</td>
<td>-0.319</td>
</tr>
<tr>
<td>pH</td>
<td>0.018</td>
<td>0.363</td>
<td>-0.849</td>
</tr>
</tbody>
</table>

Table 4.8. Validating PME and ATP during the recovery phase by comparing its calculated value with a test value T given by $\chi^2$ distribution.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>1.21</td>
<td>21.03</td>
<td>yes</td>
</tr>
<tr>
<td>ATP</td>
<td>1.34</td>
<td>21.03</td>
<td>yes</td>
</tr>
</tbody>
</table>

4.5.4 The whole model

We have been looking at the different phases during the experiment and we have evaluated them separately. Now we will try to evaluate the whole model instead of dividing it up in phases. The residuals will be plotted and both the skewness and the kurtosis will be calculated.

When all the data is collected apparently all the signals pass the skewness and the kurtosis test, including ATP. But looking at the residuals we can see that many signals are far from being randomly distributed. PME is quite constant throughout the whole experiment and passes the tests, although the mean is too negative. P has a good mean value but the residuals are not randomly distributed in the contraction phase or the recovery phase making the whole model validation useless. The exact same things happen with PCr; it is relatively constant during the resting phase but the remaining 2 minutes show patterns unlike a gaussian distribution. ATP was the only signal that did not pass the skewness nor the kurtosis test during the resting phase making it the “worst” signal at the time. But looking at the whole model perspective we can see that it has a very good mean and it passes the tests. It shows a negative skewness which indicates too many values to the right of the mean, but in general it has become
4.5 Simulating a scaled and modified model

one of the best measured signals observing the whole model. The pH signal has
the same problem as P and PCr, which can be summarized with non-randomly
distributed values during the contraction and the recovery phase. The validation
will look at the whole model, the separate signals and also the 3 different phases
during the experiment.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>95.24</td>
<td>282.51</td>
<td>yes</td>
</tr>
<tr>
<td>PME</td>
<td>6.62</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>P</td>
<td>12.97</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>PCr</td>
<td>51.11</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>ATP</td>
<td>5.08</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>pH</td>
<td>19.45</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>Resting</td>
<td>13.28</td>
<td>146.57</td>
<td>yes</td>
</tr>
<tr>
<td>Contraction</td>
<td>47.67</td>
<td>84.82</td>
<td>yes</td>
</tr>
<tr>
<td>Recovery</td>
<td>34.29</td>
<td>79.08</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 4.10. Validating the scaled and modified model by comparing the calculated
values with a test value T given by $\chi^2$ distribution.
Considering the assumptions which we based the $\chi^2$ test on, with randomly distributed variables, it follows that it is impossible to say anything about the models acceptance or rejection even though it passed the $\chi^2$ test. The closest region was the Resting phase but ATP showed both a skewness and a kurtosis problem during that period of time. Despite obvious model error, we can conclude that the minimalistic model we have created is acceptable. In the following section we will try to improve the model, and hopefully validate it with the $\chi^2$ test.

4.6 The final model

In this section the best model is being presented with statistics and plots. The weighting used to obtain the final model is discussed. The data will be split up in three separate parts; the resting, the contraction, and the recovery phase. Each of these phases will be evaluated separately and finally the model predictions are plotted and compared with measurement data.

4.6.1 Weighting the final model

The final model has been created by weighting signals in the scaled and modified model. Different weights have been used and the best combination will be presented and evaluated in the coming sections. To decide the difference between simulated models after the optimization we have used visual evaluation and common sense. The model that looked like the “best” one has been picked while the others have been discarded. It is probably possible to achieve a slightly better model by choosing different weighting-values but since the differences are very small we declare that this model is the best one we can achieve, and it has been chosen as the final model.

The weights used to obtain the final model are presented in the table below.

<table>
<thead>
<tr>
<th></th>
<th>PME</th>
<th>P</th>
<th>PCr</th>
<th>ATP</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Contraction</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Recovery</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4.11. Weighting used to generate the best model.

This combination of weights generated the visually best model. The residuals seem smaller than before and hopefully we can prove that it is a better model than without weights. We will evaluate this model the same way we did with the previous one, by plotting the residuals, calculating their skewness and kurtosis etc. The following plots show the final model’s residuals and the comparison with measurement data.
Figure 4.13. Residuals of the best model.
As we can see this model is better than the one without any weights. The residuals seem more randomly distributed then before, but unfortunately they still have non-random behavior especially during the last two minutes, which consists of the contraction and the recovery phase. The coming sections will evaluate the model statistically and hopefully it will be possible to draw conclusions from the results.

**Figure 4.14.** Plotting the final model and measurement data.
4.6.2 Resting phase

The resting phase is the first two minutes of the experiment when we make sure there is no oxygen present making the reaction anaerobic. The residuals during this phase will be plotted in a histogram and their skewness and kurtosis will be calculated for an evaluation.

![Histograms of residuals during resting phase](image)

Figure 4.15. Histogram for the final models residuals during resting phase.

<table>
<thead>
<tr>
<th>Data</th>
<th>Mean</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>-0.399</td>
<td>0.017</td>
<td>-0.784</td>
</tr>
<tr>
<td>P</td>
<td>-0.368</td>
<td>0.052</td>
<td>-1.126</td>
</tr>
<tr>
<td>PCr</td>
<td>0.252</td>
<td>0.399</td>
<td>-0.711</td>
</tr>
<tr>
<td>ATP</td>
<td>0.317</td>
<td>-1.161</td>
<td>1.794</td>
</tr>
<tr>
<td>pH</td>
<td>-0.0163</td>
<td>0.717</td>
<td>-0.038</td>
</tr>
</tbody>
</table>

Table 4.12. The skewness and the kurtosis of final model during the resting phase.

This model originates from the scaled and modified model and we will use the old model to try to show the improvements. The weighting for PME resulted in a shift to the left for the mean value without major differences in the skewness nor the kurtosis. This shift is disturbing because the mean was already suspiciously negative for being a random distribution. The P signal shows the same problem; the mean has shifted to the left, while the skewness shows a significant improvement and the kurtosis became worse. The PCr signal’s mean
moved to the left which is good but the absolute value for the difference from zero has increased which is disturbing. The skewness for PCr worsened, while the kurtosis had no significant change. The ATP which “ruined” our validation in the previous model has not changed, meaning that it will be impossible to validate the final model during the resting phase, at least not for all the 5 signals. The weighting affected the pH signal the most, making it look a lot better during the recovery phase but resulted also in a skewness problem for the residuals during the resting phase. The kurtosis value has also increased to a problematic high value compared to the previous model, barely reaching under the limit for kurtosis problem. The conclusion is that the model has become worse for the resting phase, none of the signals showed any improvement and instead the pH signal was entangled with a skewness problem and is extremely close to a kurtosis problem. A validation will be executed, primarily to use as a comparison with the previous model.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>14.74</td>
<td>146.57</td>
<td>yes</td>
</tr>
<tr>
<td>PME</td>
<td>4.35</td>
<td>36.42</td>
<td>yes</td>
</tr>
<tr>
<td>P</td>
<td>2.96</td>
<td>36.42</td>
<td>yes</td>
</tr>
<tr>
<td>PCr</td>
<td>0.81</td>
<td>36.42</td>
<td>yes</td>
</tr>
<tr>
<td>ATP</td>
<td>2.48</td>
<td>36.42</td>
<td>yes</td>
</tr>
<tr>
<td>pH</td>
<td>4.14</td>
<td>36.42</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 4.13. Validating the resting phase for the final model by comparing the calculated values with a test value T given by $\chi^2$ distribution.

Having in mind that the previous model passed the $\chi^2$ test with a very wide marginal, it is not a surprise that the final model does the same. The changes are insignificant, the overall change is minimal. ATP has the same T-value while PME, P and PCr have a smaller T-value than before. The pH signal increased its value slightly but has also joined the ATP value with skewness problems. Compared to the previous model, the only conclusion we can draw from these results is that now we can not validate the pH signal either.
4.6 The final model

4.6.3 Contraction

The second phase in the experiment is the contraction which lasts for 60 seconds under anaerobic conditions. Using the previous model the majority of the signal residuals were not randomly distributed and which caused a validation problem. But the final model shows a significant improvement for the residuals during the contraction. This phase contains 13 time points and due to the few samples it is difficult to make any conclusions. PCr is the only signal which we can confidently claim is not randomly distributed, while the other signals can be accepted. Due to the few samples, histograms are irrelevant, but since the signals have improved compared to the previous model their mean, skewness and kurtosis will be calculated and presented.

<table>
<thead>
<tr>
<th>Data</th>
<th>Mean</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>0.164</td>
<td>0.042</td>
<td>-1.251</td>
</tr>
<tr>
<td>P</td>
<td>0.470</td>
<td>0.166</td>
<td>-0.739</td>
</tr>
<tr>
<td>PCr</td>
<td>-0.526</td>
<td>0.383</td>
<td>-0.872</td>
</tr>
<tr>
<td>ATP</td>
<td>0.033</td>
<td>-0.736</td>
<td>-0.128</td>
</tr>
<tr>
<td>pH</td>
<td>0.029</td>
<td>0.145</td>
<td>-0.938</td>
</tr>
</tbody>
</table>

Table 4.14. The mean, skewness, and kurtosis for the final model during the contraction.

It was possible to discard the PCr signal by visual evaluation and the statistical evaluation showed that ATP have a skewness problem. This is a major improvement compared to the previous model, where PME was the only signal that could be validated.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>2.12</td>
<td>22.36</td>
<td>yes</td>
</tr>
<tr>
<td>P</td>
<td>1.46</td>
<td>22.36</td>
<td>yes</td>
</tr>
<tr>
<td>pH</td>
<td>6.17</td>
<td>22.36</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 4.15. Validating PME, P and pH during the contraction phase by comparing their calculated values with a test value T given by $\chi^2$ distribution.

All the tested signals passed the validation which can be regarded as a success compared to the previous signal, where PME was the only signal which could be validated at all. On the other hand the model validation for the whole contraction phase is still not possible to perform because PCr and ATP do not fulfil the criteria for randomly distributed variables.
4.6.4 Recovery phase

The recovery phase is the last 60 seconds of the experiment. We have seen that the residuals for the final model during the recovery phase look a lot better than before. But unfortunately P and PCr still show a non-random pattern and cannot be accepted for a validation.

\[
\begin{array}{c|c|c|c}
\text{Data} & \text{Mean} & \text{Skewness} & \text{Kurtosis} \\
\hline
\text{PME} & -0.586 & -0.005 & -1.363 \\
\text{P} & -0.448 & -0.117 & -1.093 \\
\text{PCr} & -0.172 & 0.138 & -1.315 \\
\text{ATP} & -0.570 & 0.181 & -0.319 \\
\text{pH} & 0.026 & -0.641 & 0.056 \\
\end{array}
\]

Table 4.16. The mean, skewness, and kurtosis for the final model during the recovery phase.

pH has improved a lot but the mean is a bit high considering the low values, though the mean is not too high to reject a Gaussian distribution, meaning it can be validated with PME and ATP.

\[
\begin{array}{c|c|c|c}
\text{Data} & T & 95\% & \text{Passed} \\
\hline
\text{PME} & 2.96 & 21.03 & yes \\
\text{ATP} & 1.29 & 21.03 & yes \\
\text{pH} & 1.63 & 21.03 & yes \\
\end{array}
\]

Table 4.17. Validating PME, ATP and pH during the recovery phase by comparing their calculated values with a test value T given by $\chi^2$ distribution.

All the signals passed the validation, but it has to be regarded as a failure that PCr and P could not be validated and we cannot use a statistical evaluation for the phase, not even with the final model.
4.6 The final model

4.6.5 The whole model

Instead of dividing the experiment into three separate phases, we will treat it as a single experiment where all the phases are combined. The same procedure was done to the scaled and modified model, where it was impossible to draw some conclusions about the validation.

![Histogram for the final model. Including PME, P, PCr, ATP and pH.](image)

By evaluating the residuals and by looking at the simulation it is possible to state that this model is better than the scaled and modified one. The PME and the ATP did not get affected much by the weighting, which is positive since their residuals looked quite random. P, PCr and also pH had better residuals in the final model, but unfortunately it was not possible to eliminate the disturbing patterns of non-random distributions. It succeeded for the P signal during the contraction phase and for pH signal during the recovery phase but the other

<table>
<thead>
<tr>
<th>Data</th>
<th>Mean</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>-0.296</td>
<td>0.468</td>
<td>0.252</td>
</tr>
<tr>
<td>P</td>
<td>-0.165</td>
<td>0.357</td>
<td>-0.198</td>
</tr>
<tr>
<td>PCr</td>
<td>-0.058</td>
<td>-0.548</td>
<td>0.021</td>
</tr>
<tr>
<td>ATP</td>
<td>-0.024</td>
<td>-0.707</td>
<td>0.711</td>
</tr>
<tr>
<td>pH</td>
<td>0.006</td>
<td>0.177</td>
<td>-0.911</td>
</tr>
</tbody>
</table>

Table 4.18. The mean, skewness, and kurtosis for the best models residuals.
phases and signals with obvious problems it could not fix. A validation will be performed for all the signals and phases, but unfortunately the whole model validation is irrelevant as a statistical test.

<table>
<thead>
<tr>
<th>Data</th>
<th>T</th>
<th>95%</th>
<th>Passed</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>49.44</td>
<td>282.51</td>
<td>yes</td>
</tr>
<tr>
<td>PME</td>
<td>9.44</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>P</td>
<td>6.26</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>PCR</td>
<td>17.01</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>ATP</td>
<td>5.10</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>pH</td>
<td>11.62</td>
<td>66.34</td>
<td>yes</td>
</tr>
<tr>
<td>Rest</td>
<td>14.88</td>
<td>146.57</td>
<td>yes</td>
</tr>
<tr>
<td>Contraction</td>
<td>21.17</td>
<td>84.82</td>
<td>yes</td>
</tr>
<tr>
<td>Recovery</td>
<td>13.39</td>
<td>79.08</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 4.19. Validating the final model by comparing the calculated values with a test value T given by $\chi^2$ distribution.

All the signals and phases passed the $\chi^2$ test quite easily. Though the result gets diminished due to the residuals are not randomly distributed, not even for the final model. The final model is definitely more appealing than the previous model and the residuals look better, but unfortunately it is not possible to validate the models statistically. As was concluded in section 4.5.4, the model itself seems very appealing even though it can not be validated. The minimal model created is able to describe the measured data much better than expected.
Chapter 5

Discussion and Conclusion

In this chapter we will discuss how the modeling process have progressed and what conclusions we have been able to make. Further objectives are also discussed because this thesis has raised many questions about modeling biochemical reactions.

5.1 Error in the model

During the parameter estimation, one of the newly introduced parameters has an unrealistic value which results in an error.

\[ [\text{G6P}] = [\text{PME}] - \text{HP}_{\text{const}} \] (5.1)

\text{HP}_{\text{const}} \text{ decreases during the optimization, and leads to a negative input of G6P in the model. However, this error does not affect the model significantly. Of course there would be minor changes if a new model is created, but in general the current model can be accepted as a decent model though it is important to have this error in mind. The error can be eliminated by putting bounds on the parameters. This procedure can be applied to all the models and will be discussed in this chapter. Another simple method to avoid this error is to use a constant \text{HP}_{\text{const}} during the experiment. The following plot illustrates how this error affects the model, and the input of G6P.}
5.2 Discussion

The different topics will be presented separately.

**Modeling tool and parameter estimation:** It has been difficult to run the parameter estimation because the toolbox is relatively new and some errors might still occur. Still, simulating the model works perfectly and it is easy to edit the plots made by the SBTB compared to some other biochemical programs. On the other hand it could be useful to combine SBTB with some other programs like COPASI, which is a free software application for simulation and analysis of biochemical networks. It is also possible to put bounds on some parameters while using COPASI or by using the add-on program for SBTB. This has not been prioritized during the work since there have been many questions about the limitations of some parameters. However, putting more effort in that area could increase the understanding of the model even more.

**The model’s observability and identifiability:** A system is said to be observable if its current state can be determined by observing its outputs only. The identifiability problem is a special case of observability, usually used on the parameters of a system. The system’s observability is quite difficult to determine since the model is non-linear, but it is of great importance to manage in the future. There are 2 popular methods to test the observability of nonlinear
control systems; the differential geometric and the algebraic approach. Further
details about observability testing can be found here [19]. Parameter
identifiability, that the parameters can be determined from the systems
input-output values, is highly appreciated if achievable. But when the minimal
model is expanded, which it should be if we are looking for a better model, then
it also follows that we have more parameters which makes parameter
identifiability less likely. A recommended method that can be applied to our
model is a probabilistic algorithm by Alexandre Sedaglovic. A Maple
implementation of this algorithm can be downloaded from the net [18].

A better model: Since this is a minimal model it can of course be expanded to
describe the contraction even better. A more detailed muscle metabolism could
be obtained by adding more parameters into the model helping the reactions to
describe the measurement data even better. Qualified help from biochemists is
strongly recommended if this step is to be realized. The implementation of the
contraction is at this stage very simple, an on-off switch based on Ca. This is of
course wrong in a biochemical view, as we can flex our muscles in different ways,
from a very intense contraction lifting a weight to a more soft contraction lifting
a lighter object. The current implementation does not cover the different stages
of the contraction and could be one of the reasons why the measurement data is
different from experiment to experiment. By using a more flexible Ca-value we
would be able to describe variations of power in the muscle.

5.3 Further objectives

In this section new ideas will be presented that can develop modeling. Focus will
be on our best model and how improvements can be done in the future.

The Ca value: As discussed earlier, it is better to equip Ca with a efficiency
value instead of implementing it as an on-off switch with value 1. Maybe it is
possible to maintain the same model and parameters while the test person can
decide the amount of strength he is using, knowing that the model will be able to
describe the signals. This Ca-value could be put as a parameter during the
optimizing phase, to determine its correct value.

Expanding the model: As we concluded before, the final model could not be
validated because the residuals were not randomly distributed. This could be a
direct consequence of using a minimal model. It would be very interesting to
expand the model; both in terms of a more realistic reaction description but also
in the expansion of phases, for example, making it describe the values after the
recovery phase. This would test how efficient the recovery phase is at present
and would constitute a small step towards a full body model. While discussing
further objectives it is important to eloquently point out that this thesis is
approaching modeling from a mathematical point of view. Our best model could
work as a platform for new models, but the construction of them must be done
by biochemists since it is hard describing biochemical reactions in a mathematical way. With an origin in the model presented in this thesis, it is possible to expand the model in many ways, depending on which part the biochemists would like to focus on.

**Optimization:** When it comes to mathematical improvements several things can be pointed out. Better, faster and more stable optimization algorithms are being implemented at this very moment around the globe, helping us simulating and optimizing future models. While studying how separate parameters interact, simulated annealing was used as an optimization method helping us find wider range of optima instead of using Downhill simplex. Unfortunately Downhill simplex is a local method and finding another algorithm is important for coming optimizations. The model’s observability is another interesting issue which might be important in future models.

### 5.4 Conclusions

The model can describe the collected data impressively well and this result can be interpreted as a success. Using this model as a platform, better models can be created taking us one step closer to a full body model. The road there is long and difficult and more research is needed in form of new collected data, faster computers with improved programs and algorithms, and not least our increasing knowledge about making and interpreting models. This fact is proved once again by the fact that an erroneous assumption made by scientists got revealed during this thesis. Future models and simulations will not only spare us many costly experiments but it will also increase our knowledge about the complex human body.
Bibliography


[18] http://www2.lifl.fr/ sedoglav/Software/ObservabilityTest/

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