Modeling Drug Transport across the Membrane of a Tumor Cell: Improving drug development for cancer treatment

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Contents

Abstract

This research was performed to determine which type of model gives a better prediction of the drugs transport into and inside a tumor cell. Two types of models have been developed with compartment modeling using the partition coefficient and concentration gradient of a compound. We compared the simulations of these models with varying the drug's partition coefficient. The model with intermediate steps to cross the membrane takes more time than the model without these steps to reach the partition in equilibrium of the drug in the several compartments. This suggests that the model including these steps give a better prediction of real life drug transport. From a certain value for the partition coefficient, the drug does not enter the cell faster if we increase this value. These results suggest that the model with the intermediate steps is most effective for modeling data for different compounds to test whether they would be suitable drugs to treat cancer.

Keywords— Tumor Cell, Membrane, Drug Transport, Partition Coefficient, Compartment Modeling

1 Introduction

Nowadays cancer is a very common problem in society and exists in various kinds of different degrees. If cancer is formed by a tumor, it can usually be treated with chemotherapy. In this project we study chemotherapeutic drugs and their movements into and inside one tumor cell. Given the wide use of anticancer drugs, it is of great importance to understand how it moves into a cell and the regions within. In this thesis we refer to these regions with compartments. It is useful to understand the effect of the character of a cell membrane on the transport of a drug, or compound, to come across. We will establish a mathematical model that corresponds to reality to receive a better understanding of this effect. Developing a mathematical model can be used to improve the development of drugs for cancer treatment. Such a model will give a strong interpretation of the behavior of a compound without the need for experiments. This in turn will ensure that both time and money will be saved. Researchers found that the results of experiments do not match with a model which assumes that drugs cross the membrane without intermediate steps. The problem is that the drugs need more time to arrive at the cell and to force the cell to apoptosis. Apoptosis means that the cell commits suicide. Therefore, we will develop a model that considers two steps to cross the membrane. This model will mainly be based on the findings of a study conducted by Eytan [4]. We will also develop a model that does not take the intermediate steps of crossing the membrane into account. This model will be modified from a model developed by Leedale et al. [9] in such a way that we are able to compare it with the model we mentioned earlier.

The aim of this study is to find if the model that considers intermediate steps to cross the membrane gives a better prediction of the time the compound needs to move into a cell and fights the tumor. We will do that by developing two types of models, one with the intermediate steps and one without. For the first model we develop a system of ordinary differential equations (ODEs) starting with analyzing the movement through the two layers of the membrane and the cytoplasm. In a more advanced model, we will also look at the transport into organelles such as the nucleus and mitochondria. This expanded model also includes the process of drug binding to DNA. Once the drug is inside the nucleus it will bind to free DNA receptors such that it blocks an important enzyme so that the tumor cell commits suicide. In one of the chapters of this thesis we will explain this phenomenon in more detail. With analytical methods and numerical simulations, we will analyze these models and compare them to the numerical simulations of the other model. This model considers no intermediate steps to pass the membrane. It is based on the pH partition hypothesis and the partition coefficient of a compound. Due to the partition hypothesis the uncharged drug molecules will be equally divided over the several compartments. However, the compound will not be uniformly distributed because of the partition coefficient. This represents the ratio of the concentration drugs between two compartments at equilibrium. With compartmental modeling we will analyze the local processes in the different compartments of both models.

We aim to reinforce the understanding of the effect of the structure of the membrane on the movement of drugs by accurately simulating the process of these movements. Therefore, literature research has been done to find credible values corresponding to a particular compound for the parameters we use in our models. These models will be analyzed, expanded, analyzed again and finally we will compare the two types of models with each other. In our results we expect to receive a better prediction of the transport of the compound for the model that considers two intermediate steps to cross the membrane. We already know that the other model is too fast in comparison to the real experiments. If we implement multiple steps in our model to enter from outside the cell, it is plausible to expect that it will indeed take longer for the drug to enter the cell. As a result, it will also take longer for the drug to reach the cell nucleus and bind to the DNA causing the cell to die.

2 Drug Uptake

A tumor consists of a clump of cells. In this study we analyze the movement of a drug into one of these cells in a medium. Tumor cells embody a cell membrane surrounding the cytoplasm with organelles including the nucleus, mitochondria, and many others. The membrane has a phospholipid bilayer with hydrophilic heads and hydrophobic tails, see figure 1. This bilayer consists of an outer- and inner leaflet on the extracellular and intracellular side of the cell respectively. The surfaces of the leaflets are negatively charged and the hydrophobic tails between the leaflets form a lipid core. Because of these properties, for a drug to cross such a membrane it should be positively charged so that it can locate at the surface of the leaflets. In addition, the compound must be at least partly hydrophobic to pass the lipid core of the membrane. The partition into the membrane depends on the lipid solubility of the drug is the most critical factor for a compound to cross the membrane. A drug that is relatively hydrophilic has a harder time penetrating the chemical barrier without any transporter, according to Leedale et al. [9].

To analyze the movement of a drug into and inside a tumor cell, we will develop a model that determines the distribution of the total concentration of the compound over different parts of the cell. Using some of the features of a drug we can tell something about its movements and the time that equilibrium is reached. These features are for example the adsorption and desorption rates of the drug and the time the drug needs to cross the membrane by flip-flop events. In this project we will develop two types of models and compare them with each other. The first model considers two steps to cross the membrane and the second one assumes that the compound moves into the cell without an intermediate step to cross the membrane. At the beginning we will model the transport of a drug from outside the cell into the cytoplasm, so we do not consider any of the organelles nor DNA binding yet. In a later stadium we will extend both models so that they also deal with these facts. We compare the models by analyzing them both and then look at the differences in time and the ratios in concentration when equilibrium is reached. All simulations are performed in Matlab_2022b.

For the model where the movement of drugs into a cell that considers two steps to cross the membrane, the method described by Eytan [4] is used. The difference between the procedure of Eytan and the model described here is that in our first model we do not consider drug binding yet. This will be implemented in a later model where we also include other factors, for example adding some organelles of the cell. So, first we observe the movement of the drug to the outer leaflet of the plasma membrane, this movement is expressed by the adsorption rate of the compound. Then the compound will flop from the outer leaflet across the lipid core formed by the hydrophobic tails of the leaflets to the inner leaflet, this phenomenon is called a flip-flop event. Then the drug is finally adsorbed by the cytoplasm. When the compound locates at the surface of the inner leaflet again can flop back to the outer leaflet and then moves away from the cell. The movement of the latter appearance is expressed by the desorption rate of the compound. The total process is visualized in figure 2.

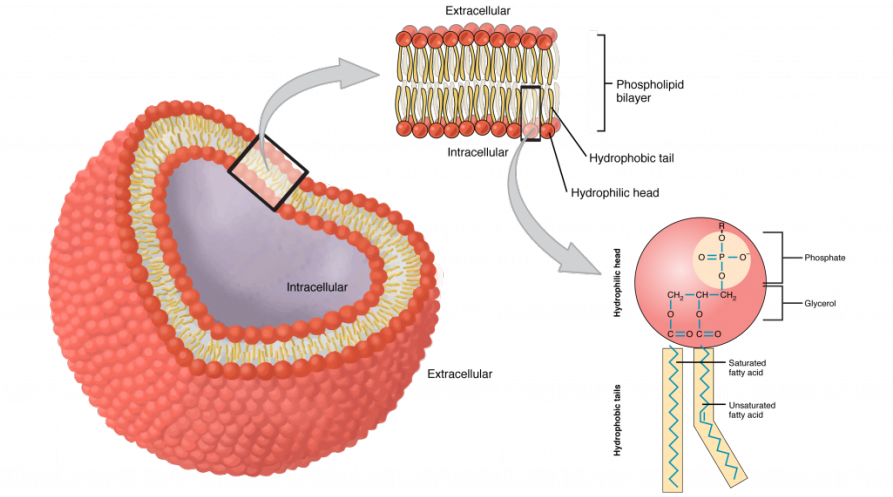


Figure 1: **Sketch of the membrane of a tumor cell.** The red balls on the outside and the yellow strings on the inside of the layers form the hydrophilic heads and the hydrophobic tails of the phospholipid bilayer respectively. The layers on the extracellular and intracellular sides of the cell are called the outer- and inner leaflet respectively.

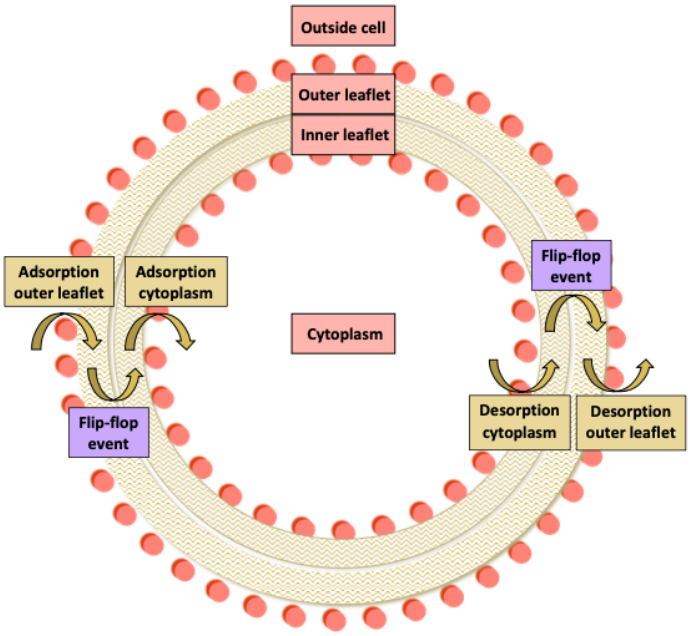


Figure 2: **This figure shows a sketch of the movements of a compound into a tumor cell.** The cell membrane consists of two leaflets around the cell and within these layers we have the cytoplasm. The red boxes represent the compartments of the model, the yellow boxes are the adsorption and desorption rates of the several compartments and the flip-flop events are represented by the purple boxes.

2.1 Doxorubicin

Focusing on the characters we want the compound to have, we should first decide which drug to study. From the situation explained above we know that we should search for a drug that has the nature to cross a membrane with a phospholipid bilayer without the need of a specific transporter. Therefore, we analyze the drug doxorubicin (DOX). This compound is a front runner of anticancer drugs and is broadly used, because it is one of the most effective drugs developed against tumors [13]. However, according to El-Kareh and Secomb [8] the effectiveness of DOX is offset due to its cardiotoxicity. This limits the lifetime dose a patient may receive.

The reason that doxorubicin is qualified as a drug that can be used to treat cancer is because it blocks a specific enzyme called topoisomerase 2. This enzyme is in the nucleus of a cancer cell and is needed for the cell to divide and grow. In order to be effective, the drug should block these enzymes so that the cancer cell can no longer grow and eventually will die [3].

In figure 3 we can see the chemical structure of DOX. Here the hydrophilic part of the molecule is bold. The rest of the molecule is hydrophobic. Since doxorubicin is positively charged and the surface of a plasma membrane is negatively charged, it has high affinity with membranes. Note that the hydrophobicity and the charge are not uniformly distributed, therefore the compound localizes at the surface of the membrane and not in its lipid core [4]. Due to this fact and the very low lipophilicity of DOX, the compound cannot diffuse through the membrane. As mentioned before, this must happen by a so-called flip-flop mechanism. Since doxorubicin is partly hydrophobic, it can cross the lipid core of the membrane by this mechanism [6]. The flip-flop mechanism will be given a formal definition in section 2.1. At room temperature the average time period that DOX locates at the one of both leaflets of the membrane before flopping to the other is 0.7 minutes, according to Eytan [4].

For pharmacy it is important to find a variant of doxorubicin so that it crosses the membrane as soon as possible and still can block the enzyme that causes the cell to divide and grow. Other important features to consider are having as few side effects as possible and low cardiotoxicity, for the well-being of the patients. Although, we will not bear the last-mentioned features in mind for this project. In this project we will develop a model and simulate it for doxorubicin. This model can later be used to test the effects of variants of doxorubicin without the need for experimental testing. The variant that moves the fastest through the membrane and bind to the DNA can be selected and further investigated.

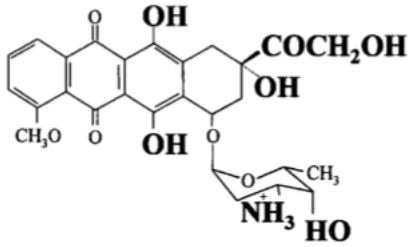


Figure 3: **A 2D sketch of the chemical structure of doxorubicin.** The groups in bold show the hydrophilic parts and the parts that are not in bold are the hydrophobic parts of a molecule of doxorubicin.

2.2 Experiments LUMC

The LUMC has done some experiments with the compound azidodoxorubicin (ADOX), to analyze the distribution and movements inside the cell for a time period of four minutes, see figure 4. In the upper series we see the distribution of the compound (white) in the lysosomes (light blue) compared to the nucleus (dark blue). Note that initially the concentration of the compound is higher in the lysosomes than in the nucleus. That is because the lysosomes takes up ADOX faster in comparison to the nucleus [14]. However, during the observed time we see that the nucleus also obtained a certain concentration of the compound. In contrast to the first series the second one shows the saturability between the mitochondria (light blue) and nucleus (dark blue). In this series we see that the compound initially will be in the mitochondria and later moves on to the nucleus as well.

The reason that researchers of the LUMC experimented with ADOX instead of DOX, is because it lights up better in the cells and therefore the movements are more visible. This makes it easier to analyze the transport of the compound. Even though an analogue of doxorubicin is used, there are only some small differences, for example the drug resistance index values of ADOX. Those of doxorubicin are significantly higher than that of azidodoxorubicin. Another difference is that ADOX exhibited higher activity and less toxicity than DOX, according to Geng et al. [7]. However, these differences have no significant impact on the speed of transport of the drug molecules through a tumor cell.

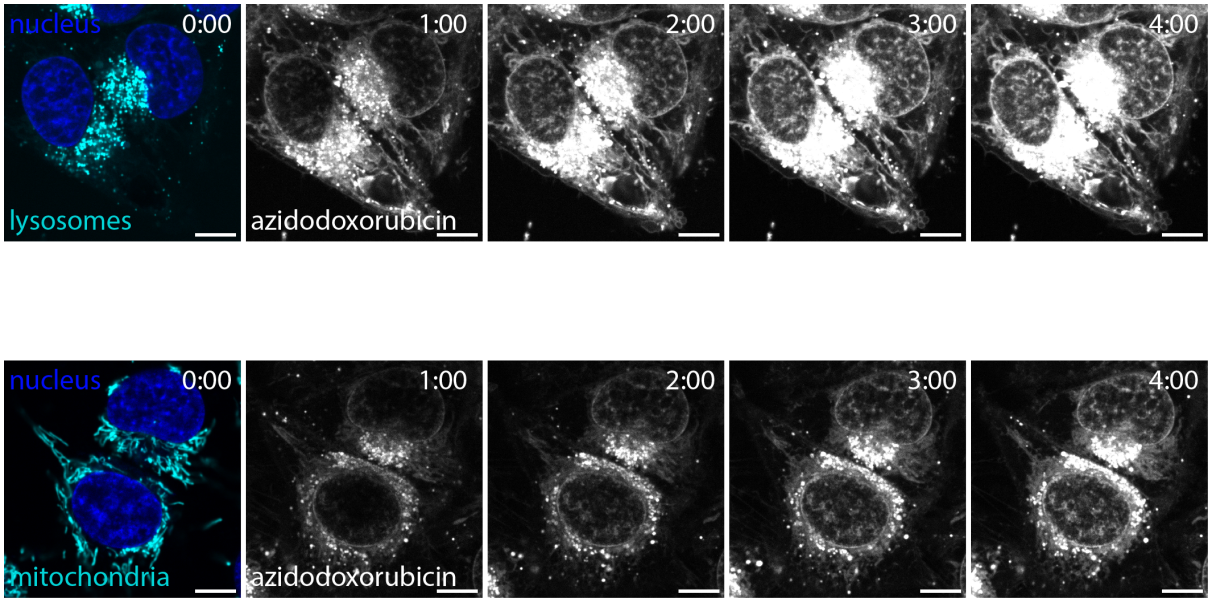


Figure 4: **Both series show that ADOX initially resides mainly in either the lysosomes or the mitochondria and only later transports into the nucleus.** In the first picture of both series the nucleus is highlighted in dark blue, whereas in the upper and lower series the lysosomes and mitochondria are both represented in light blue respectively. The following pictures in both series show the movements of the compound ADOX in white.

3 Basic Model

In this section we develop the model of the process which is described in section 2 where we consider two steps to cross the membrane and merely observe the transport of a drug into the cell. From now on we will call this model the "Basic Model". We model the flow of drugs on a cellular level by treating the several parts in and around the cell as compartments. The compound will be tracked as it enters and leaves each compartment at the rate proportional to the concentration of drugs in the various compartments. The model we will develop is called a compartment model. Such a model is formally defined in the following definition.

Definition 3.1 (Compartment model)**.** A *compartment model* is a mathematical representation of a local process in several compartments [12].

For the Basic Model the distribution of a compound in the areas outside the tumor cell, the outer- and inner leaflet of the plasma membrane and the cytoplasm are represented in terms of its concentration denoted by c_o, c_{om}, c_{im} and c_c respectively. These four areas can be regarded as the compartments of our compartment model. The total concentration drugs is expressed by c_{tot} and is defined as the total sum of the four mentioned compartments, i.e.

$$c_{tot} := c_o + c_{om} + c_{im} + c_c$$

We assume that before the process begins, the total drug concentration is completely outside the cell and the other three compartments are therefore empty of drug molecules. In addition, we assume that there will not be any compound added to the medium before we are at steady state, nor will the compound be removed from any of the areas we analyze. Conclusively, we assume that the total concentration remains constant throughout the process.

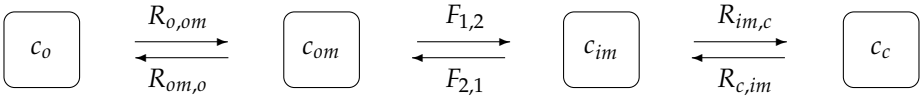
The rates in our model that are proportional to the concentration of drugs are the adsorption and desorption rates and the flip-flop events. These constants correspond with the local processes of a compartment model and are defined in the following definitions which are more or less self-explanatory.

Definition 3.2 (Adsorption rate)**.** The *adsorption rate* is the physical process where the compound in compartment l sticks to the surface of compartment k. The adsorption rates are denoted by $R_{l,k}$.

Definition 3.3 (Desorption rate)**.** The *desorption rate* is the reverse process of the adsorption rate, i.e. the release of an adsorbed compound from surface of compartment k to compartment l. The desorption rates are denoted by $R_{k,l}$.

Definition 3.4 (Flip-flop events)**.** The *flip-flop events* represent the speed of movement of a compound from layer i to layer j [6]. The flip-flop events are denoted by $F_{i,j}$.

In the following sketch we can see the transport of the compound through the various compartments:



The dimensions of the compartments are $[c_k] = ML^{-1}$ for $k = o, om, im, c$. Here M is the weight in μg and L is the volume in ml. The reason that we consider such small weight is because a drug molecule in general is expressed with this metric prefix. In addition, we use ml for the volume since the cell has a volume with the factor 10^{-5}m^3 and organelles have a volume with the factor 10^{-6}m^3 . Since $\text{m}^3 = 1000\,\text{l}$, the volume of the cell and an organelle can be best expressed in ml. The local processes all have the dimension $[R_{l,k}] = [F_{i,j}] = T^{-1}$ where T is the time in min. We consider minutes since this correspond with the time the drug takes to move through the membrane into the cell.

Under some assumptions about the transport of drugs in general we can derive the system of ODEs for the Basic Model. Remark that concentrations and rates cannot have negative values. Thereby, we assume that rates must have some value greater than zero too. It will not be interesting to look at parameters which are equal to zero. Because if one of the rates would be zero then the compound has no speed and therefore does not move. Though it is possible that the variables have zero concentration if their sum equals the total concentration. So, we assume that all variables are positive and that all parameters are strictly positive. Since we do not take the acidity into account in this model already, we suppose that the acidity is the same in all compartments. Thereby, we do not take active transport into account. In research experiments it is believed that only uncharged molecules can pass the cell membrane. Since all the values of all parameters are based on these experiments it is reasonable to assume that in this model only uncharged molecules can cross the membrane.

In the simplest and most suitable way to express the process in a system of ODEs we achieve the following model:

$$\begin{cases} \frac{dc_o}{dt} = -R_{o,om}c_o + R_{om,o}c_{om} \\ \frac{dc_{om}}{dt} = R_{o,om}c_o - (R_{om,o} + F_{1,2})c_{om} + F_{2,1}c_{im} \\ \frac{dc_{im}}{dt} = F_{1,2}c_{om} - (F_{2,1} + R_{im,c})c_{im} + R_{c,im}c_c \\ \frac{dc_c}{dt} = R_{im,c}c_{im} - R_{c,im}c_c \end{cases} \tag{3.1}$$

We go through each equation one by one and explain explicitly what each term describes.

1. The first equation describes the change of the concentration drugs outside the cell in the course of time.
   - $-R_{o,om}c_o$ is the decrease of the compound's concentration outside the cell by the adsorption of drug molecules by the outer leaflet.
   - $+R_{om,o}c_{om}$ is the increase of the compound's concentration outside the cell by the desorption of drug molecules from the outer leaflet.
2. The second equation describes the change of the concentration drugs in the outer leaflet in the course of time.
   - $+R_{o,om}c_o$ is the increase of the compound's concentration outside the cell by the adsorption of drug molecules by the outer leaflet.
   - $-(R_{om,o} + F_{1,2})c_{om}$ is the decrease of the compound's concentration in the outer leaflet by the desorption of the drug molecules from the outer leaflet and by the flip-flop event from the outer leaflet to the inner leaflet respectively.
   - $+F_{2,1}c_{im}$ is the increase of the compound's concentration in the outer leaflet by the flip-flop event from the inner leaflet to the outer leaflet.
3. The third equation describes the change of the concentration drugs in the inner leaflet in the course of time.
   - $+F_{1,2}c_{om}$ is the increase of the compound's concentration in the inner leaflet by the flip-flop event from the outer leaflet to the inner leaflet.
   - $-(F_{2,1} + R_{im,c})c_{im}$ is the decrease of the compound's concentration in the inner leaflet by the flip-flop event from the inner leaflet to the outer leaflet and by the desorption of drug molecules from the inner leaflet.
   - $+R_{c,im}c_c$ is the increase of the compound's concentration in the inner leaflet by the adsorption of drug molecules by the inner leaflet.
4. The fourth equation describes the change of the concentration drugs in the cytoplasm in the course of time.
   - $+R_{im,c}c_{im}$ is the increase of the compound's concentration in the cytoplasm by the desorption of drug molecules from the inner leaflet.
   - $-R_{c,im}c_c$ is the decrease of the compound's concentration in the cytoplasm by the adsorption of drug molecules by the inner leaflet.

Recall that the total concentration stays constant and is defined as $c_{tot} := c_c + c_{om} + c_{im} + c_c$. Hence, we can easily derive the concentration of the fourth compartment when the total concentration and the concentration drugs in the other three compartments are known. So, we can eliminate the last equation.
Therefore, the system of equations of the Basic Model is:

$$\begin{cases} \frac{dc_o}{dt} = -R_{o,om}c_o + R_{om,o}c_{om} \\ \frac{dc_{om}}{dt} = R_{o,om}c_o - (R_{om,o} + F_{1,2})c_{om} + F_{2,1}c_{im} \\ \frac{dc_{im}}{dt} = F_{1,2}c_{om} - (F_{2,1} + R_{im,c})c_{im} + R_{c,im}(c_{tot} - c_o - c_{om} - c_{im}) \\ c_{tot} = c_o + c_{om} + c_{im} + c_c \end{cases} \tag{3.2}$$

With initial condition $c_o(0) = c_{o(0)}, c_{om}(0) = c_{im}(0) = c_c(0) = 0$, because of the assumption that the initial distribution is that the compounds' total concentration will be outside the cell and all other compartments will not contain any of the drug molecules.

3.1 Dimensionless Model

The Basic Model now consists of combinations of variables with units μg ml^{-1} and parameters with units min^{-1}. The outcomes of each of the equations then have a unit of μg ml^{-1} min^{-1}. To make it easier to interpret the results without the influence of the dimension, we want to modify the model such that it is completely dimensionless. Therefore, we scale the variables and time to achieve such a system.

From now on we will move to normalized variables, i.e., we divide c_o, c_{om}, c_{im} and c_c by c_{tot}. Doing so, the variables lose their dimension, and we obtain the following scaled variables:

$$x_1 := \frac{c_o}{c_{tot}} \qquad x_2 := \frac{c_{om}}{c_{tot}} \qquad x_3 := \frac{c_{im}}{c_{tot}} \qquad x_4 := \frac{c_c}{c_{tot}}$$

Since the total sum is constant in the course of time, see section 3 and all original variables are divided by c_{tot} the new total sum becomes $\sum_{i=1}^{4} x_i = 1$.

Now we scale the time such that $t = t_0\hat{t}$, where t_0 is a dimension carrying parameter and $\hat{t}$ is dimensionless. For this model we define $t_0 := \frac{1}{R_{o,om}}$. Because

$$\frac{dx_n}{d\hat{t}} = \frac{dx_n}{dt} \cdot \frac{dt}{d\hat{t}} = t_0 \cdot \frac{dx_n}{dt},$$

for $n = 1, 2, 3$. It is clear that $\frac{dx_n}{d\hat{t}}$ has no dimension for all n .

So, if we scale the original parameters with the new time, then we receive new parameters for the dimensionless system. These are defined as follows:

$$P_o := \frac{R_{om,o}}{R_{o,om}}, \qquad I_i := \frac{F_{i,j}}{R_{o,om}}, \qquad J_{k,l} := \frac{R_{k,l}}{R_{o,om}},$$

for $i, j = 1, 2$ and $k, l = im, c$.

One of these parameters has a special meaning. This parameter is called a partition coefficient.

Definition 3.5 (Partition coefficient)**.** The *partition coefficient* is the ratio of the compounds' concentration in one compartment compared to the other when they are at steady state. This can also be expressed by the ratio of the adsorption rate constant and the desorption rate constant of one of the compartments [4], i.e.

$$P_k := \frac{\text{adsorption rate constant } R_{l,k}}{\text{desorption rate constant } R_{k,l}}.$$

The partition coefficient can be used to demonstrate the extent of drug affinity toward membranes, see [4]. A higher partition coefficient means that the compound has greater lipophilicity, hence more drug affinity towards membranes.We will use this coefficient for the simulation of cellular drug transport across the cell membrane.

Rewriting the Basic Model with the scaled variables and parameters gives the following dimensionless system:

$$\begin{cases} \frac{dx_1}{d\hat{t}} = -x_1 + P_o x_2 \\ \frac{dx_2}{d\hat{t}} = x_1 - (P_o + I_1)x_2 + I_2 x_3 \\ \frac{dx_3}{d\hat{t}} = I_1 x_2 - (I_2 + J_{im,c})x_3 + J_{c,im}(1 - x_1 - x_2 - x_3) \\ x_1 + x_2 + x_3 + x_4 = 1 \end{cases} \tag{3.3}$$

With initial condition $x_1(0) = 1, x_2(0) = x_3(0) = x_4(0) = 0$, since we assumed in section 3 that the total concentration drugs is outside the cell before the process has started. Note that due to the normalization, the total sum now equals one.

We will perform further analysis on this model.

3.1.1 Analysis Basic Model

In this section we analyze the dimensionless model we have just developed. To study the behavior of the transport of a compound into a cell, it is important to always know the distribution of the concentration. We want to know if at a certain time the partition of the concentration drugs over the several compartments remains the same and stays the same for the remaining time. The steady state or equilibrium of the system we derive says something about this distribution of the concentration drugs in the different compartments when there is no change in concentration at a certain time. So, our goal now is to find the steady state of the dimensionless system of the Basic Model. Then we can find under which conditions the partition of the concentration drugs does not change any more.

We start with finding the nullclines of the system (3.3). These are the lines or planes where there is no change in any of the quantities. We assemble each of the equations to zero as follows:

$$\frac{dx_1}{d\hat{t}} = 0 \qquad \frac{dx_2}{d\hat{t}} = 0 \qquad \frac{dx_3}{d\hat{t}} = 0 \qquad \frac{dx_4}{d\hat{t}} = 0$$

Then, we find the equilibrium or equilibria by finding the combinations of x_1, x_2, x_3 and x_4 such that all equations of the system equal zero simultaneously. Recall that the sum of these differential equations is zero. Therefore, we only need to find the nullclines for the first three differential equations. If these are all zero, the last one must be zero as well. So, we must solve the following set of equations for x_1, x_2 and x_3:

$$-x_1 + P_o x_2 = 0$$

$$x_1 - (P_o + I_1)x_2 + I_2 x_3 = 0$$

$$I_1 x_2 - (I_2 + J_{im,c})x_3 + J_{c,im}(1 - x_1 - x_2 - x_3) = 0$$

The point(s) where all differential equations are simultaneously zero, are called the steady states or equilibria. Note that since the system is nonhomogeneous, the trivial equilibrium is not included in the set of steady states. For this system we can find one steady state which we define by x_*:

$$x_* = \left(\frac{P_o I_2 J_{c,im}}{I_1(J_{im,c}+J_{c,im})+I_2 J_{c,im}(P_o+1)}, \frac{I_2 J_{c,im}}{I_1(J_{im,c}+J_{c,im})+I_2 J_{c,im}(P_o+1)}, \frac{I_1 J_{c,im}}{I_1(J_{im,c}+J_{c,im})+I_2 J_{c,im}(P_o+1)}, \frac{I_1 J_{im,c}}{I_1(J_{im,c}+J_{c,im})+I_2 J_{c,im}(P_o+1)} \right)^\top$$

We leave the simple calculations to derive this equilibrium to the reader.

Note that when we divide each of the rows of the steady state by $J_{c,im}$ we find:

$$\tilde{x}_* = \left(\frac{P_o I_2}{I_1\left(\frac{J_{im,c}}{J_{c,im}}+1\right)+I_2(P_o+1)}, \frac{I_2}{I_1\left(\frac{J_{im,c}}{J_{c,im}}+1\right)+I_2(P_o+1)}, \frac{I_1}{I_1\left(\frac{J_{im,c}}{J_{c,im}}+1\right)+I_2(P_o+1)}, \frac{I_1 \frac{J_{im,c}}{J_{c,im}}}{I_1\left(\frac{J_{im,c}}{J_{c,im}}+1\right)+I_2(P_o+1)} \right)^\top$$

Remark that

$$\frac{J_{im,c}}{J_{c,im}} = \frac{\frac{R_{im,c}}{R_{o,om}}}{\frac{R_{c,im}}{R_{o,om}}} = \frac{R_{im,c}}{R_{c,im}}.$$

This is how we defined the partition coefficient in definition 3.5, in section 3 thus $\frac{J_{im,c}}{J_{c,im}} = P_c$.

Hence the equilibrium can also be written as:

$$\tilde{\tilde{x}}_* = \left(\frac{P_o I_2}{I_1(P_c+1)+I_2(P_o+1)}, \frac{I_2}{I_1(P_c+1)+I_2(P_o+1)}, \frac{I_1}{I_1(P_c+1)+I_2(P_o+1)}, \frac{I_1 P_c}{I_1(P_c+1)+I_2(P_o+1)} \right)^\top \tag{3.4}$$

This vector represents the ratios of the concentration of the compound between the different compartments at steady state. Note that since all parameters are strictly positive and the sum of the four rows equals one. Hence this equilibrium is well defined and never becomes zero, so indeed the trivial equilibrium is not included in our set of steady states.

3.1.2 Stability Analysis Basic Model

Now we will locally linearize to the variables x_1, x_2 and x_3, and determine the (local) stability of the equilibrium we found in the previous section. The behavior near the equilibrium is approximately equal to the behavior of the linear system near that equilibrium. This is very important to examine, since we want to know if the distribution of a drug remains the same as t goes to infinity from a certain time point or if this ratio of the concentration can change again. This has great impact on the efficiency of the compound forcing a tumor cell to apoptosis (cell death).

In this paragraph we mainly follow the method described by Braun [2] chapter 4.2 to determine the stability of the equilibrium given in equation (3.4).

We rewrite our system in the form $\dot{x} = Df(x_*) + g$ where we have the three dimensional vectors $x = (x_1, x_2, x_3)^\top$ and $g = (0, 0, J_{c,im})$ and the (3×3)-matrix $Df(x_*)$ is the Jacobian of the dimensionless system of ODEs:

$$Df(x_*) = \begin{pmatrix} -1 & P_o & 0 \\ 1 & -P_o - I_1 & I_2 \\ -J_{c,im} & I_1 - J_{c,im} & -I_2 - J_{im,c} - J_{c,im} \end{pmatrix} \tag{3.5}$$

where x_* is the equilibrium of the system (3.4).

Now we will calculate the determinant of $\lambda I - Df(x_*)$ to find the characteristic polynomial of the system (3.3):

$$\det(\lambda I - Df(x_*)) = \begin{vmatrix} \lambda + 1 & -P_o & 0 \\ -1 & \lambda + P_o + I_1 & -I_2 \\ J_{c,im} & -I_1 + J_{c,im} & \lambda + I_2 + J_{im,c} + J_{c,im} \end{vmatrix}$$

$$= (\lambda + 1)((\lambda + P_o + I_1)(\lambda + I_2 + J_{im,c} + J_{c,im}) + I_2(-I_1 + J_{c,im})) + P_o(-(\lambda + I_2 + J_{im,c} + J_{c,im}) + I_2 J_{c,im})$$

$$\begin{aligned} = \lambda^3 + [P_o + I_1 + I_2 + J_{im,c} + J_{c,im} + 1]\lambda^2 + [P_o(I_2 + J_{im,c} + J_{c,im}) + I_1(J_{im,c} + J_{c,im} + 1) \\ + I_2(J_{c,im} + 1) + J_{im,c} + J_{c,im})]\lambda + [I_1(J_{im,c} + J_{c,im}) + I_2 J_{c,im}(1 + P_o)] \end{aligned} \tag{3.6}$$

Since this is a rather large polynomial, we will not find the exact eigenvalues.
Instead we will only search for the features of these eigenvalues.

Note that the characteristic polynomial is a third-degree polynomial, so we have three eigenvalues. If we write out the linear terms for arbitrary λ_i, where $i = 1, 2, 3$ and such that λ is the same as above we receive the following equality's:

$$(\lambda - \lambda_1)(\lambda - \lambda_2)(\lambda - \lambda_3) = \lambda^3 - [\lambda_1 + \lambda_2 + \lambda_3]\lambda^2 + [\lambda_1(\lambda_2 + \lambda_3) + \lambda_2\lambda_3]\lambda - \lambda_1\lambda_2\lambda_3, \tag{3.7}$$

where λ_i for $i = 1, 2, 3$ are the eigenvalues of the Jacobian $Df(x_*)$.
In the following step we compare the coefficients of the characteristic polynomial with the coefficients we found in equation (3.7) so that we can say something about the sign of the eigenvalues:

$$-\lambda_1\lambda_2\lambda_3 = I_1(J_{im,c} + J_{c,im}) + I_2 J_{c,im}(1 + P_o) \tag{3.8}$$

$$\lambda_1(\lambda_2 + \lambda_3) + \lambda_2\lambda_3 = P_o(I_2 + J_{im,c} + J_{c,im}) + I_1(J_{im,c} + J_{c,im} + 1) + I_2(J_{c,im} + 1) + J_{im,c} + J_{c,im} \tag{3.9}$$

$$-(\lambda_1 + \lambda_2 + \lambda_3) = P_o + I_1 + I_2 + J_{im,c} + J_{c,im} + 1 \tag{3.10}$$

Recall that all parameters are strictly positive, hence we can prove that the following propositions are valid:

Proposition 3.1.1. *If the parameters of the dimensionless system of the Basic Model all have positive values, then the equilibrium is asymptotically stable.*

If all eigenvalues of $Df(x_*)$ have negative real part, then the equilibrium (3.7) is stable. If at least one eigenvalue of $Df(x_*)$ has positive real part, then the steady state is unstable. Braun also claims that the solution is asymptotically stable if all eigenvalues of $Df(x_*)$ have negative real part. Note that since the parameters are strictly positive, none of the eigenvalues could be zero. So, we do not need to consider the cases where the eigenvalues are zero.

Proof. Note that since the parameters of the dimensionless system are all positive, we know from equations (3.8), (3.9) and (3.10) that the eigenvalues must apply to all three of the following conditions:

$$\lambda_1\lambda_2\lambda_3 < 0 \tag{3.11}$$

$$\lambda_1(\lambda_2+\lambda_3)+\lambda_2\lambda_3 > 0 \tag{3.12}$$

$$\lambda_1+\lambda_2+\lambda_3 < 0 \tag{3.13}$$

From (3.11) it follows that we can assume that the eigenvalues satisfy either one of the following combinations:

(a.1) All eigenvalues λ_i are real and negative;

(a.2) All eigenvalues λ_i are real and one of the eigenvalues is negative and the other two are positive eigenvalues;

(a.3) One of the eigenvalues is real and the other two are complex with negative real part;

(a.4) One of the eigenvalues is real and the other two are complex with positive real part.

If all the eigenvalues are as in **(a.1)** or **(a.3)**, then the proposition is trivially valid.
So, we must check if it is possible to have eigenvalues as in **(a.2)** or **(a.4)**.

Suppose that λ_1 is real and negative and λ_2, λ_3 are real and positive.
We find from expression (3.11) that $\lambda_1 > -\frac{\lambda_2\lambda_3}{\lambda_2+\lambda_3}$.

We use this expression to determine the sign of $\lambda_1+\lambda_2+\lambda_3$:

$$\lambda_1+\lambda_2+\lambda_3 > -\frac{\lambda_2\lambda_3}{\lambda_2+\lambda_3}+\lambda_2+\lambda_3 = \frac{-\lambda_2\lambda_3+(\lambda_2+\lambda_3)^2}{\lambda_2+\lambda_3} = \frac{\lambda_2^2+\lambda_3^2+\lambda_2\lambda_3}{\lambda_2+\lambda_3} > 0$$

Since $\lambda_2, \lambda_3 > 0$, both the nominator and the denominator are evidently positive. Thus, the sum of the three eigenvalues is positive when two of the eigenvalues are real and positive. This gives a contradiction with condition (3.11). Without loss of generality the same is true when either λ_2 or λ_3 is negative and real, and the corresponding two eigenvalues are real and positive. Thus a combination of eigenvalues as in **(a.2)** cannot occur.

Now we assume that λ_1 is real and negative and λ_2, λ_3 are complex with positive real part.
As above we find that the sum is positive:

$$\frac{\lambda_2^2+\lambda_3^2+\lambda_2\lambda_3}{\lambda_2+\lambda_3} = \frac{\lambda_2^2+\lambda_3^2+(Re(\lambda_2)+iIm(\lambda_2))(Re(\lambda_3)+iIm(\lambda_3))}{(Re(\lambda_2)+iIm(\lambda_2))+(Re(\lambda_3)+iIm(\lambda_3))}$$

Note that $Re(\lambda_3) = Re(\lambda_2)$ and $Im(\lambda_3) = -Im(\lambda_2)$, therefore

$$= \frac{\lambda_2^2+\lambda_3^2+(Re(\lambda_2)+iIm(\lambda_2))(Re(\lambda_2)-iIm(\lambda_2))}{(Re(\lambda_2)+iIm(\lambda_2))+(Re(\lambda_2)-iIm(\lambda_2))} = \frac{\lambda_2^2+\lambda_3^2+Re(\lambda_2)^2+Im(\lambda_2)^2}{2Re(\lambda_2)} > 0$$

This also gives a contradiction with condition (3.11). Without loss of generality, the same is true when either λ_2 or λ_3 is negative and real, and the corresponding two eigenvalues complex and have positive real part. Thus, a combination of eigenvalues as in **(a.4)** also cannot occur.

Hence, we conclude that all eigenvalues of $Df(x_*)$ have strictly negative real part and thus by Braun [2] the equilibrium is (locally) stable. Conclusively, once the compound's concentration is partitioned such as in the equilibrium, then it does not change anymore even though time goes to infinity. □

Since we know that the system either satisfies to one of the combinations **(a.1)** or **(a.3)** it turns out that if all parameters of the system of the Basic Model are strictly positive, then the system cannot have one real eigenvalue and two complex eigenvalues with negative real part. Hence, the equilibrium will never be oscillating stable.

Proposition 3.1.2. *If the parameters of the dimensionless system of the Basic Model all have positive values, then the three corresponding eigenvalues are real.*

Proof. The sign of the discriminant of the characteristic polynomial, derived from the Jacobian, plays an important role when we want to decide whether the eigenvalues are all real or the eigenvalues can be complex as well. With Cardano's Formula 3.7 we will prove that it is not possible to have eigenvalues which are of the form as in **(a.3)**.

If we write a general cubic as $g(\lambda) = a\lambda^3 + b\lambda^2 + c\lambda + d$ for some parameters a, b, c and d, then the discriminant of a general cubic can be found with the formula

$$\Delta_g = 18abcd - 4b^3d + b^2c^2 - 4ac^3 - 27a^2d^2 \tag{3.14}$$

Recall from (3.6) on page 11 the characteristic polynomial corresponding to the dimensionless system.
Hence,

- $a = 1$
- $b = [P_o + I_1 + I_2 + J_{im,c} + J_{c,im} + 1]$
- $c = [P_o(I_2 + J_{im,c} + J_{c,im}) + I_1(J_{im,c} + J_{c,im} + 1) + I_2(J_{c,im} + 1) + J_{im,c} + J_{c,im}]$
- $d = [I_1(J_{im,c} + J_{c,im}) + I_2 J_{c,im}(1 + P_o)]$

When we substitute a, b, c and d as above, then the discriminant becomes extremely large. Therefore, we want to rewrite the polynomial and use Cardano's formula to determine if we can have complex eigenvalues when all parameters are strictly positive.

Thus, we transform the characteristic polynomial to a so called depressed cubic which is of the form:

$$d(\lambda) = t^3 + pt + q.$$

We can find this cubic by the following transformations, where a, b, c, d are the coefficients of the general cubic (3.14):

$$\eta = \lambda - \frac{b}{3a}, \qquad p = \frac{3ac - b^2}{3a^2}, \qquad q = \frac{2b^3 - 9abc + 27a^2d}{27a^3}.$$

The discriminant of the depressed cubic d is

$$\Delta_d = -(4p^3 + 27q^2)$$

Now we can apply Cardano's formula, see 3.7 to the discriminant of the depressed cubic to determine if it is possible to have strictly positive parameters such that the equilibrium of the Basic Model has two complex eigenvalues.

Definition 3.6 (Discriminant of Cardano)**.** To test whether the roots of a depressed cubic have real and imaginary roots, Cardano uses the *discriminant of Cardano*:

$$\Delta_{dCardano} = \frac{q^2}{4} + \frac{p^3}{27}$$

Theorem 3.7 (Cardano's Formula [16])**.** *There are three possibilities:*

- *If* $\Delta_{dCardano} > 0$ *is positive, the cubic has one real root and two complex conjugate roots.*
- *If* $\Delta_{dCardano} < 0$ *is negative, the cubic has three real roots.*
- *If* $\Delta_{dCardano} = 0$*, the cubic has three real roots with a double or a triple root.*

Remark that since all parameters are strictly positive, the discriminant will never equal zero. Hence, we only must check whether the discriminant of Cardano is positive or negative.

Clearly, we have that $q^2 > 0$, so we only need to analyze the behavior of p^3.
Note that

$$\begin{cases} \Delta_{dCardano} > 0, \text{ if } p^3 > 0; \\ \Delta_{dCardano} > 0, \text{ if } p^3 < 0 \text{ and } 27q^2 > -4p^3; \\ \Delta_{dCardano} < 0, \text{ else .} \end{cases}$$

Therefore, we must check when p^3 is greater than zero or when $p^3 < 0$ and $27q^2 > -4p^3$ occurs. To check this we used the Satisfiability Modulo Theories (SMT) solver, see [19] to determine if one of these possibilities are satisfied for strictly positive parameters. With this solver we concluded that there is no combination of (positive) values for the parameters such that one of these two conditions hold. Conclusively, by 3.7 it is not possible to have one real eigenvalue and two complex eigenvalues.

Hence all eigenvalues are real and by our earlier findings strictly negative. □

Thus, in case that all values for the parameters are positive, the equilibrium of the Basic Model is (locally) stable but never oscillating stable.

3.2 Numerical Simulation Basic Model

The main goal of this simulation is to research the transport of a drug into a cell through a membrane such that the model considers two steps to cross it. We want to compare these results with a model that do not take these two extra steps into account and see which model gives a better prediction of the movement of a drug into a cell.

In this section, we will study the dimensionless system of the Basic Model using numerical simulations. For this we use known data from the compound doxorubicin. By some literature studies we found some of the values we need for the parameters we use. According to Alves et al., the partition coefficient (P) is equal to 8195 with an uncertainty of 406 [1]. Therefore, we choose to let the value of the partition coefficient be 8000. By Eytan [4] it follows that in both ways the flip-flop events for doxorubicin take 0.7 minutes. Since we do not know the exact values for the other parameters, we make some assumptions based on that of Eytan's [4].

Eytan assumes that the adsorption rate of the outer leaflet 3.2 $R_{o,om}$ can be chosen arbitrarily large and the desorption rate of the outer leaflet 3.3 $R_{om,o}$ is dependent on the adsorption rate of the outer leaflet and the partition coefficient, $R_{om,o} = \frac{R_{o,om}}{P}$. The rates $R_{o,om}$ and $R_{im,c}$ are equally large and the adsorption rate of the inner leaflet is $R_{c,im} = R_{im,c}P$. We also assume that the adsorption rates of both leaflets of the membrane are too large to be measured. Therefore, the time for the drug to bind to the leaflets is chosen relatively small in comparison to the flip-flop events, namely 0.01 minutes. Note that this value is just an assumption and not experimentally proven. With the values for the partition coefficient, the adsorption rate of the drug to the membrane and the time the drug needs to flop between the leaflets of the membrane we can derive all the values we need. Since we assume that it takes 0.01 minutes for the drug to be adsorbed by the membrane the adsorption rate $R_{o,om} = \frac{1}{0.01} = 100 \text{ min}^{-1}$. Consequently, the desorption rate of the outer leaflet is $R_{om,o} = \frac{100}{8000} = 0.0125 \text{ min}^{-1}$. Recall that the flip-flop events happen in 0.7 minutes for both ways we have $F_{1,2} = F_{2,1} = \frac{1}{0.7} = 1.4 \text{ min}^{-1}$. By the assumptions of Eytan $R_{im,c} = R_{o,om} = 100 \text{ min}^{-1}$ and $R_{c,im} = 100 \times 8000 = 8 \cdot 10^5 \text{ min}^{-1}$. Lastly we let the total concentration drugs be $20\mu\text{g ml}^{-1}$ as Eytan and Kuchel [5]. Knowing these values we can easily determine the values for the parameters we have in the dimensionless system of the Basic Model, by dividing all these values by the adsorption rate of the outer leaflet $R_{o,om}$. In table 1 we stated the scaled values we used for the simulation of the dimensionless system of the Basic Model.

Table 1: Parameter values of doxorubicin of the dimensionless system of the Basic Model.

Parameters	**Values**
P_o	$1.25 \cdot 10^{-4}$
I_1	0.014
I_2	0.014
$J_{im,c}$	1
$J_{c,im}$	8000
c_{tot} $(\mu\text{g ml}^{-1})$	20

From the plots we receive by simulating these values, we can analyze the distributions in the compartments for a given time. We analyze the plots until equilibrium is reached. The most important information we want to derive from the plots, are the speed at which the steady state is reached and the distribution of the drugs over the compartments at equilibrium. These results will be compared to the results of the model that does not consider two steps to cross the membrane. In figure 5, we show the plot we achieved using the values given in table 1. The Matlab code for this simulation can be found in Appendix A.

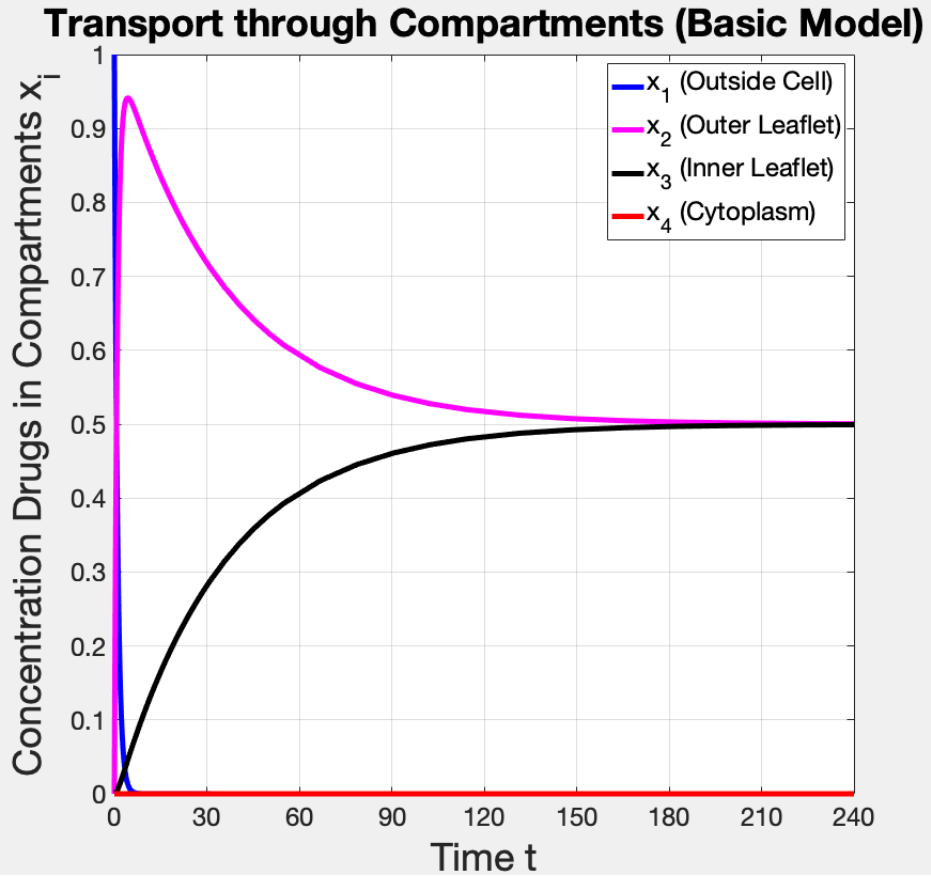


Figure 5: **In equilibrium, the drugs concentration is mainly within the membrane of the cell.** Each graph represents the change in compounds' concentration over time in the various compartments. For this plot we used the values from table 1 and simulated them in Matlab.

The graphs in figure 5 represent the concentrations of the drug in the several compartments over 240 time points, then equilibrium is reached. We observe that at the very beginning, the graph of x_1 extremely decreases in a very small time. Later this graph decreases less and less and finally converges to a relatively small value. In contrast to the first one, the graph corresponding to x_2 increases extremely fast at the very beginning and when a maximum is reached, the graph drops again and converges to a relatively large value compared to x_1. The third graph x_3 is increasing at an increasing rate when x_2 has reached is maximum value and converges to the same value as x_2. Lastly, the graph corresponding to x_4 seems to be constant. However, when we calculate the equilibrium of the system, we know that it converges to a value very close to zero, so it has slowly risen to a small value.

3.2.1 Interpretation Numerical Analysis Basic Model

The fact that x_1 decreases extremely fast at the beginning can be explained by the fact that then the total concentration is outside the cell and moves rapidly to the outer leaflet. From the outer leaflet the molecules will move to the inner leaflet at a relatively slow rate, due to the time needed for the drugs to flop from one leaflet to the other. Therefore, we can see a high peak in the graph corresponding to the outer leaflet and when enough time has passed, we see that the drug molecules move to the inner leaflet which causes the decrease of x_2 and the increase of x_3. As was predicted by Eytan we see that both leaflets of the membrane contain a relatively high drug concentration because doxorubicin has high affinity to membranes. This also causes the fact that the graph x_4 which corresponds to the cytoplasm, almost remains to be zero. Since the compound will immediately be adsorbed by the inner leaflet again once it arrives at the cytoplasm. Since the drug will mostly be obtained by the membrane, it seems less relevant to look at the partition coefficient between the compartments outside the cell and the cytoplasm. In section 7 we will go into further detail about this phenomenon when we compare it to the model where we do not consider the intermediate steps to cross the membrane.

3.3 Solution Basic Model

Recall from the nonhomogeneous equation (3.3) in section 3.1. By the method used for inhomogeneous equations described as in [2] chapter 3.12, we can find the solution of the initial-value problem

$$\dot{x} = Df(x_*)x + g(x) \tag{3.15}$$

where $Df(x_*)$ is the Jacobian of the system which was given in equation (3.5) and the vector $x = (x_1, x_2, x_3)^\top$ and the nonhomogeneous part of the system is $g(x) = (0, 0, J_{c,im})^\top$.

Let x_i for $i = 1, 2, 3, 4$ be linearly independent solutions of the homogeneous equation $Df(x_*)x$. The general solution of the nonhomogeneous equation is of the form

$$x = \sum_{i=1}^{3} \gamma_i e^{\lambda_i t} v_i + \beta$$

where β is a particular solution $\psi(t)$ we need to "guess".

First we solve the homogeneous part of (3.15), namely $Df(x_*)x$. Let the eigenvalues $\lambda_1, \lambda_2, \lambda_3$ be such that they are strictly negative and real. For these eigenvalues the corresponding eigenvectors can be found with the standard eigenvector methods. However, since the results for the eigenvectors for arbitrary eigenvalues are so extensive, we will not mention them explicitly.

Once we found the eigenvectors we can determine the particular solution by applying the method described in chapter 3.12 of the book written by Braun [2]. The next step is that we substitute β for x in equation (3.15) and solve this to find the vector β. Note that in our case, β is not dependent on time, hence

$$\frac{d\beta}{dt} = Df(x_*)\beta + \begin{pmatrix} 0 \\ 0 \\ J_{c,im} \end{pmatrix}.$$

Thus $\beta = -Df(x_*)^{-1} \begin{pmatrix} 0 \\ 0 \\ J_{c,im} \end{pmatrix}$, since $\frac{d\beta}{dt} = 0$.

Then after some calculations, which can be found in Appendix B, we find

$$\beta = \begin{pmatrix} \frac{P_o I_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{I_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{I_1}{I_1(P_c+1)+I_2(P_o+1)} \end{pmatrix},$$

which corresponds with the equilibrium of the dimensionless system (3.4).

The following step is to find the constants γ_i. We can do that using the initial condition x_0 and the particular solution β . Let V be the matrix of the eigenvectors and $\gamma = (\gamma_1, \gamma_2, \gamma_3)^\top$. Since $x_0 = x(0) = V\gamma + \beta$ we can find the constants γ_i by solving $\gamma = V^{-1}(x_0 - \beta)$, where V^{-1} is the inverse matrix of the eigenvectors to the corresponding eigenvalues.

Recall that the initial condition is $x_0 = (1, 0, 0)^\top$, hence:

$$(x_0 - \beta) = \begin{pmatrix} \frac{I_1(P_c+1)+I_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{-I_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{-I_1}{I_1(P_c+1)+I_2(P_o+1)} \end{pmatrix}$$

If the values are known, then it is easy to determine the solution of the Basic Model with the above formulas. We give an example of this using the values we used for the numerical simulation in section 3.2. The Matlab code can be found in Appendix A.

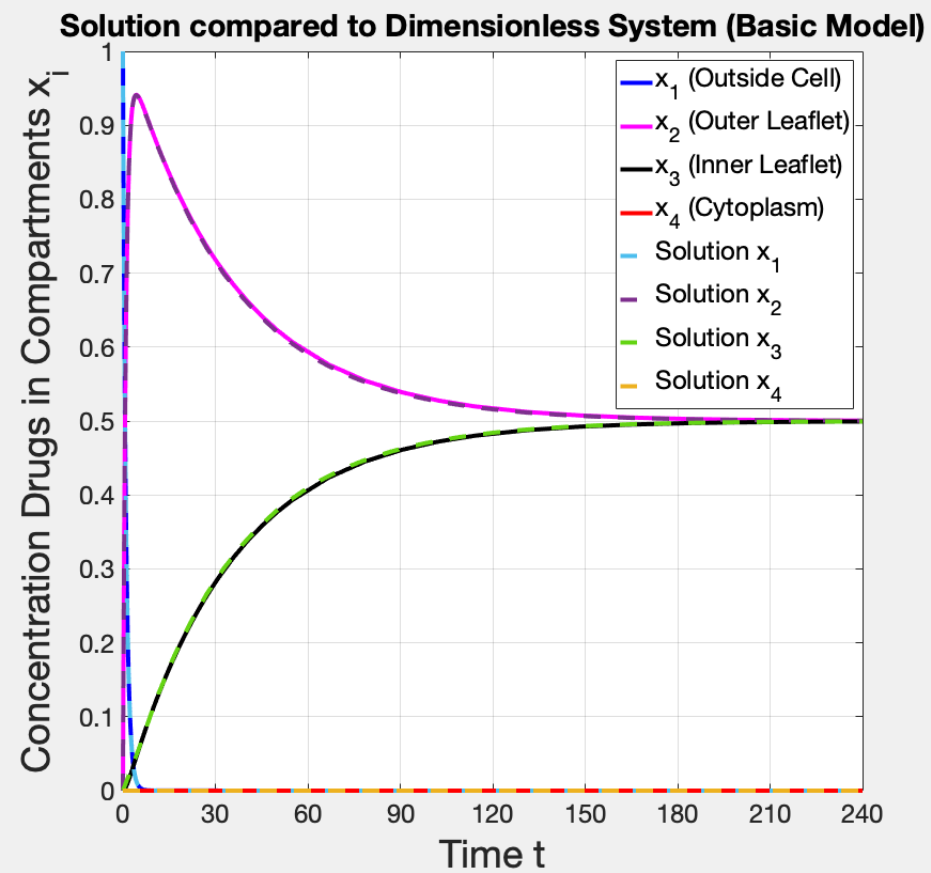


Figure 6: **The solutions give the same graphs as the equations of the dimensionless system of the Basic Model.** By Braun's method [2] we compute the solution of the Basic Model. From this solution we determine the separate solutions and compare them with the graphs corresponding to the graphs of the equations of the Basic Model.

The dashed graphs in figure 6 show the solutions to the four ODEs of the dimensionless system of the Basic Model for the values used in the numerical analysis, see table 1. The lines show the equations for the dimensionless system of the Basic Model. Hence, we can conclude that, when we apply these formulas, we indeed find the solutions corresponding to the equations of our system.

For the values we used the explicit formula of the solution of the Basic Model is:

$$x(t) = 6.2 \cdot 10^{-5} e^{-8001t} \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} + 0.015 e^{-t} \begin{pmatrix} 68.021 \\ -69.021 \\ 1 \end{pmatrix} - 0.515 e^{-0.029t} \begin{pmatrix} 0 \\ -1 \\ 1 \end{pmatrix} + \begin{pmatrix} 6.2 \cdot 10^{-5} \\ 0.499938 \\ 0.499938 \end{pmatrix} \tag{3.16}$$

Note that the eigenvectors are linearly independent, since there is no linear combination such that $a_1 v_1 + a_2 v_2 + a_3 v_3 = 0$ when $a_1, a_2, a_3 \neq 0$. According to Braun the solutions of the equations are linearly independent if and only if the eigenvectors are linearly independent. Thus, the solution is well defined.

Also recall from section 3.1.2 that all eigenvalues of $Df(x_*)$ have negative real part. Hence, every solution $x(t)$ of $\dot{x} = Df(x_*)x + \beta$ approaches β as t goes to infinity. In our case this is exactly the equilibrium of the system. Therefore, every solution of our system of ordinary differential equations is asymptotically stable.

Now, let us determine with the first and second derivatives tests if the results match with the graphs of the plot in figure 6. The derivation of these results are written out in Appendix B.

The first derivative of the solution in equation (3.16) is:

$$x'(t) = e^{-8001t} \begin{pmatrix} 0 \\ 0 \\ -0.496 \end{pmatrix} + e^{-t} \begin{pmatrix} -1.020 \\ 1.035 \\ 0.015 \end{pmatrix} + e^{-0.029t} \begin{pmatrix} 0 \\ -0.0148 \\ 0.0148 \end{pmatrix}$$

When we solve $x'(t) = 0$ for t then we find the minima and maxima of the first three graphs. To do that, we solve this for each row of the solution separately. Solving these solutions numerically in Matlab receive the following results:

- The solution corresponding to the outside of the cell is monotonically decreasing.
- The solution corresponding to the outer leaflet either has a minimum or maximum at $t = 4.3$.
- The solution corresponding to the inner leaflet is monotonically increasing.
- The solution corresponding to the cytoplasm increases monotonously slowly.

The second derivative of the solution is:

$$x''(t) = e^{-8001t}\begin{pmatrix} 0 \\ 0 \\ 3968 \end{pmatrix} + e^{-t}\begin{pmatrix} 1.020 \\ -1.035 \\ -0.015 \end{pmatrix} + e^{-0.029t}\begin{pmatrix} 0 \\ 4.3 \cdot 10^{-4} \\ -4.3 \cdot 10^{-4} \end{pmatrix}$$

From the second derivative we can conclude that the solution corresponding to the outer leaflet has a maximum at $t = 4.3$. The concentration at this point in the outer leaflet is 0.94. So, we can conclude that the maximum concentration drugs that the outer leaflet can obtain is 94% of the total concentration drugs.

If we compare the results of the first and second derivative tests with our plot in figure 6, we see that these results correspond to the courses of the graphs.

3.4 Sensitivity Analysis Basic Model

In this paragraph we will examine the influence of the partition coefficient on the time and the value when x_2 reaches its maximum. We expect that for a smaller value of the partition coefficient, this top will be lower because the compound will move earlier to the next compartments. The time could either increase or decrease because if the partition coefficient is smaller, then one would expect that it takes longer to reach the maximum value. On the other hand, the maximum value is expected to be smaller, which would mean that the time will decrease because the maximum will be reached sooner. We expect the opposite if we increase the value of the partition coefficient.

The values of the partition coefficient will be varied in two ways. First we will research the values within the range which is given by Alves et al. [1] with steps of 200. Recall that the partition coefficient is 8195 with an uncertainty of 406. Therefore, we want to examine values within a range between 7789 and 8601. We will enlarge this range by observing value between 7600 and 8800 to be sure that we take all values into account within the range that is determined by Alves et al. In the following table, the time that x_2 reached it's maximum and the corresponding maximum value are given for the values we investigate.

Table 2: Partition coefficient values of doxorubicin and the required time to reach the maximum concentration in the outer leaflet with its corresponding value.

P	t_{max}	x_2
7600	4.345189	0.940528
7800	4.345145	0.940556
8000	4.345172	0.940541
8200	4.345173	0.940542
8400	4.345179	0.940543
8600	4.345182	0.940545
8800	4.345157	0.940563

Note that the changes in time and maximum values are very small. Therefore, we can conclude that within these range of values, the partition coefficient has no large impact on the time and value where the maximum of the graph is reached. This is probably caused by the fact that the outer leaflet has a maximum concentration it can adsorb. Hence it does not matter how far we increase the partition coefficient from a certain value. Both the time it reaches its maximum and the maximum value stay the same.

Now we will research the influence of the partition coefficient such that we divide and multiply the chosen partition coefficient $(P = 8000)$ with factors ten. This way we can examine the effect of P on the time and value of the concentration drugs in the outer leaflet for much smaller and much larger values. In the following table, the time and corresponding maximum values when x_2 reaches its top are given for different P.

Table 3: Partition coefficient values of doxorubicin and the required time to reach the maximum concentration in the outer leaflet with its corresponding value.

P	t_{max}	x_2
0.8	–	–
8	4.078675	0.843586
80	4.332821	0.931119
800	4.344634	0.939961
8000	4.345172	0.940542
$8 \cdot 10^4$	4.345234	0.940597

The values for $P = 0.8$ could not be calculated numerically because the values are too small. However, for the other values we can see that both the time and the maximum value increase as we increase the partition coefficient. Although note that for a certain value the time and value will not increase any more, see figure 7. As mentioned before, this could be cause by the fact that the outer leaflet has a maximum concentration drug it can obtain. In the following plot one can see the changes of the time and values when x_2 has reached its for the several values. The scatter plot shows the exact values, and the lines roughly show the course of increase in time and values. Note that the lines show an indication of what the time and values could be and are not explicitly calculated. The Matlab code can be found in Appendix A.

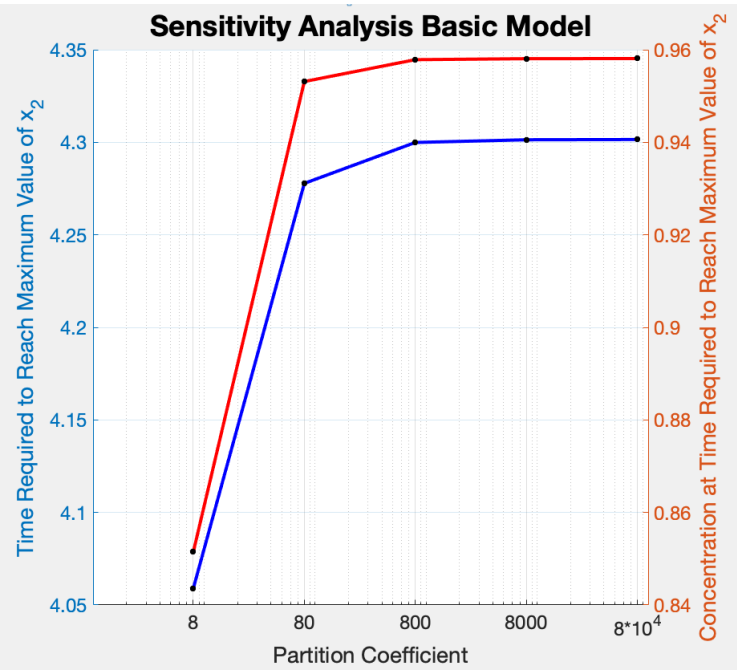


Figure 7: **From a certain value of the partition coefficient, both the time and the maximum value no longer change.** We plot the required time to reach the maximum and the corresponding value against different values of P ranging them from 8 to $8 \cdot 10^4$ with increments of factors of 10. The blue and red lines represent the trend lines between the points. Note that the y-axes do not start at zero to give a better indication of the proportions between the time and maximum value.

4 Binding DNA

This chapter describes how an extension of the Basic Model (3.1) is derived. The difference between this model and the Basic Model is that it also includes the nucleus and mitochondria. Both organelles contain free receptors, i.e., DNA molecules to which the compound could bind. However, because we do not yet know much about drug binding to mitochondrial DNA (mtDNA), the model will only look at DNA binding in the nucleus. The reason we want to look at this is because it is very important for pharmacy to understand how fast a compound transports to the nucleus and bind to DNA. To give a credible idea of what happens we also included the mitochondria, since we know that the compound moves into these organelles faster than into the nucleus, see section 2.2. Therefore, a model such as this one will give a representative interpretation of what will happen in biological experiments.

Now, let us explain how doxorubicin fights tumor cells. Once DOX enters the nucleus it causes breakage of DNA strands and inhibition of DNA synthesis when it intercalates with DNA base pairs, see figure 9. By doing that, DOX blocks an enzyme, called topoisomerase II, which results in double-strand breaks of the DNA. When this happens, cells activate a DNA damage respond. If the repair machinery to these breaks fails, then the DNA repair program initiates apoptosis [13].

The movement of the compound into and inside the cell including the nucleus, mitochondria and binding to receptors in the nucleus is shown in figure 8. The blue organelle represents the nucleus and the grey one corresponds to one of the mitochondria a tumor cell contains. The yellow strings in the nucleus embody the free- and bound receptors. The variables are the compounds' concentration in the several compartments and in the sketch represented by the red boxes. The yellow boxes in the picture are the adsorption 3.2 and desorption rates 3.3 of the compartments. The flip-flop events 3.4 are the purple boxes and the blue ones represent the binding on and binding of rates 4.1 of the compound. In the following section we go into further detail about the meaning of the variables and parameters mentioned in the boxes of the sketch in figure 8 and establish a simple but suitable model for this process.

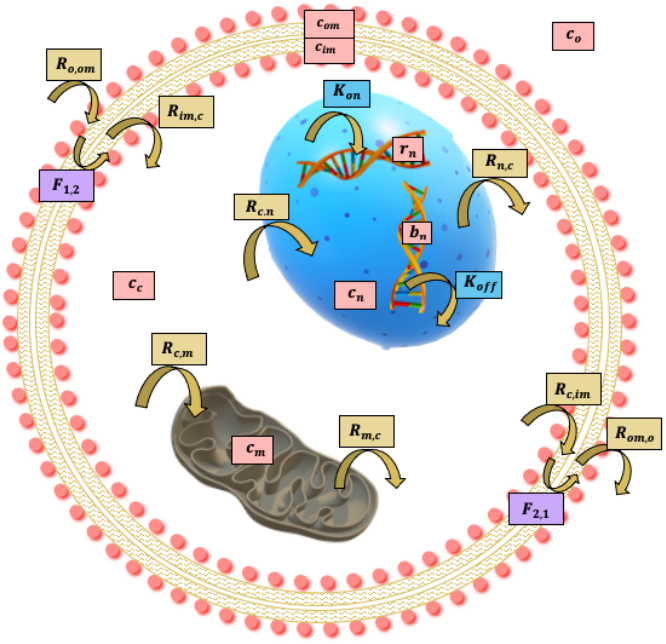


Figure 8: **This figure shows a sketch of the movements of a compound into and inside a tumor cell, including DNA binding.** The membranes are represented by the two strings around the cell, within these leaflets is the cytoplasm, the nucleus and mitochondria are given by the blue and grey objects respectively. The red boxes represent the variables of the system, the yellow ones the adsorption and desorption rates of the several compartments and the flip-flop events and the binding rates are represented by the purple and blue boxes respectively.

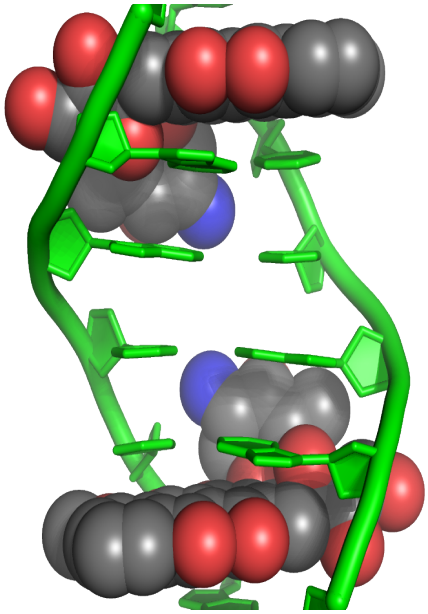


Figure 9: **Noncovalent complex of doxorubicin with a DNA molecule.** The green strings represent the DNA strands and the structures in black, red, and blue represents the molecules of doxorubicin that intercalates with the base pairs.

4.1 Expanded Model

In the context of what is described in the previous section, we will set up a more extensive model. Also, for this model we will consider two steps to cross the membrane. This model will be able to show in a better way how the compound transports into the cell and moves between the different compartments within the cell. From now on we will call this model the "Expanded Model".

In the following lines we will describe the variables and parameters we used to set up this extensive model. Recall the compartments that we defined in section 3, we use these variables again and add the compartments c_n, c_m, r_n and b_n as new variables for this model. These variables stand for the concentration drugs in the nucleus, the mitochondria and for the free- and bound receptors in the nucleus respectively. We define the new total concentration as the sum of the compound's concentration in each of the compartments which may contain drugs, i.e.

$$C_{tot} := c_o + c_{om} + c_{im} + c_c + c_n + c_m + b_n$$

Note that the concentration of free receptors is not included, since this compartment obviously does not contain any of the concentration drugs. Thereby we define a total concentration that includes all receptors within the nucleus, i.e.

$$r_{ntot} := r_n + b_n$$

Both total sums are assumed to be constant during in the course of time and for the same reasons as in section 3 we assume that all variables are real and positive including zero.

Next to the adsorption rates, desorption rates and the flip-flop events we used in the Basic Model, see section 3, we have implemented the adsorption and desorption rates of the nucleus and the mitochondria, namely $R_{c,n}, R_{n,c}, R_{c,m}$ and $R_{m,c}$ respectively. We also introduce some other parameters, which we call the binding on and binding of rates to DNA. Before can understand what, the binding rates are, we need a few definitions.

Observe the following two reactions: $c_n + r_n \xrightarrow{K_{on}} b_n$ $\qquad$ $c_n + r_n \xleftarrow{K_{off}} b_n$

In these reactions the K_{on} and K_{off} represent the proportionality constants. Here the flux reactions are $K_{on}[r_n][c_n]$ and $K_{off}[b_n]$ respectively. In the meaning of this context, the flux reaction is the number of times the reaction takes place in one direction per minute. Now we can properly define the binding on and binding off rates of a compound.

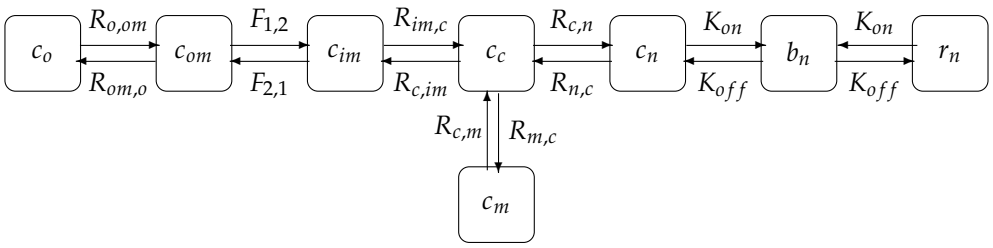
Definition 4.1 (Binding on / Binding off rates)**.** The *binding on-* and *off rates* of the compound to DNA are represented by the proportionality constants in the following reactions:

$$c_n + r_n \underset{K_{off}}{\overset{K_{on}}{\rightleftharpoons}} b_n$$

Here the binding on and binding off rates are denoted by K_{on} and K_{off} respectively.

The variables for this model all have the dimension ML^{-1} where M is the weight in μg and L is the volume in ml. So, we have the same dimensions as we had in the Basic Model, and for the same reasons these units are suitable to use for a compound's concentration. For most of the parameters we again use the dimension T^{-1} where T is the time in min. However, note that since the flux reaction of $c_n + r_n \to b_n$ is $K_{on}[r_n][c_n]$ and the units of both r_n and c_n are μg ml^{-1} the proportionality constant K_{on} should have a dimension of $LM^{-1}T^{-1}$. Also, here L is volume in ml, M is weight in μg and T is the time in min.

Here follows a sketch of the compartment model for the Expanded Model with the nucleus, mitochondria, free- and bound receptors included:



Under the same assumptions we mentioned in section 3 the system of ODEs for the Expanded Model is as follows:

$$\begin{cases} \frac{dc_o}{dt} = -R_{o,om}c_o + R_{om,o}c_{om} \\ \frac{dc_{om}}{dt} = R_{o,om}c_o - (R_{om,o} + F_{1,2})c_{om} + F_{2,1}c_{im} \\ \frac{dc_{im}}{dt} = F_{1,2}c_{om} - (F_{2,1} + R_{im,c})c_{im} + R_{c,im}c_c \\ \frac{dc_c}{dt} = R_{im,c}c_{im} - (R_{c,im} + R_{c,n} + R_{c,m})c_c + R_{n,c}c_n + R_{m,c}c_m \\ \frac{dc_n}{dt} = R_{c,n}c_c - (R_{n,c} + K_{on}r_n)c_n + K_{off}b_n \\ \frac{dc_m}{dt} = R_{c,m}c_c - R_{m,c}c_m \\ \frac{dr_n}{dt} = -K_{on}r_nc_n + K_{off}b_n \\ \frac{db_n}{dt} = K_{on}r_nc_n - K_{off}b_n \end{cases} \tag{4.1}$$

The change of the concentration drugs and the concentration of receptors in these compartments are determined in the same way as in the Basic Model. Therefore, we will not go into further detail this time and leave it to the reader to think about what happens in each of these equations.

Note that since the total sums for the concentration drugs and the concentration of receptors are constant in the course of time. Therefore, if we know six of the differential equations, we can derive the concentrations in the two remaining compartments. However, do note that the two concentrations we want to derive should neither be in both sums nor should they be part of the same sum. Because it is convenient for making the system dimensionless in the following section, we will eliminate the equations that correspond to the changes in the concentration of drugs within the nucleus and the change in concentration of the free receptors inside the nucleus.

$$\begin{cases} \frac{dc_o}{dt} = -R_{o,om}c_o + R_{om,o}c_{om} \\ \frac{dc_{om}}{dt} = R_{o,om}c_o - (R_{om,o} + F_{1,2})c_{om} + F_{2,1}c_{im} \\ \frac{dc_{im}}{dt} = F_{1,2}c_{om} - (F_{2,1} + R_{im,c})c_{im} + R_{c,im}c_c \\ \frac{dc_c}{dt} = R_{im,c}c_{im} - (R_{c,im} + R_{c,n} + R_{c,m})c_c + R_{n,c}(C_{tot} - c_o - c_{om} - c_{im} - c_c - c_m - b_n) + R_{m,c}c_m \\ \frac{dc_m}{dt} = R_{c,m}c_c - R_{m,c}c_m \\ \frac{db_n}{dt} = K_{on}(r_{ntot} - b_n)(C_{tot} - c_o - c_{om} - c_{im} - c_c - c_m - b_n) - K_{off}b_n \\ C_{tot} = c_o + c_{om} + c_{im} + c_c + c_n + c_m + b_n \\ r_{ntot} = r_n + b_n \end{cases} \tag{4.2}$$

With initial condition $c_o(0) = c_{o(0)}$, $c_{om} = c_{im}(0) = c_c(0) = c_n(0) = c_m(0) = 0$, $r_n(0) = r_{n(0)}$, $b_n(0) = 0$.

We apply the same method to the above system (4.2) as for the Basic Model to make the system dimensionless. So, we divide each of the drugs' concentrations in all the compartments of the cell and the bound receptors by the total concentration C_{tot} the same way as for the Basic Model. We will scale the free receptors as well, i.e., r_n divided by r_{ntot}. Doing this for all t we receive the following dimensionless variables:

$$y_1 := \frac{c_o}{C_{tot}} \qquad y_2 := \frac{c_{om}}{C_{tot}} \qquad y_3 := \frac{c_{im}}{C_{tot}} \qquad y_4 := \frac{c_c}{C_{tot}}$$
$$y_5 := \frac{c_n}{C_{tot}} \qquad y_6 := \frac{c_m}{C_{tot}} \qquad y_7 := \frac{r_n}{r_{ntot}} \qquad y_8 := \frac{b_n}{C_{tot}}$$

As in section 3.1 we scale the time t such that $t = t_0\hat{t}$ where t_0 and $\hat{t}$ are defined the exact same way as for the Basic Model. So next to the parameters $P_o, I_1, I_2, J_{im,c}$ and $J_{c,im}$ we already found we also receive the following parameters by scaling the new parameters we received for the Expanded Model:

$$J_{c,n} := \frac{R_{c,n}}{R_{o,om}}, \quad J_{n,c} := \frac{R_{n,c}}{R_{o,om}}, \quad J_{c,m} := \frac{R_{c,m}}{R_{o,om}}, \quad J_{m,c} := \frac{R_{m,c}}{R_{o,om}}, \quad B_{on} := \frac{K_{on}r_{ntot}}{R_{o,om}}, \quad B_{off} := \frac{K_{off}}{R_{o,om}}$$

Remark that B_{on} is defined by not only the parameter K_{on} but is also multiplied with the total concentration of the receptors. The reason we do this is because we define $x_7 = \frac{r_n}{r_{ntot}}$, hence $r_n = r_{ntot}x_7$. If we substitute this in our dimension carrying system then we have the term $K_{on}r_{ntot}x_7$ in a few of our equations of system (4.1).

Note that B_{on} is dimensionless as well. So with our new scaled variables and parameters we can develop the dimensionless system of ODEs for the Expanded Model, see equation (4.3).

$$\begin{cases} \frac{dy_1}{d\hat{t}} = -y_1 + P_o y_2 \\ \frac{dy_2}{d\hat{t}} = y_1 - (P_o + I_1)y_2 + I_2 y_3 \\ \frac{dy_3}{d\hat{t}} = I_1 y_2 - (I_2 + J_{im,c})y_3 + J_{c,im} y_4 \\ \frac{dy_4}{d\hat{t}} = J_{im,c} y_3 - (J_{c,im} + J_{c,n} + J_{c,m})y_4 + J_{n,c} y_5 + J_{m,c} y_6 \\ \frac{dy_5}{d\hat{t}} = J_{c,n} y_4 - (J_{n_c} + B_{on} y_7)y_5 + B_{off} y_8 \\ \frac{dy_6}{d\hat{t}} = J_{c,m} y_4 - J_{m,c} y_6 \\ \frac{dy_7}{d\hat{t}} = -\frac{C_{tot}}{r_{ntot}} B_{on} y_5 y_7 + \frac{C_{tot}}{r_{ntot}} B_{off} y_8 \\ \frac{dy_8}{d\hat{t}} = B_{on} y_5 y_7 - B_{off} y_8 \end{cases} \tag{4.3}$$

Such that $y_1 + y_2 + y_3 + y_4 + y_5 + y_6 + y_8 = 1$ and $y_7 + \frac{C_{tot}}{r_{ntot}} y_8 = 1$.

Note that we have a lot of parameters. We want as few parameters as possible so we need less information, but we still be able to perform a reliable analyses on a given compound. To lose one of the equations we define new variables $(a_i y_i)$ and substitute them for y_i the in system (4.3) for all $i = 1, 2, \ldots, 8$.

$$\begin{cases} \frac{da_1 y_1}{d\hat{t}} = -a_1 y_1 + P_o a_2 y_2 \\ \frac{da_2 y_2}{d\hat{t}} = a_1 y_1 - (P_o + I_1)a_2 y_2 + I_2 a_3 y_3 \\ \frac{da_3 y_3}{d\hat{t}} = I_1 a_2 y_2 - (I_2 + J_{im,c})a_3 y_3 + J_{c,im} a_4 y_4 \\ \frac{da_4 y_4}{d\hat{t}} = J_{im,c} a_3 y_3 - (J_{c,im} + J_{c,n} + J_{c,m})a_4 y_4 + J_{n,c} a_5 y_5 + J_{m,c} a_6 y_6 \\ \frac{da_5 y_5}{d\hat{t}} = J_{c,n} a_4 y_4 - (J_{n,c} + B_{on} a_7 y_7)a_5 y_5 + B_{off} a_8 y_8 \\ \frac{da_6 y_6}{d\hat{t}} = J_{c,m} a_4 y_4 - J_{m,c} a_6 y_6 \\ \frac{da_7 y_7}{d\hat{t}} = -\frac{C_{tot}}{r_{ntot}} B_{on} a_5 y_5 a_7 y_7 + \frac{C_{tot}}{r_{ntot}} B_{off} a_8 y_8 \\ \frac{da_8 y_8}{d\hat{t}} = B_{on} a_5 y_5 a_7 y_7 - B_{off} a_8 y_8 \end{cases} \tag{4.4}$$

Since y_i is dimensionless, also the constants (a_i) obviously should not have a dimension to keep the system dimensionless. We let $a_i = \frac{1}{B_{on}}$ for all $i = 1, 2, \ldots, 8$. Since B_{on} is defined as a dimensionless parameter, we may use this factor as a constant to multiply the equations on both sides with.

Recall that $y_1 + y_2 + y_3 + y_4 + y_5 + y_6 + y_8 = 1$ and $y_7 + \frac{C_{tot}}{r_{ntot}} y_8 = 1$. Hence the concentrations for the fifth and seventh equation can be easily determined if we know the concentrations in the other compartments. Therefore, we can eliminate these two equations from the model:

$$\begin{cases} \frac{dy_1}{d\hat{t}} = -y_1 + P_o y_2 \\ \frac{dy_2}{d\hat{t}} = y_1 - (P_o + I_1)y_2 + I_2 y_3 \\ \frac{dy_3}{d\hat{t}} = J_{im,c} y_2 - (I_2 + J_{im,c})y_3 + J_{c,im} y_4 \\ \frac{dy_4}{d\hat{t}} = J_{im,c} y_3 - (J_{c,im} + J_{c,n} + J_{c,m})y_4 + J_{n,c}(1 - y_1 - y_2 - y_3 - y_4 - y_6 - y_8) + J_{m,c} y_6 \\ \frac{dy_6}{d\hat{t}} = J_{c,m} y_4 - J_{m,c} y_6 \\ \frac{dy_8}{d\hat{t}} = (1 - y_1 - y_2 - y_3 - y_4 - y_6 - y_8)(1 - \frac{C_{tot}}{r_{ntot}} y_8) - B_{off} y_8 \\ y_1 + y_2 + y_3 + y_4 + y_5 + y_6 + y_8 = 1 \\ y_7 + \frac{C_{tot}}{r_{ntot}} y_8 = 1 \end{cases} \tag{4.5}$$

Note that this system is still dimensionless and that the parameter B_{on} is not part of the system anymore, so now we have 10 parameters instead of 11. Thus, we received our goal of losing one of the parameters. The system defined in (4.5) will be used for the numerical simulation and analyses of the model.

4.2 Numerical Simulation Expanded Model

With this simulation we do not only want to research the movement of a drug into the cell considering two steps to cross the membrane, but also the movements inside the cell. Thereby, we want to investigate the rate of drug binding to and from DNA. In section 7 we will compare this model with the model that does not take the two steps into account to go through the membrane. The results of this analysis are very important because it will give a strong prediction of the behavior of a drug inside a cell. Therefore, it is not needed to do real experiments with several compounds to see which one can be used best to treat cancer.

For this simulation we use the same values for the partition coefficient P and the other parameters we used for the simulation of the Basic Model, see section 3.2. We also did some literature studies to find values for the adsorption and desorption rates of doxorubicin into and out of the nucleus and mitochondria. However, we were not successful in finding these values. Therefore we used the idea of the research experiments performed by the LUMC which was described in section 2.2. Recall that at the beginning the mitochondria contained more of the compound than the nucleus. Hence, we assume that the adsorption rate of the compound to mitochondria is higher than that of the nucleus. So we define $R_{c,n} := 4R_{c,im}$ and $R_{c,m} := 5R_{c,im}$, then obviously the adsorption rate of the mitochondria is higher than that of the nucleus. Since these adsorption rates are higher than that of the inner leaflet, the compound will also move away from the inner leaflet into these two organelles. Then we define the desorption rate of both the mitochondria and the nucleus as their adsorption rates divided by the partition coefficient P as we did to determine the desorption rate of the outer leaflet, i.e. $R_{n,c}P := R_{c,n}$ for the nucleus and $R_{m,c}P := R_{c,m}$ for the mitochondria. These desorption rates are representative because for the nucleus the desorption rate should be much smaller than its desorption rate caused by the fact that most of the drug will bind to the DNA rather than move outside the nucleus again. For the mitochondria we assume that the drug moves into the mitochondria faster than it moves out. Note that since we eliminated the parameter which is dependent on the binding on rate of the compound to the DNA in the dimensionless system, we do not need to know this value for the simulation but for doxorubicin can be found in article [15]. Since the binding off rate from the DNA of the compound is this small that it is negligible, we assemble this value to zero. In table 4 we see the parameters which are used for the numerical simulation.

Table 4: Parameter values of doxorubicin of the dimensionless system of the Expanded Model

Parameters	**Values**
P_o	$1.25 \cdot 10^{-4}$
I_1	0.014
I_2	0.014
$J_{im,c}$	1
$J_{c,im}$	8000
$J_{c,n}$	$3.2 \cdot 10^4$
$J_{n,c}$	4
$J_{c,m}$	$4 \cdot 10^4$
$J_{m,c}$	5
B_{off}	0
C_{tot} (μg ml^{-1})	20
r_{ntot} (μg ml^{-1})	20

We analyze the distributions in the several compartments of the cell by simulating these values numerically. Because it takes a very long time to reach the steady state, we do not show the full plot. This way we can better observe the more interesting movements of the drug in the different compartments. Since the system is nonlinear, we could not determine the stability of the equilibrium nor the solution for this system. Contrary to the Basic Model we are now more in the time half of the total concentration receptors is bound than in the time we are at steady state. We assume that at this point the cell does not have enough DNA to survive and will die. The time that half a concentration of bound receptors is reached will be compared with the time of the model that does not consider the steps of the membrane. These results can be compared to experimental research, then we want to see which of these models gives a more reliable prediction. In figures 10 and 11, we show the received plots after the numerical simulation where the latter plot is zoomed in. The Matlab code for this simulation can be found in Appendix A.

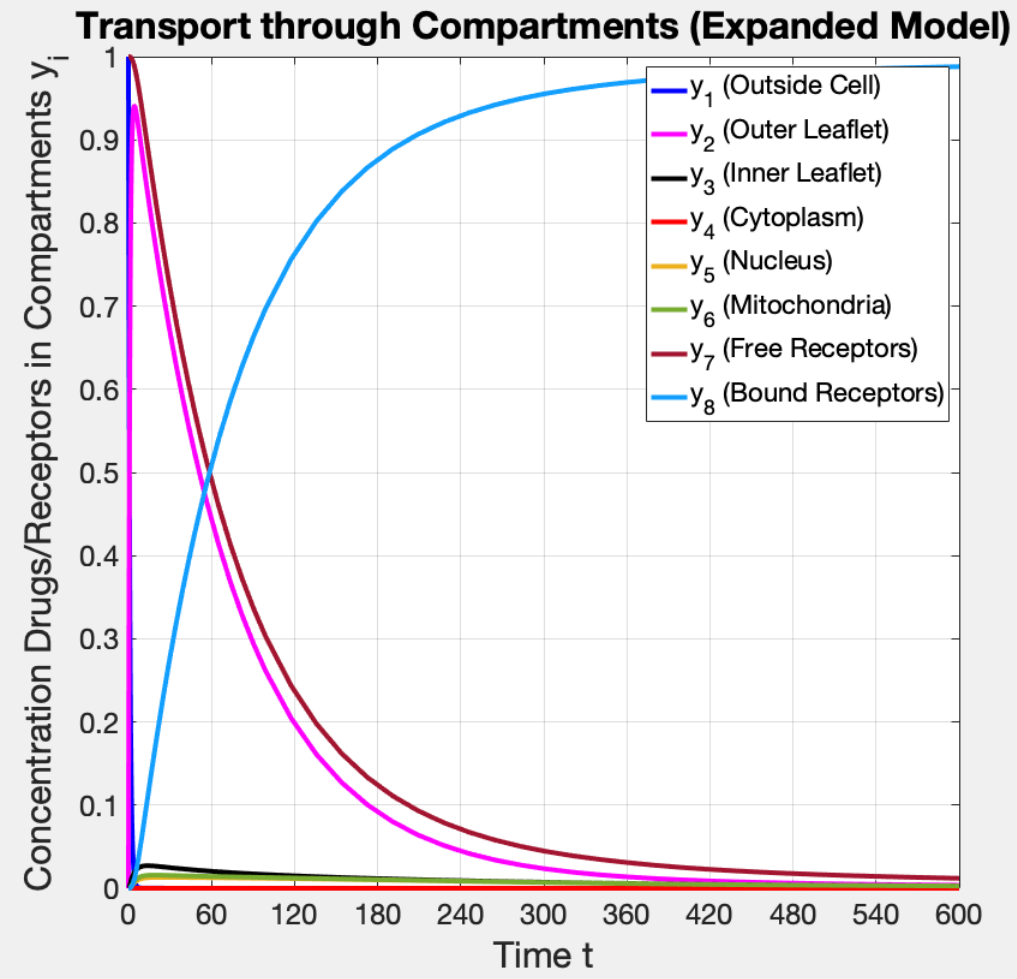


Figure 10: **The time point to reach half a concentration of bound receptors is 59.** Each graph represents the change in compounds' concentration over time in the various compartments. For this plot we used the values from table 4 and simulated them in Matlab.

The graphs in figure 10 represent the compound's concentration in the different compartments over 600 time points. As we observed in the Basic Model x_1 decreases extremely fast from one to zero in about eight time points as can be seen in figure 11. We also observe that x_2 reaches a maximum as we saw in the Basic Model and later it also decreases to zero. In contrast to the Basic Model, x_3 increases to a maximum and then slowly decreases till it becomes zero again. We see the same kind of movements in the graphs for x_5 and x_6, although their maxima are a bit smaller as can be better seen in figure 11. The line corresponding to x_4 remains constantly zero in the course of time. The graphs x_7 and x_8 are inverses of each other such that x_7 converges to zero and x_8 to one.

Since the binding off rate is chosen to be zero, the equilibrium is $(0,0,0,0,0,0,0,1)^\top$. Note that indeed $y_1+y_2+y_3+y_4+y_5+y_6+y_8=1$ and $y_7+\frac{C_{tot}}{r_{ntot}}=0+\frac{20}{20}\cdot 1=1$ and that this steady state is within the range of the values for the concentrations of the several compartments. If we let the binding off rate of doxorubicin be arbitrarily small, then we have two equilibria. One of those two are within the range of the values for the concentrations. However, the other steady state contains values below zero and above one, so we should not consider this equilibrium for the analysis of the simulation.

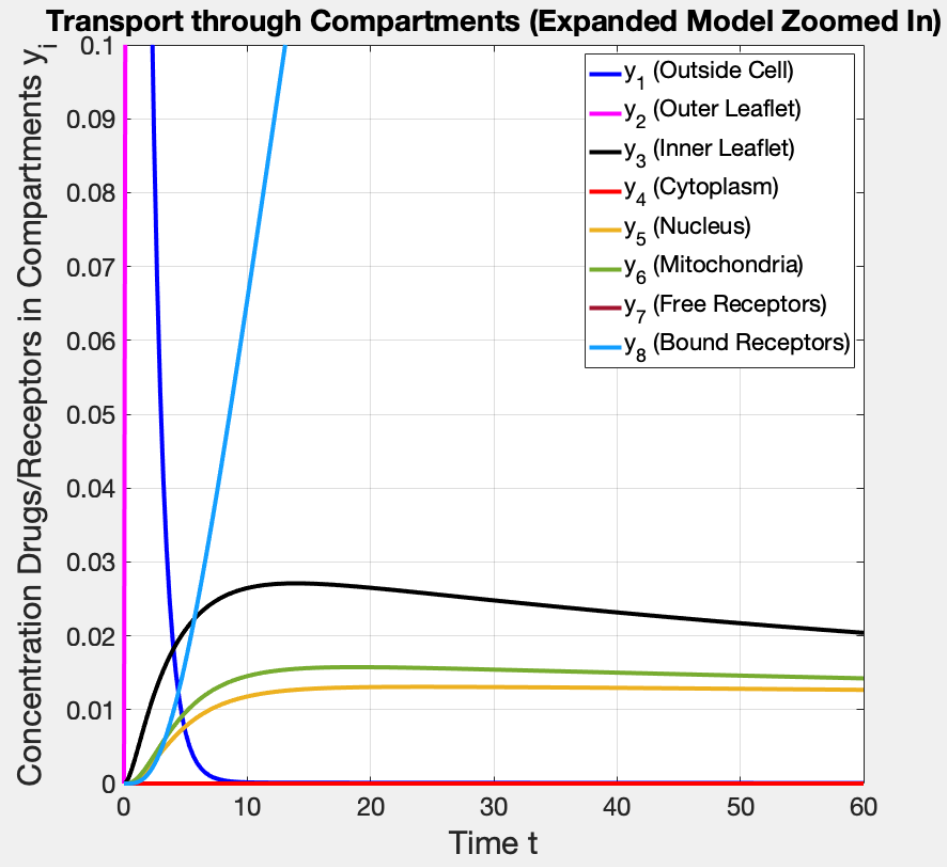


Figure 11: **The inner leaflet, nucleus and mitochondria reach a maximum value and then slowly converge to zero.** We zoom in at the original plot by ranging the concentration drugs from 0 to 0.1 to receive a better picture.

4.2.1 Interpretation Numerical Analysis Expanded Model

The fact that x_1 decreases at this high pace is for the same reasons as we concluded for the Basic Model just as the top that x_2 reaches. However, this time x_2 decreases to zero caused by the fact that in this model the compound also has high affinity to the membranes of the nucleus and mitochondria. Thereby, the compound will bind to the free receptors in the nucleus and since we assume that the binding off rate is negligible, we indeed expect that when enough time has passed, the whole concentration of the compound will be bound to the receptors in the nucleus. For this reason, the concentration in the inner leaflet also reaches a maximum and at the end will have zero concentration as well. Because the drug has such high affinity with membranes, the concentration in the cytoplasm is practically zero for the whole course of time. Because the compound moves faster to the mitochondria than to the nucleus the concentration in the mitochondria increases earlier than that of the nucleus and reaches a higher maximum. However, also the concentrations in these two organelles will decrease until there is no concentration of the compound in either one of them. Also, this phenomenon is caused by the fact that we assume that the drug does not unbind from the receptors anymore. Therefore, we also see that the free receptors drop to a concentration of zero and that the bound receptors will rise until they cover the total concentration of receptors in the nucleus. Here we assumed that the total concentration drugs is the same as the total concentration of receptors. Therefore, we see that the total concentration drugs will bind to the receptors. So, the concentration of free receptors becomes zero and the total concentration drugs will be in the bound receptors. If the total concentration of free receptors is higher than the total concentration drugs, then we see that all the drugs are bound to the receptors. However, some of the concentration free receptors we be left in the nucleus. When there is more drug available than free receptors, the total concentration of receptors will be bound at the end. In this case the drugs will can also be obtained by one of the other compartments than the receptors.

4.3 Sensitivity Analysis Expanded Model

In this section we will analyze the influence of the partition coefficient (P) on the time that half the concentration of receptors is bound. We suppose that when the concentration of the bound receptors is above 50%, the tumor cell dies. If we take a look at figure 10 of the numerical simulation of the Expanded Model, we see that at time point 59 the concentration of the bound receptors is half the total concentration of receptors. The goal is to decrease the time before the cell dies, since we want the tumor to shrink as soon as possible.

We expect that for smaller values of P, the time to reach half a concentration of bound receptors will increase since if P is smaller, then it is harder for the compound to come across the membranes of the tumor cell and the nucleus. Therefore, it will take more time for the drug to arrive at the nucleus where it can bind to the free receptors. If we let P be larger, then it will move faster into and inside the cell. From the cell it will move quickly into the nucleus where it can bind to the free receptors. Hence if the partition coefficient of the compound is larger, we expect that the drug needs less time to bind to a receptor.

To investigate the influence of the partition coefficient, we simulate the model for different values of P. From the plots we receive for these values of P we can read the time the compound takes to bind to half of the initial concentration of total receptors. In the following plot we see the changes of time when we vary the value of the partition coefficient in a range of 7600 to 8800 with steps of 200. The reason we do this is because according to Alves et al. [1] the partition coefficient ranges between 7789 and 8601 and this way we consider all values for P. On the y-axis we range the time between 0 and 600 so that it gives a strong representation at the time when half the concentration of bound receptors is reached compared to the time the system is at steady state.

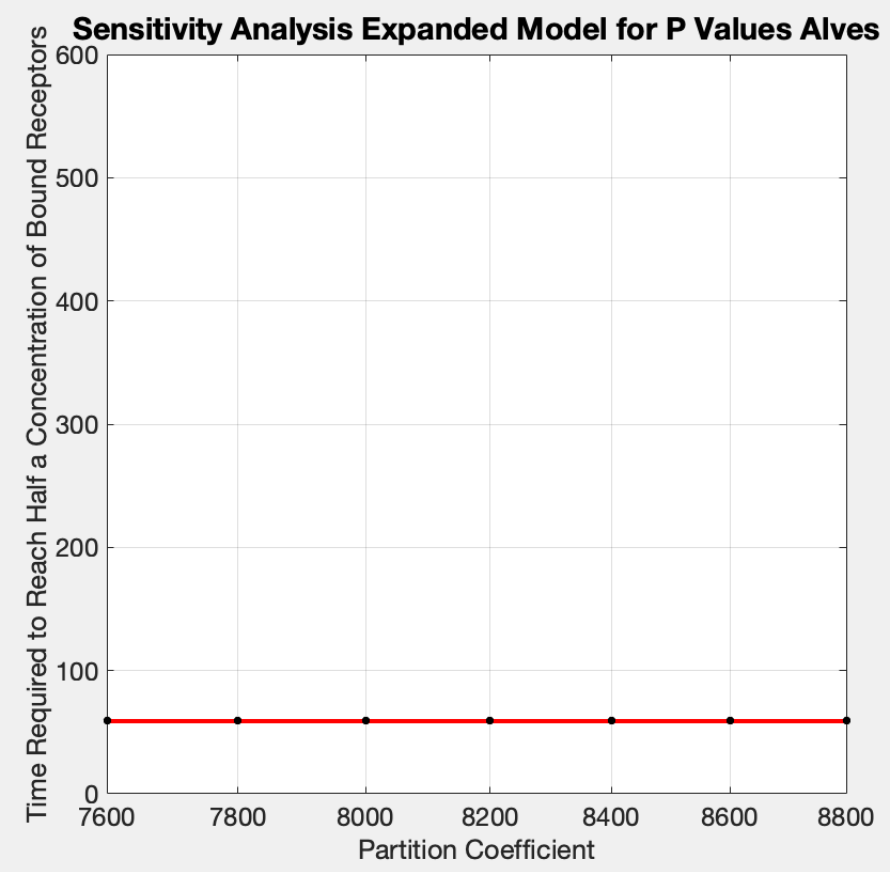


Figure 12: **If the partition coefficient is within a range of** 7600 **and** 8800**, then the time required to reach half a concentration of bound receptors does not change.** We plot the required time to reach half a concentration of bound receptors against different values of P ranging from 7600 to 8800 with increments of 200. The red line represents the trend line between the points.

In figure 12 we can see that within this range of values for P, the time to reach half the concentration of bound receptors does not change at all. This can be explained by the fact that when the cell or nucleus obtained a maximum concentration, they cannot adsorb any more drug molecules. Therefore, it does not matter how high the partition coefficient is. When the maximum concentration in a compartment is reached the compound will not move into this compartment anymore. Since we do not see much difference within this range, we observed the influence of values for much lower and much higher values as well. The values we examined where values between 0.8 and $8 \cdot 10^4$ that differ from each other by a factor of 10. In figure 13 we see the times when half a concentration of receptors is bound for these values of P. In this plot we can clearly see that, the time to reach half the concentration of bound receptors will not decrease any further when P reaches a value between 8 and 80. However, do note that for very small values of P, the compound needs more time to bind the free receptors as we expected. Remark that the red lines between the dots in figures 12 and 13 just give an indication of what the values might be between the values we have calculated explicitly. The Matlab code for both plots can be found in Appendix A.

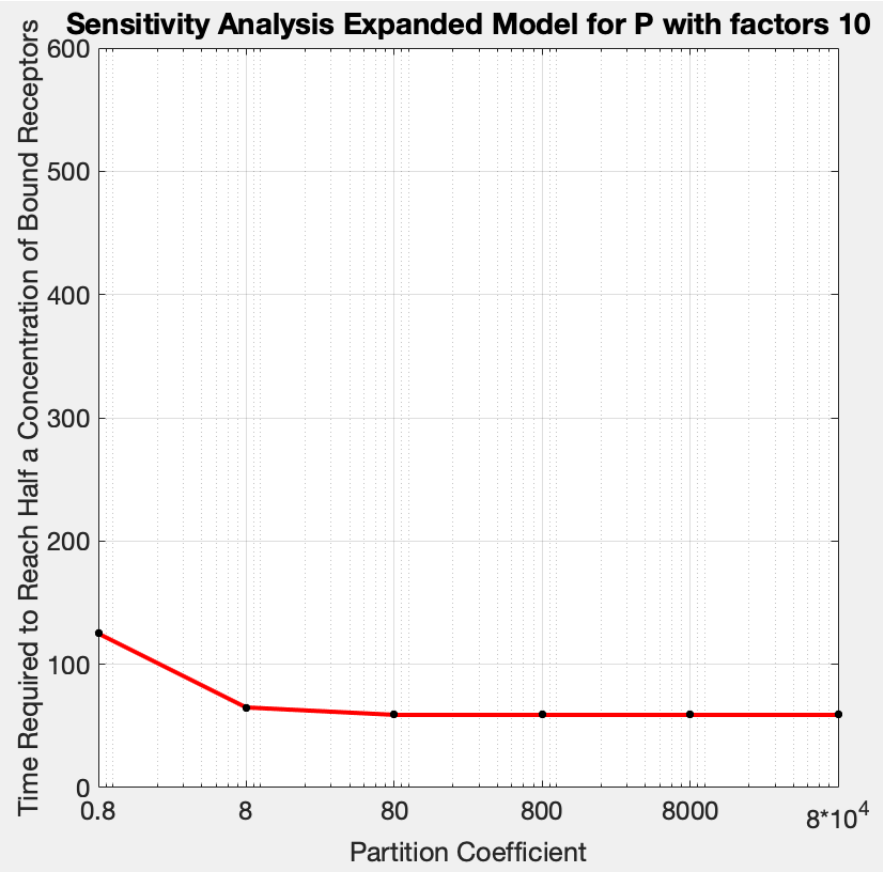


Figure 13: **For relatively small partition coefficient values the time to reach half a concentration of bound receptors is greater.** We plot the required time to reach half a concentration of bound receptors against different values of P ranging from 0.8 to $8 \cdot 10^4$ with increments of factors of 10. The red line represents the trend line between the points.

4.4 Sensitivity Analysis Dimensionfull System Expanded Model

In the previous section we examined how the plot changes when we vary the partition coefficient P of the dimensionless system of the Expanded Model (4.5). Now we will study the effect on the plot when we multiply both the adsorption and desorption rates from outside the cell with some factor. Then the partition coefficient obviously remains the same. In table 5 we give the values of next to the factors we multiplied these rates with. Our aim is to find out what the influence is of the adsorption and desorption rates from the outer leaflet on the uptake of doxorubicin by the tumor cell.

Table 5: Multiplication factors of the adsorption and desorption rates of doxorubicin and the required time to reach half a concentration of bound receptors, we denote this time with $t_{\frac{1}{2}}$.

Factor	$t_{\frac{1}{2}}$ **in minutes**
0.001	1.45
0.1	0.6
1	0.5
10	0.5
100	0.5
1000	0.5
$1 \cdot 10^4$	0.5

In figure 14 we will see that from a certain multiplication factor, there is no difference in time when half of the concentration of receptors is bound. Again, the line through the dots only gives an indication of what the values between the determined values are. Also note that we compare the times that half a concentration of bound receptors is reached with the time the system is at steady state. When the outer leaflet cannot obtain any more drug molecules, it does not matter how much we increase the values for the adsorption and desorption rates, the drug uptake by the cell will not go any faster. Therefore, there is no difference in time from when the multiplication factor is greater than one. However, when we multiply the rates with a factor smaller than 1 the time increases rather fast to reach half the concentration of bound receptors. This suggests that when the adsorption rate in comparison to the desorption rate is relatively small, then the compound needs more time to be adsorbed by the outer leaflet. Hence it will take longer for the drug to bind the receptors of the tumor cell. The Matlab code can be found in Appendix A.

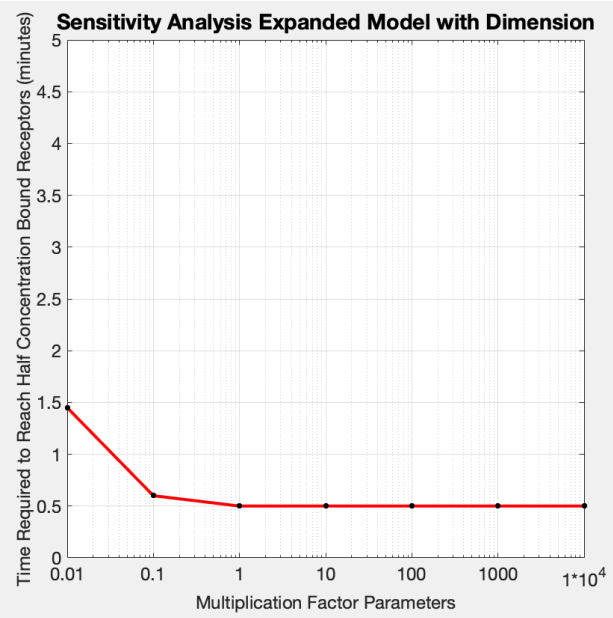


Figure 14: **For relatively small adsorption and desorption rates the time to reach half a concentration of bound receptors is greater.** We plot the required time to reach half a concentration of bound receptors against different multiplication factors of the adsorption and desorption rates with increments of factors of 10. The red line represents the trend line between the points.

5 pH Model

In the previous chapters we described how a compound transports into and inside the cell considering two steps to cross the membrane. The goal of this chapter is to develop a new model where we immediately move from outside the cell into the cytoplasm. In section 7 this model will be compared with the previous models. From this we want to deduce which of these models give a better interpretation of real-life experiments.

Recall that the previous models describe the movements of a drug into and inside a tumor cell where the focus especially lies on the fact that we consider two steps to cross the membrane. The model we will develop in this section assumes that the drug transports into the cell at once driven by a concentration gradient. For this model we consider the pH partition hypothesis. So only uncharged drug molecules can cross the membrane and at equilibrium the concentrations of the uncharged molecules are equal on both sides of the membrane. However, note that due to ionization the total concentration drugs outside the cell may be very large in comparison to the cytoplasm.The extent of ionization depends upon the pH and pK_a of the medium from where the drug is being adsorbed. In this context pK_a is the negative logarithm of the equilibrium constant of a drug under physiological conditions. In case we know these values, we can determine the ratio of the charged and uncharged concentrations of the drug [17]. This can be done by the Henderson-Hasselbalch equation.

Definition 5.1 (Henderson-Hasselbalch Equation)**.** The *Henderson-Hasselbalch equation* can be defined as follows:

$$\text{pH} = p\text{K}_\text{a} + \log\left(\frac{[A^-]}{[HA]}\right), \text{ for acidic compounds}$$

$$\text{pH} = p\text{K}_\text{a} + \log\left(\frac{[B]}{[BH^+]}\right), \text{ for basic compounds}$$

By the fact that all parameters we use for the numerical simulations are derived from research experiments, we assume that merely uncharged molecules cross the membrane. Therefore, we will not use this equation for our model. Although, this equation can be used if the reader wishes to calculate the ratio between the charged and uncharged molecules.

Let us start with developing a model for drug transport from outside the cell into the cytoplasm not considering organelles or DNA binding yet. As before we will use the variables c_o and c_c to represent the compartments outside the cell and the cytoplasm. For this model we define the total concentration drugs by $c_t := c_o + c_c$ which again will assumed to remain constant in the course of time. At the beginning of the process, the total concentration drugs is assumed to be outside the cell.

This model will be based on the system of partial differential equations for the interior and exterior drug concentrations at the cell membrane boundary described in an article written by Leedale et al. [9]. With the help of the supplementary material given by this article we rewrite this system into a system of ordinary differential equations.

According to Leedale et al., the transport of drugs into the cytoplasm can be represented by equation (5.1) where K_{in} represents the transport rate into the cell per μl per minute and V_{med} the volume in μl of the media.

$$\frac{dA_{cell}}{dt} = K_{in} V_{med} c_o \tag{5.1}$$

where A_{cell} is the concentration drugs in the cytoplasm expressed in moles per 10^6 cells. Since we want to compare this model to the Basic Model which only considers the transport in one cell instead of 10^6 cells. Therefore, we must divide the right side of the equation by 10^6 and the intracellular volume V_{cell} with units of μl per 10^6 cells. Hence the transport of the compound in one cell can be expressed as

$$\frac{dA_{cell}}{dt} = \frac{K_{in} V_{med} c_o}{10^6 V_{cell}} \tag{5.2}$$

According to Leedale et al., the uptake rate of the cell can also be defined by $P_{diff} c_o$, where P_{diff} is the passive diffusion clearance. Therefore according to Leedale et al., $K_{in} V_{med} = P_{diff} = 10^{0.6316 \times \log D_{7.4} - 0.3143}$ where $\log D_{7.4}$ is a partition coefficient measure of lipophilicity at a physiologically relevant pH [9]. From now on will use this measure for our simulations.

The partition coefficient of the compound between the compartments outside the cell and the cytoplasm plays an important role in the uptake of drugs. It represents the distribution of the compound when the system is at steady state. Recall the definition 3.5 given in section 3, in this context the distribution of the compound in equilibrium is $c_c = P \cdot c_o$. Hence the transport drugs outside the cell is given by

$$\frac{dA_{med}}{dt} = -\frac{1}{P}\frac{P_{diff}c_c}{10^6 V_{cell}}$$

Note that the dimensions of this system are not the same as we had in the previous model. Therefore, we need to scale them using the molecular mass of the compound. According to Leedale et al., P_{diff} has units of μl min^{-1} 10^{-6} cells, the concentrations c_o and c_c are expressed in μmol and V_{cell} in μl 10^{-6}. Hence the change in concentrations is expressed in μmol μl^{-1} min^{-1}. Recall from section 3 that we expressed these terms in units of μg ml^{-1} min^{-1}, so we will multiply the equations in system (5.2) with one over thousand times the molecular weight (W) of the compound. Applying these changes to the system described in the supplementary material of Leedale's article provides the system of ordinary differential equations in (5.3).

$$\begin{cases} \frac{dc_o}{dt} = \frac{WP_{diff}}{10^9 V_{cell}}(-c_o + \frac{1}{P}c_c) \\ \frac{dc_c}{dt} = \frac{WP_{diff}}{10^9 V_{cell}}(c_o - \frac{1}{P}c_c) \end{cases} \tag{5.3}$$

With initial condition $c_o(0) = c_{o(0)}$ and $c_c(0) = 0$. From now on we refer to this model with "pH Model".

5.1 Dimensionless pH Model

We will also make this model dimensionless to achieve a better interpretation of the results. Recall the method we used in section 3.1, the same method will be used to derive the dimensionless system of the pH model. Therefore, we will not explain in detail how we derived the new variables and parameters. The normalized variables are

$$z_1 := \frac{c_o}{c_t} \qquad\qquad z_2 := \frac{c_c}{c_t}$$

and we scale the time t such that $t = t_0\hat{t}$ where $t_0 = \frac{10^9 V_{cell}}{WP_{diff}}$. Then we obtain the dimensionless system

$$\begin{cases} \frac{dz_1}{d\hat{t}} = -z_1 + \frac{1}{P}z_2 \\ \frac{dz_2}{d\hat{t}} = z_1 - \frac{1}{P}z_2 \end{cases} \tag{5.4}$$

Recall that the total sum of the compound's concentration was constant, meaning that $z_1 + z_2 = 1$ for all time. Therefore, we can rewrite the system as follows:

$$\begin{cases} \frac{dz_1}{d\hat{t}} = -(1 + \frac{1}{P})z_1 + \frac{1}{P} \\ z_1 + z_2 = 1 \end{cases} \tag{5.5}$$

with initial condition $z_1(0) = 1$ and $z_2(0) = 0$. Note that since P is strictly positive, we will not divide by zero. So, the system is well defined. The nullclines of system (5.5) are $z_1 = \frac{1}{P+1}$ and $z_2 = 1 - z_1$, hence the steady state of the pH Model is $\mathrm{z}_* = \left(\frac{1}{P+1}, \frac{P}{P+1}\right)^\top$.

We can solve the system by explicitly using the eigenvalue-eigenvector method of finding solutions described by Braun [2] chapter 3.8. The characteristic polynomial of system (5.4) is

$$\det(\lambda I_2 - B) = \begin{vmatrix} \lambda + 1 & -\frac{1}{P} \\ -1 & \lambda + \frac{1}{P} \end{vmatrix} = (\lambda + 1)(\lambda + \frac{1}{P}) - \frac{1}{P} = \lambda^2 + (1 + \frac{1}{P})\lambda$$

hence the eigenvalues are $\lambda_1 = 0$ and $\lambda_2 = -(1 + \frac{1}{P})$. According to Braun [2] chapter 4.3 Theorem 2(c), the stability of the equilibrium cannot be determined since one of the eigenvalues equals zero. The corresponding eigenvectors to the eigenvalues are $v_1 = (\frac{1}{P}, 1)^\top$ and $v_2 = (-1, 1)^\top$. With the initial condition from equation (5.4) and these eigenvalues and eigenvectors we find the solution of (5.4):

$$z(t) = \left(\tfrac{1}{1+P}, \tfrac{P}{1+P}\right)^\top + e^{-(1+\frac{1}{P})t}\left(\tfrac{P}{1+P}, -\tfrac{P}{1+P}\right)^\top$$

The calculations for determining this solution can be found in Appendix B.

5.2 Numerical Simulation pH Model

The goal of this section is to examine the transport of drugs into a cell without considering the intermediate steps to pass the membrane. The only parameter we use for this model is the partition coefficient (P). Later we will compare this model to the Basic Model so we will use the same value as for the Basic Model, i.e., $P = 8000$. Thereby we will assume that the total concentration drugs is $c_t = 20\mu\text{g ml}^{-1}$, however note that we do not need this value for the simulation of the dimensionless system.

Recall from the section above that the stability of the steady state cannot be determined since one of the eigenvalues is zero. However, if we observe figure 15 and let the time t be very large, then the graphs seem to remain constant after a period of time. So we assume that the equilibrium is at least locally stable. Now we are interested in the time that equilibrium is reached and in the partition of the concentration drugs at steady state. These results can be compared to the Basic Model, which is done in section 7.

The partition of the concentration drugs over 10 time points is represented in figure 15. Since the sum of the corresponding differential equations equals one, they are inverses of each other. Recall that the ratio of the concentration in these constants is defined by the partition coefficient P. Hence we want to see that for the partition coefficient of doxorubicin z_1 converges to $\frac{1}{P} = \frac{1}{8000}$, if we consider the partition coefficient found by Alves et al. [1]. Since this value is this small it is hard to see that z_1 indeed converges to $\frac{1}{8000}$. Therefore we will look at a zoomed in plot, see figure 16, and see that z_1 indeed converges to this value as z_2 converges to one. The Matlab code for this numerical simulation can be found in Appendix A.

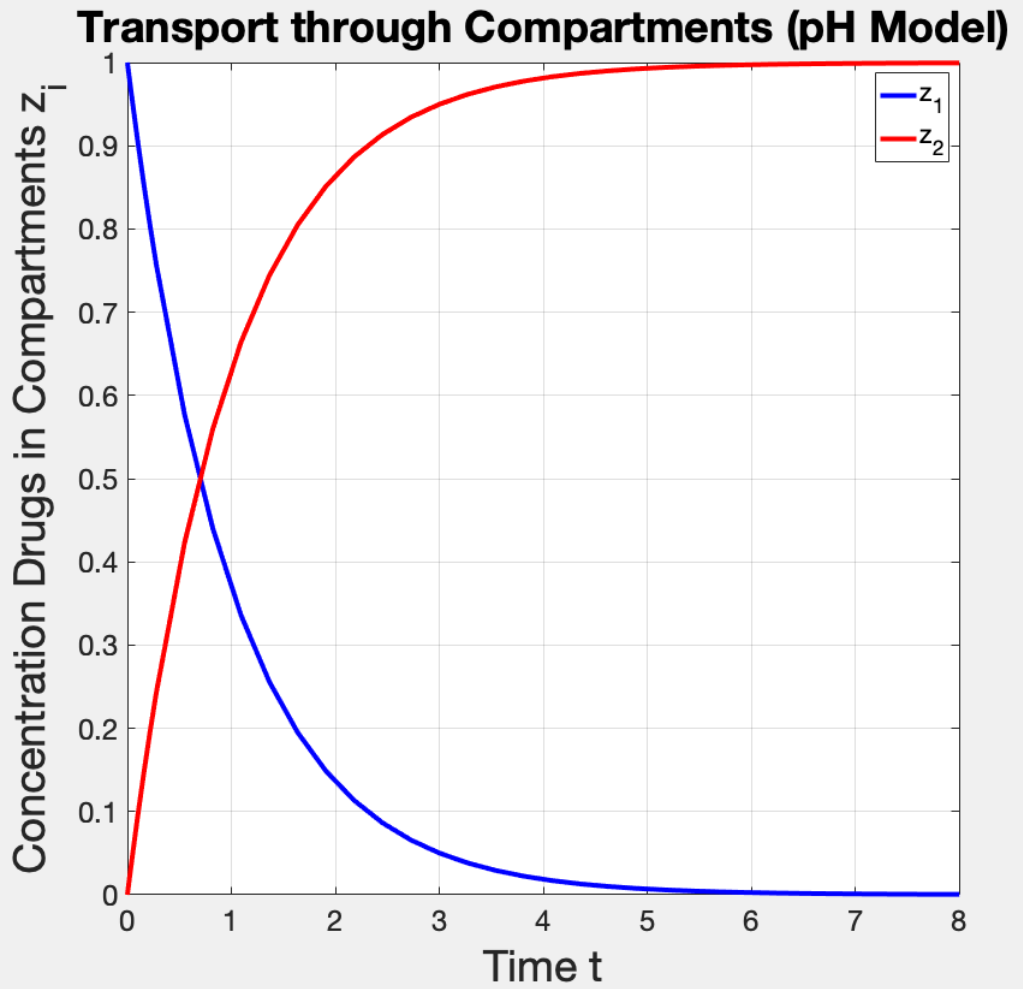


Figure 15: **In equilibrium, the drugs concentration is mainly in the cytoplasm.** Each of the graphs in the plot represent the compounds' concentration at a certain time in the various compartments. For this plot we used $P = 8000$, from Alves et al. [1] and simulated in Matlab.

Figure 16: **In equilibrium, the concentration drugs outside the cell is $\frac{1}{P}$ times the concentration in the cytoplasm.** We zoom in at the original plot by ranging the concentration drugs from 0 to $3 \cdot 10^{-4}$ to receive a better picture.

5.2.1 Interpretation Numerical Analysis pH model

The course of these graphs can be explained by the fact that at the start of the process the compartment outside the cell contains the total concentration drugs. Since the partition coefficient we chose for this numerical simulation is rather large, the concentration drugs moves relatively quick into the cell. As the compound moves into the cell, the concentration drugs in the cytoplasm will rise until the maximum concentration it can obtain is reached. Since multiple sources use other values for the partition coefficient of doxorubicin, we have little doubt if this partition coefficient is representative enough. Therefore, we calculated the equilibrium values and the times equilibrium was reached for multiple P values.

5.3 Sensitivity Analysis pH Model

Since we simulated the dimensionless system of the pH Model, there is only one parameter we can analyze in this section. This parameter would be the partition coefficient of the compound doxorubicin. Now we examine the model where we only observe the partition of the compartments outside the cell and the cytoplasm. Therefore, we are interested in the time that equilibrium is reached and how the compound is distributed over these two compartments. As mentioned above, we are not sure which partition coefficient to use since multiple sources found or used different values for the partition coefficient. Recall that the system (5.3) contains the parameter $P_{diff} = 10^{0.6316 \times \log D_{7.4} - 0.3143}$. This one is dependent on the partition coefficient measure of lipophilicity. According to Leedale et al. [9] the value of $\log D_{7.4}$ ranges between 1 and 5 meaning that the partition coefficient could range between values of 10 and 10^5. Hence, we investigate values for the partition coefficient within this range. We expect that for larger P the equilibrium will be reached earlier than for smaller P since the compound has higher lipophilicity. Hence the drug moves quicker through the membrane into the cell and equilibrium will be reached sooner. In table 6 we see the time equilibrium was reached and the corresponding partition of the compound's concentration in both compartments at steady state.

Table 6: Partition coefficient values of doxorubicin and the required time to reach equilibrium with the corresponding partition of the drug at steady state, we denote this time with t_{eq}.

P	t_{eq}	**Equilibrium**
10	10	$(0.0909, 0.9090)$
100	10	$(0.0099, 0.9901)$
1000	18	$(0.0010, 0.9990)$
$1 \cdot 10^4$	20	$(0.0001, 0.9999)$
$1 \cdot 10^5$	24	$(0.00001, 0.99999)$

In contradiction to our expectations we see in figure 17, that for smaller P the time that the system reaches equilibrium is less than the time for larger P. This is probably caused by the partition of the compound over both compartments. In table 6 we see that the larger P is the smaller will the concentration be outside the cell and the larger it is in the cytoplasm. Because it takes longer to reach such low or high concentrations in the compartments outside the cell and in the cytoplasm respectively, the time that the system is at steady state is more than the time needed for smaller P. For smaller P the drug is more distributed, hence the time that equilibrium is reached is less than the time for larger P. The Matlab code can be found in Appendix A.

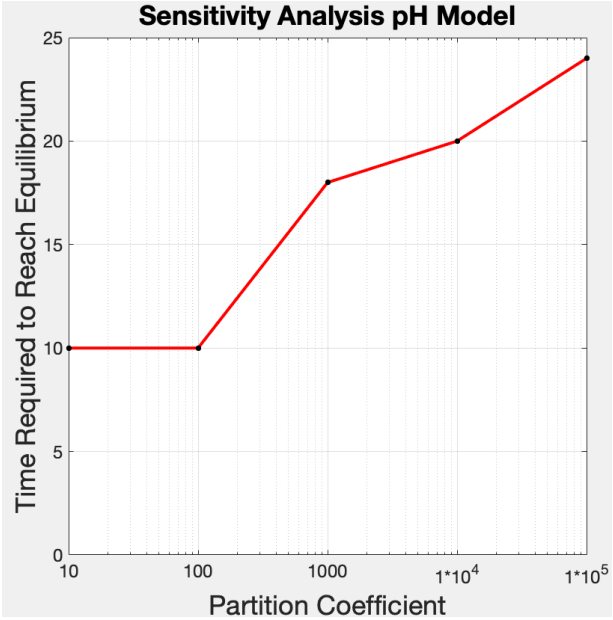


Figure 17: **For increasing partition coefficient values the time required to reach equilibrium increases.** We plot the required time to reach the steady state against different values of P ranging from 10 to 10^5 with increments of factors of 10. The red line represents the trend line between the points.

6 Expanded pH Model

We will also extend this model to analyze it and compare it to the Expanded Model. These certain characters of these models will be compared such as the timescale and the time half the total concentration of receptors is bound.

Note that the equations for the compartments of the nucleus, mitochondria, free- and bound receptors are exactly the same as the corresponding equation in the system of the Expanded Model (4.1). The only changes here are that we do not have equations for the outer- and inner leaflet. This is because we do not consider the membrane in this model. In addition, we substitute the equations we developed in the pH model by the equations for the compartments outside the model and the cytoplasm, see the system given in equation (5.4). We call this system the "Expanded pH Model".

$$\begin{cases} \frac{dc_o}{dt} = \frac{WP_{diff}}{10^9 V_{cell}}(-c_o + \frac{1}{P}c_c) \\ \frac{dc_c}{dt} = \frac{WP_{diff}}{10^9 V_{cell}}(c_o - \frac{1}{P}c_c) - (R_{c,n} + R_{c,m})c_c + R_{n,c}c_n + R_{m,c}c_m \\ \frac{dc_n}{dt} = R_{n,c}c_c - (R_{n,c} + K_{on}r_n)c_n + K_{off}b_n \\ \frac{dc_m}{dt} = R_{c,m}c_c - R_{m,c}c_m \\ \frac{dr_n}{dt} = -K_{on}r_n c_n + K_{off}b_n \\ \frac{db_n}{dt} = K_{on}r_n c_n - K_{off}b_n \end{cases} \tag{6.1}$$

With initial condition $c_o(0) = c_{o(0)}, c_c(0) = c_n(0) = c_m(0) = 0, r_n = r_{n(0)}, b_n(0) = 0$.
We define the total concentration drugs as $C_t := c_o + c_c + c_n + c_m + b_n$ and the total concentration receptors in the nucleus by $r_{nt} := r_n + b_n$. We normalize these variables to lose the dimensions as follows:

$$u_1 := \frac{c_o}{C_t} \qquad u_2 := \frac{c_c}{C_t} \qquad u_3 := \frac{c_n}{C_t} \qquad u_4 := \frac{c_m}{C_t} \qquad u_5 := \frac{r_n}{r_{nt}} \qquad u_6 := \frac{b_n}{C_t}$$

We scale the time t such that $t = t_0\hat{t}$ where $t_0 = \frac{10^9 V_{cell}}{WP_{diff}}$ as before. If we define $V := \frac{10^9 V_{cell}}{WP_{diff}}$ then we receive the following new dimensionless parameters

$$G_{c,n} := R_{c,n}V \qquad G_{n,c} := R_{n,c}V \qquad G_{c,m} := R_{c,m}V$$
$$G_{m,c} := R_{m,c}V \qquad H_{on} := K_{on}r_{nt}V \qquad H_{off} := K_{off}V$$

By substitution of the new defined variables and parameters in equation (6.1) we receive a dimensionless system.

$$\begin{cases} \frac{du_1}{d\hat{t}} = -u_1 + \frac{1}{P}u_2 \\ \frac{du_2}{d\hat{t}} = u_1 - \frac{1}{P}u_2 - (G_{c,n} + G_{c,m})u_2 + G_{n,c}u_3 + G_{m,c}u_4 \\ \frac{du_3}{d\hat{t}} = G_{n,c}u_2 - (G_{n,c} + H_{on}u_5)u_3 + H_{off}u_6 \\ \frac{du_4}{d\hat{t}} = G_{c,m}u_2 - G_{m,c}u_4 \\ \frac{du_5}{d\hat{t}} = -\frac{C_t}{r_{nt}}H_{on}u_5u_3 + \frac{C_t}{r_{nt}}H_{off}u_6 \\ \frac{du_6}{d\hat{t}} = H_{on}u_5u_3 - H_{off}u_6 \end{cases} \tag{6.2}$$

As we did for the parameter B_{on} in the Expanded Model (4.5), we can eliminate the parameter H_{on}. We leave it to the reader to check the calculations. Then the final dimensionless system of the Expanded pH Model is presented by the system in equation (6.3).

$$\begin{cases} \frac{du_1}{d\hat{t}} = -u_1 + \frac{1}{P}u_2 \\ \frac{du_2}{d\hat{t}} = u_1 - \frac{1}{P}u_2 - (G_{c,n} + G_{c,m})u_2 + G_{n,c}(1 - u_1 - u_2 - u_4 - u_6) + G_{m,c}u_4 \\ \frac{du_4}{d\hat{t}} = G_{c,m}u_2 - G_{m,c}u_4 \\ \frac{du_6}{d\hat{t}} = (1 - \frac{C_t}{r_{nt}}u_6)(1 - u_1 - u_2 - u_4 - u_6) - H_{off}u_6 \\ u_1 + u_2 + u_3 + u_4 + u_6 = 1 \\ u_5 + \frac{C_t}{r_{nt}}u_6 = 1 \end{cases} \tag{6.3}$$

With initial condition $u_1(0) = u_5(0) = 1$ and $u_2(0) = u_3(0) = u_4(0) = u_6(0) = 0$.
Because this system is nonlinear we will not solve it explicitly.

6.1 Numerical Analysis Expanded pH Model

The aim of performing this analysis is because we want to examine the time doxorubicin needs to force a tumor cell to apoptosis. Also, here we assume that when half the total concentration receptors is bound, then the cell dies. Again for the partition coefficient (P) we will use the one within the range of possible partition coefficients found by Alves et al. [1]. Note that this P also is the partition corresponding to a pH of 7.4 which is the pH we want to investigate in this simulation. The reason we assume this pH is because this value is physiologically relevant, according to Leedale et al. [9]. All the adsorption rates and desorption rates will have the same values as we used in the simulation of the Expanded Model, see section 4.2. Then we also need the molecular weight (W) of doxorubicin which is by definition equal to 543.52 g mol^{-1}. Recall that $P_{diff} = 10^{0.6316 \times \log D_{7.4} - 0.3143}$, so we need to know the values of $\log D_{7.4}$ which is a partition coefficient measure. Since $P = 8000$, we have $\log D_{7.4} = \log(8000) = 3.9$, thus $P_{diff} = 142\mu$l min^{-1} 10^{-6}. According to Menochet et al. [10] the volume of one cell is $V_{cell} = 3.9\mu$l per 10^6 cells. Hence, $V = 0.05$ mol min g^{-1}. Recall from the previous section that the scaled variables are found by multiplying V with the adsorption and desorption rates we use for this model. For both the total concentrations of the drugs and the receptors we will use 20μg ml^{-1} as we did for the Expanded Model, so that the comparison between this model is credible. The exact values we use can be found in table 7.

Table 7: Parameter values of doxorubicin of the dimensionless system of the Expanded pH Model

Parameters	**Values**
P	8000
$G_{c,n}$	$1.6 \cdot 10^5$
$G_{n,c}$	20
$G_{c,m}$	$2.0 \cdot 10^5$
$G_{m,c}$	25
H_{off}	0
C_t (μg ml^{-1})	20
r_{nt} (μg ml^{-1})	20

By simulating these values numerically, we can analyze the distribution of the compound's concentration in the various compartments of the cell. These values will be observed until equilibrium is (almost) reached. Also, this system is nonlinear so we cannot say much about the stability of the steady state. As for the Expanded Model we want to investigate the time that half a concentration of bound receptors is reached. The plot, see figure 18 we receive from the simulation will be compared to the plot of the Expanded Model, see figure 10. The Matlab code for this simulation can be found in Appendix A.

Let us explain what happens to each of the graphs in figure 18 one by one. The graph corresponding to x_1 decreases extremely fast to at the beginning of the process and converges to zero as t goes to infinity. The line x_2 practically remains constantly zero in the course of time. In the following graph we observe a fast increase until it reaches a maximum and decreases slowly to zero. The same happens to the graph x_4, which lies exactly on top of x_3. Then x_7 drops relatively fast at the beginning and later decreases at a slower rate until it converges to zero. The graph x_8 is the inverse of x_7, meaning that it increases fast at the start of the process and later still increases but at a slower pace until equilibrium is reached.

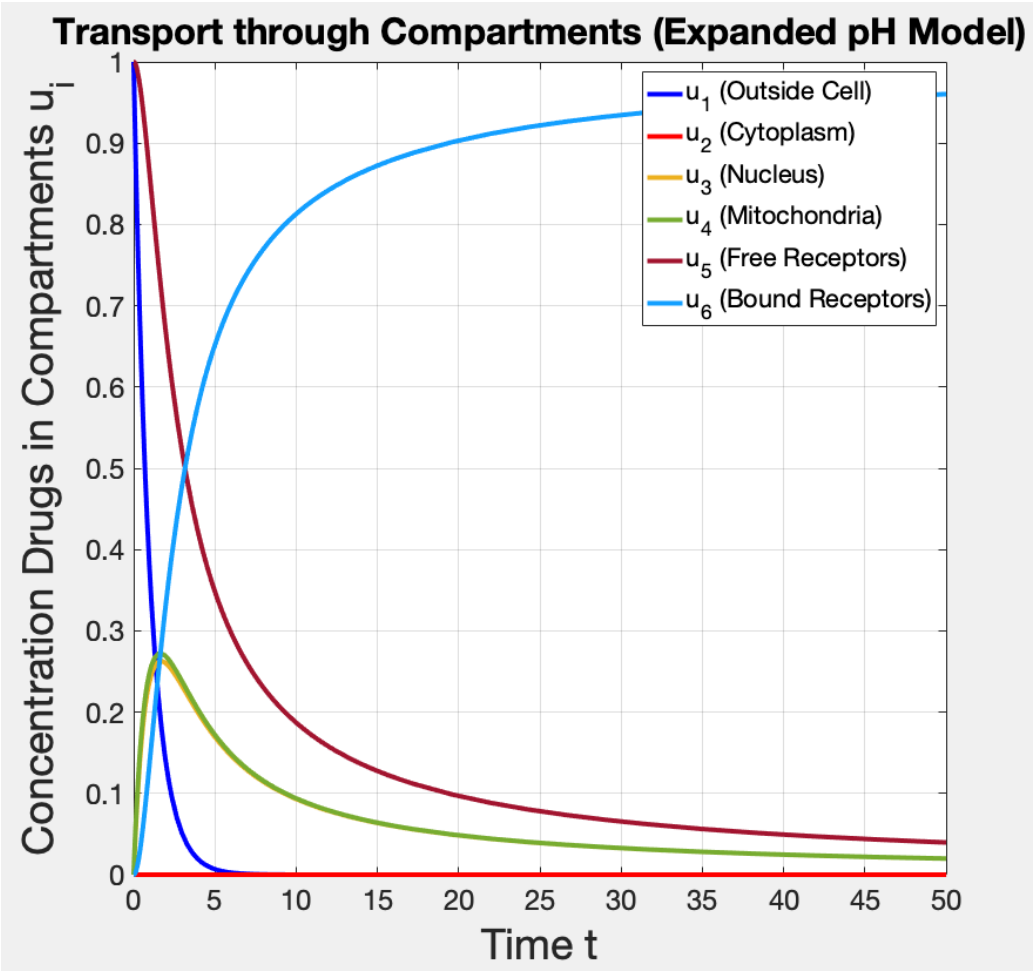


Figure 18: **The time point to reach half a concentration of bound receptors is 2.1.** Each graph represents the change in compounds' concentration over time in the various compartments. For this plot we used the values from table 8 and simulated them in Matlab.

6.1.1 Interpretation Numerical Analysis Expanded pH Model

Since the total concentration is outside the cell and then moves into the cell, the graph x_1 decreases. When most of the concentration has moved into the cell, we can see that it takes longer for the drugs to move into the cytoplasm. Because the compound has such high affinity to membranes, it will immediately move from the cytoplasm to either the nucleus or the mitochondria. Hence x_2 which corresponds to the concentration in the cytoplasm remains to be zero. Thereby, we see that the graphs corresponding to the nucleus and mitochondria increase. The reason that the concentration of free receptors decrease is because the drug binds to them and forms bound receptors which increase at the same rate as the concentration free receptors decreases. Because the number of bound receptors increases, the concentrations in the nucleus and mitochondria will drop over the course of time until they are left without any of the concentration of the compound. The reason that the concentration of bound receptors increase until it contains the total concentration bound receptors is because we assume that the drug cannot unbind from the DNA anymore. If we suppose that a small change of the drug moves away from the bound receptors again, then we will see that the total concentration will be divided over the several compartments depending on the chosen binding off rate. Also note that in this case we assume the total concentration drugs to be the same as the total concentration of receptors. Therefore, we see that all the drugs bind to the receptors, hence the concentration of free receptors becomes zero. In the case that the total concentration of free receptors is higher than the total concentration drugs we see that all the drugs are bound to the receptors, but there also are some free receptors in the nucleus left. When the total concentration of receptors is higher than that of the drugs than all the receptors will be bound at the end, but the drugs will also be included in one of the other compartments that are not part of the receptors.

6.2 Sensitivity Analysis Expanded pH Model

Recall from section 5 that $D_{7.4}$ is a partition coefficient measure at a certain pH value. So, for simplicity we used a pH value that is physiologically relevant to perform the simulations of the model. However, in fact the acidity in the several compartments may differ. For example outside the cell the pH value will be around 7, that of the cytoplasm has a pH of 7.5 and in the mitochondria it will have a pH value around 8 [18]. This indicates that in these several compartments, the compound we analyze has different pH values in the course of time. Also recall that only the uncharged molecules of the compound will penetrate the membrane and move into the cell. According to Eytan and Kuchel [5] doxorubicin has a pH value of 8.6 and at this value the drug is mainly uncharged. Hence the movement of doxorubicin into the cell will probably be much faster at this pH value since there are more molecules to cross the membrane. So, we have done some literature studies to find the corresponding partition coefficients of the pH values. Nevertheless, it turned out that there is not a lot of information in literature about these values. Eytan and Regev did find some partition coefficients for several pH values [6]. However, note that these values are very different from the partition coefficient we used for the rest of our simulations. The pH values with the corresponding partition coefficients and the time that half the concentration of bound receptors is reached can be found in table 8.

Table 8: pH values with corresponding partition coefficient values of doxorubicin and the required time to reach half a concentration of bound receptors, we denote this time with $t_{\frac{1}{2}}$.

pH	P	$t_{\frac{1}{2}}$	**Source**
7.4	8000	2.1	[1]
7.5	1	5	[6]
8.6	8	2.35	[6]
9.7	20	2.2	[6]

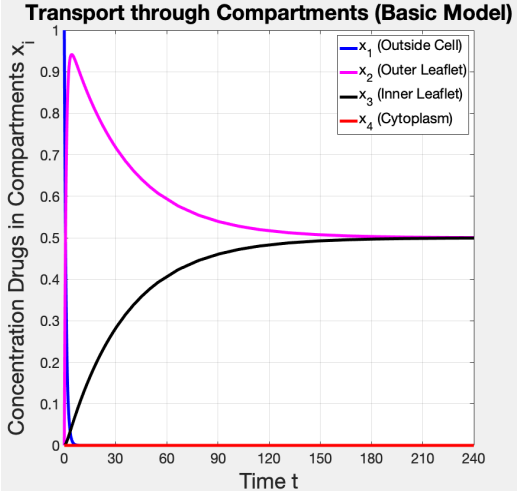
In table 8 we see that for lower pH values the time to force a tumor cell to apoptosis is more than for larger pH values. This can be explained by the fact that the compound doxorubicin moves faster within an area that has the same acidity as the compound itself. Also note that if we compare the time that half the concentration of receptors is bound between the pH values of 7.4 and 9.7 the difference in time is very small even though the corresponding partition coefficients differ greatly from each other. From this we can conclude that also for relatively small values of P, the time the compound needs to kill a tumor cell will almost be the same as for very large P values. This means that if the actual partition coefficient at the various pH values would all be greater than 20, we would not see a lot of difference in the time that half the concentration of bound receptors is reached. However, if the partition coefficients are smaller than 20, then we do observe some small difference. Therefore it is important to find out what the exact values of the partition coefficients are to the corresponding pH values.

7 Results

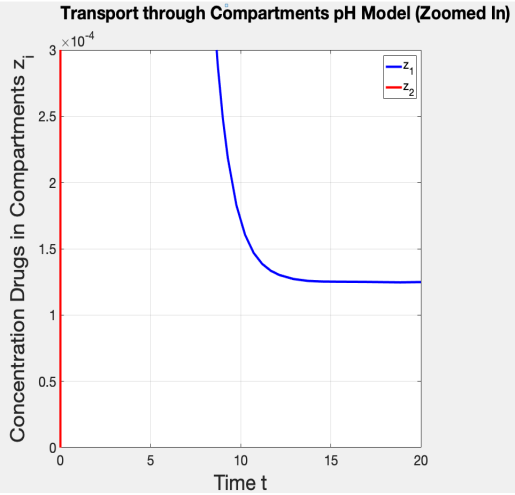
The aim of this study is to find out which of the models considering intermediate steps to cross the membrane or not, gives more accurate results when we compare them with experimental research. Therefore, we analyzed four different models. The first model shows the movement of drug transport into the cell that uses two intermediate steps to cross the membrane and arrive at the cytoplasm. To clearly understand what happens in this process we left out transport into the organelles that are inside the cell and DNA binding. In the second model these are implemented to obtain a better idea of the behavior of some compound inside a tumor cell. The next model describes the movement of a compound into a cell that does not consider intermediate steps to cross the membrane. In this model the compartments representing organelles as well as DNA binding are not included. These features have been added in the fourth and final model. We will now compare the first and third models and the second and fourth models with each other, to investigate what the exact difference are between these models.

Let us take a look at the figures 19a and 19b. In the first one of these figures the time the system needs to reach steady state is about 240 time points. Note that we do not express units for the time, since we observe the dimensionless system of both models. However, if we observe the second plot corresponding to the pH model, then we see that the equilibrium is already reached after about 15 time points, see figure 16. We can also see a clear difference in the equilibria of both systems. Namely, the steady state of the first model shows that most of the total concentration of the drug will be obtained by the membrane and the concentration outside the cell and in the cytoplasm is relatively small. In the pH model we see that again there is only a very small concentration drug left in the compartment outside the cell. Though, the concentration drugs in the nucleus contains almost the total concentration of the compound which was introduced at the beginning of the process.

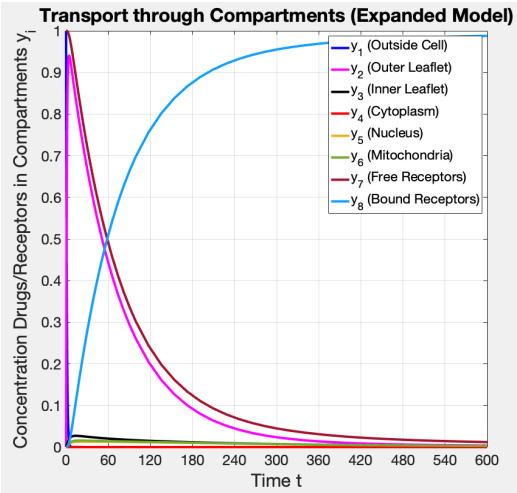
Now we will examine the differences between the expanded models. The time that the system of the Expanded Model reaches equilibrium is almost 30 times higher than that of the Expanded pH Model. This difference is not clearly visible in figures 19c and 19d, because if we would look at a greater range of time then we would not be able to observe the more important changes in the distribution of the drugs at the beginning of the process very well. We are more interested in the time that half a concentration of bound receptors is reached. In figure 19c we see that this time is about 59 time points for the Expanded Model. This time is much higher than the time the Expanded pH Model needs to force the tumor cell to apoptosis, namely 2.1 time points as can be seen in figure 19d. However, note that the compound's concentration outside the cell in both plots decrease extremely fast at the very beginning of the process and that for both models the concentration of the drugs in the cytoplasm practically remains zero in the course of time. On the other hand, we see that the maxima of the nucleus and the mitochondria, before they slowly converge to zero in both cases, are lower in the Expanded Model than in the Expanded pH Model. Though, the partition of the compound over the compartments is again the same for both models. The bound receptors contain the total concentration drugs, and the other compartments contain nothing of the compound's concentration.



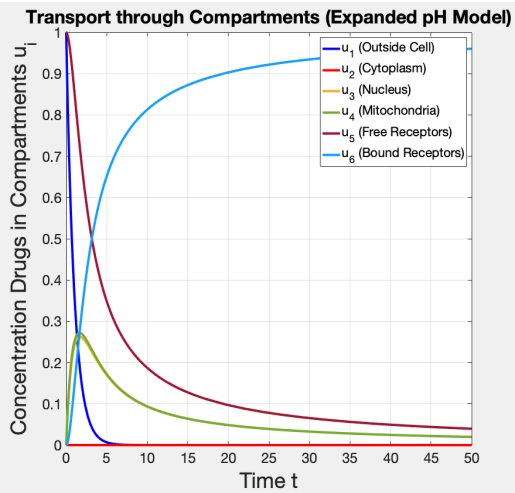
(a) The time required to reach equilibrium is 240.



(b) The time required to reach equilibrium is 15.



(c) The time required to reach half a concentration of bound receptors is 59.



(d) The time required to reach half a concentration of bound receptors is 2.1.

Figure 19: **The Basic Model requires more time to reach equilibrium than the pH Model and the Expanded model requires more time to reach half a concentration of bound receptors than the Expanded pH Model.**

8 Discussion & Conclusion

To obtain a better understanding of drug uptake by a cell is very important for pharmacy. The research aim of this thesis is develop two types of models considering different ways to show drug transport into and inside a tumor cell. We will compare them with each other to find out which type gives a better interpretation of reality. With the right model we obtain a better idea of the behavior of the compound in and around cells. If the model gives a suitable prediction of the movements of the drug, it can be used to improve the development of drugs for cancer treatment.

We know from earlier research that the pH model does not match with the research experiments, since the equilibrium is reached to soon if we consider this model. Hence, we can conclude from the results of the previous section that the Basic Model will give a better indication of the time the drug needs to move into the cell. This finding is exactly in line with our expectations.

There is a possibility that our results may differ from reality since we are not sure what value to use for the partition coefficient of doxorubicin. This is caused by the fact that different values have been found for the partition coefficient from different literature studies. For example Alves et al. [1] mention that the partition coefficient must lie within a range of 8195 ± 406 for a pH of 7.4. On the other hand, according to Eytan and Regev [6] the partition coefficients for a pH of $7.5, 8.6$ and 9.7 are $1, 8$ and > 20 respectively. These values obvious differ a lot from the partition coefficient found by Alves et al. Thereby, Menochet et al. [11] show partition coefficients for other compounds which are all within a range between 0 and 1, which makes us wonder if 8000 would be the right choice for the partition coefficient. It would be interesting to find out which value for the partition coefficient of doxorubicin is most applicable to represent the drug's transport into a tumor cell.

Also note that we "guessed" a lot of the parameter used in the expanded models, since literature does not provide the values, we would have wanted to use for our simulations. Examples of the parameters are the adsorption and desorption rates of the nucleus and the mitochondria. Although, we are not completely sure of the values we used for our simulations, it follows from the sensitivity analyses that changing the values in most cases does not lead to major differences in the model we have developed. Hence both the Basic Model and the Expanded Model that consider two intermediate steps to cross the membrane will give a strong interpretation of the movements of a compound into and inside a cell. So, these results implicate that the Basic and Expanded Model can be used by researchers to investigate the behavior of different compounds without having to conduct experiments.

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A Matlab Code

Parameter Class

```
classdef M
    properties(Constant = true)
        %initial conditions
        initial_con = [1 0 0] %dimensionless system Basic Model
        initial_vol = [20 0 0] %dimensionfull system Basic Model
        init_con = [1 0 0 0 0 0] %dimensionless system Expanded Model
        init_vol = [20 0 0 0 0 0 20 0] %dimensionfull system Expanded Model

        %Parameter values dimensionfull systems
        R_oom = 1e2 %(1 / 0.01 min), assumption
        R_omo = 1.25e-2 %(R_oom / P), Eytan
        f_12 = 1.43 %(1 / 0.7 min), Eytan
        f_21 = 1.43 %(1 / 0.7 min), Eytan
        R_imc = 1e2 %(R_imc = R_oom), Eytan
        R_cim = 8e5 %(R_imc * P), Eytan

        R_cn = 3.2e6 %(R_cim * 4), assumption
        R_nc = 4e2 %(R_cn / P), assumption
        R_cm = 4e6 %(R_cim * 5), assumption
        R_mc = 5e2 %(R_cm / P), assumption
        K_on = 0.26678e6 %Schneider
        K_off= 0 %negligible

        %Parameter values dimensionless systems
        P_o = 1.25e-4 %(R_omo / R_oom)
        I_1 = 1.43e-2 %(F_12 / R_oom)
        I_2 = 1.43e-2 %(F_21 / R_oom)
        J_imc = 1 %(R_imc / R_oom)
        J_cim = 8e3 %(R_cim / R_oom)
        P_c = 1.25e-4 %(R_imc / R_cim)

        J_cn = 3.2e4 %(R_cn / R_oom)
        J_nc = 4 %(R_nc / R_oom)
        J_cm = 4e4 %(R_cm / R_oom)
        J_mc = 5 %(R_mc / R_oom)
        B_on = 0.26678e6*20/1e2 %(K_on * r_ntot / R_oom)
        B_off =0 %(B_off / R_oom)

        %Parameter values pH model
        V_cell = 3.9e-6 %Menochet
        logD = 3.9 %for partition coefficient 8000 at 7.4, Alves
        P_diff = 10^(0.6316*3.9-0.3143) %Leedale
        P = 8000 %Alves
        W = 543.52 %Molecular Weight Doxorubicin
        V = 0.05 %(10^9*V_cell)/(W*P_diff)
        G_cn = 1.6e5 %(V * R_cn)
        G_nc = 20 %(V * R_nc)
        G_cm = 2e5 %(V * R_cm)
        G_mc = 25 %V * R_mc)
        H_off = 0 %(V * K_off)

```

```
        t0 = 0 %starting time of both models
        t_maxi = 2 %end time dimensionfull system Basic Model
        t_max = 240 %end time dimensionless system Basic Model
        T_maxi = 10 %end time dimensionfull system Expanded Model
        T_max = 600 %end time dimensionless system Expanded Model


        c_tot = 20 %total concentration drugs Basic Model
        C_tot = 20 %total concentration drugs Expanded Model
        r_ntot = 20 %total concentration receptors Expanded Model
        c_t = 20; %total concentration drugs pH Model
        C_t = 20; %total concentration drugs Expanded pH Model
        r_nt = 20; %total concentration recptors Expanded pH Model
    end
end
```

Code System Basic Model

```
%dimensionfull system of ODEs for Basic model
dxdt = @(t,x) [(-(M.R_oom * x(1)) + M.R_omo * x(2));
    (M.R_oom * x(1) - (M.R_omo + M.f_12) * x(2) + M.f_21 * x(3));
(M.f_12 * x(2) - (M.f_21 + M.R_imc) * x(3) + M.R_cim * (M.c_tot-x(1)-x(2)-x
    (3)))];

%dimensionless system of ODEs for Basic Model
dxdt = @(t,x) [(-x(1) + M.P_o*x(2));
    (x(1) - (M.P_o+M.I_1)*x(2)+ M.I_2*x(3));
    (M.I_1*x(2) - (M.I_2+M.J_imc)*x(3) + M.J_cim*(1-x(1)-x(2)-x(3)))];

%plotting the ode
[t,xa] = ode15s(dxdt,[M.t0 M.t_max], M.initial_con);
plot(t,xa(:,1))
hold on
plot(t,xa(:,2))
plot(t,xa(:,3))
plot(t,1-xa(:,1)-xa(:,2)-xa(:,3))
hold off
title('Transport through Compartments (Basic Model)')
legend('x_1 (Outside Cell)','x_2 (Outer Leaflet)','x_3 (Inner Leaflet)','
    x_4 (Cytoplasm)')
xlabel('Time t')
ylabel('Concentration Drugs in Compartments x_i')
grid on
axis([M.t0 M.t_max 0 1], 'square')
```

Code Solution Basic Model

```
%values of the solution corresponding to values used for the numerical
    simulation of the Basic Model
eul = vpa(exp(sym(1)),25); %Euler's number
%eigenvalues
lambda_1 = -8001;
lambda_2 = -1;
lambda_3 = -0.029;
%eigenvectors
```

```
vector1=[0;0;1];
vector2=[68.021; -69.021; 1];
vector3=[0;-1;1];
%constants of solution
gamma1 = 6.2e-5;
gamma2 = 0.014700388;
gamma3 = -0.514699088;
%particular solution
beta = [0.000062; 0.499938; 0.499938];

%solution functions for dimensionless system Basic Model
gsol1 = @(t) gamma1*eul.^(lambda_1*t).*vector1(1)+ gamma2*eul.^(lambda_2*t)
    .*vector2(1)+gamma3*eul.^(lambda_3*t).*vector3(1)+beta(1);
gsol2 = @(t) gamma1*eul.^(lambda_1*t).*vector1(2)+ gamma2*eul.^(lambda_2*t)
    .*vector2(2)+gamma3*eul.^(lambda_3*t).*vector3(2)+beta(2);
gsol3 = @(t) gamma1*eul.^(lambda_1*t).*vector1(3)+ gamma2*eul.^(lambda_2*t)
    .*vector2(3)+gamma3*eul.^(lambda_3*t).*vector3(3)+beta(3);

%plotting the graphs of the solution
fplot(@(t) gsol1(t),[M.t0 M.t_max])
hold on
fplot(@(t) gsol2(t),[M.t0 M.t_max])
fplot(@(t) gsol3(t),[M.t0 M.t_max])
fplot(@(t) 1-gsol1(t)-gsol2(t)-gsol3(t),[M.t0 M.t_max])
hold off
title('Solution of Basic Model')
legend('Solution x_1', 'Solution x_2', 'Solution x_3', 'Solution x_4')
xlabel('Time t')
ylabel('Concentration Drugs in Compartments x_i')
grid on
axis([M.t0 M.t_max 0 1], 'square')
```

Code Sensitivity Analysis Basic Model

```
%partition coefficient values and corresponding maximum times and values x2
Q = [8 80 800 8000 8*10^4];
timeQ = [4.0786753; 4.3328209; 4.3446344; 4.3451717; 4.3452341];
concQ = [0.843586; 0.931119; 0.939961; 0.940542; 0.940597];
%scatter plot and trendline
hold on
yyaxis left
plot(Q,timeQ)
scatter(Q,timeQ)
title('Sensitivity Analysis Basic Model')
xlabel('Partition Coefficient')
ylabel('Time Required to Reach Maximum Value of x_2')
yyaxis right
plot(Q,concQ)
scatter(Q,concQ)
ylabel('Concentration at Time Required to Reach Maximum Value of x_2')
set(gca, 'Xscale', 'log','XTick', Q, 'XTickLabel', Q)
xticks([0.8 8 80 800 8*10^3 8*10^4])
xticklabels({'0.8', '8', '80','800', '8000','8*10^4'})
hold off
grid on
axis('square')
```

Code System Expanded Model

```
%dimensionfull system of ODEs for Expanded Model
dy2dt = @(t,y) [(-M.R_oom*y(1)+M.R_omo*y(2));
    (M.R_oom*y(1)-(M.R_omo+M.f_12)*y(2)+M.f_21*y(3));
    (M.f_12*y(2)-(M.f_21+M.R_imc)*y(3)+M.R_cim*y(4));
    (M.R_imc*y(3)-(M.R_cim+M.R_cn+M.R_cm)*y(4)+M.R_nc*y(5)+M.R_mc*y(6));
    (M.R_cn*y(4)-(M.R_nc+M.K_on*y(7))*y(5)+M.K_off*y(8));
    (M.R_cm*y(4)-M.R_mc*y(6));
    (-M.K_on*y(7)*y(5)+M.K_off*y(8));
    (M.K_on*y(7)*y(5)-M.K_off*y(8))];

%dimensionless system of ODEs for Expanded Model
dy1dt = @(t,y) [(-y(1) + M.P_o*y(2));
    (y(1) - (M.P_o+M.I_1)*y(2) + M.I_2*y(3));
    (M.I_1*y(2) - (M.I_2+M.J_imc)*y(3) + M.J_cim*y(4));
    (M.J_imc*y(3) - (M.J_cim + M.J_cn + M.J_cm)*y(4) + M.J_nc*(1-y(1)-y(2)-
        y(3)-y(4)-y(5)-y(6)) + M.J_mc*y(5));
    (M.J_cm*y(4) - M.J_mc*y(5));
    ((1-y(6)*M.C_tot/M.r_ntot)*(1-y(1)-y(2)-y(3)-y(4)-y(5)-y(6))- M.B_off*y
        (6))];

%plotting the ode
[t,yb] = ode15s(dy1dt,[M.t0 M.T_max],M.init_con);
plot(t,yb(:,1))
hold on
plot(t,yb(:,2))
plot(t,yb(:,3))
plot(t,yb(:,4))
plot(t,1-yb(:,1)-yb(:,2)-yb(:,3)-yb(:,4)-yb(:,5)-yb(:,6))
plot(t,yb(:,5))
plot(t,1-(M.C_tot/M.r_ntot)*yb(:,6))
plot(t,yb(:,6))
hold off
title('Transport through Compartments (Expanded Model)')
legend('y_1 (Outside Cell)','y_2 (Outer Leaflet)','y_3 (Inner Leaflet)','
    y_4 (Cytoplasm)','y_5 (Nucleus)','y_6 (Mitochondria)','y_7 (Free
    Receptors)','y_8 (Bound Receptors)')
xlabel('Time t')
ylabel('Concentration Drugs/Receptors in Compartments y_i')
grid on
axis([M.t0 M.T_max 0 1], 'square')
```

Code Sensitivity Analysis Expanded Model

```
%partition coefficient values and the corresponding required times to reach
     half a concentration of bound receptors
P= [7600 7800 8000 8200 8400 8600 8800];
timeP = [59; 59; 59; 59; 59; 59; 59;];
%scatter plot and trendline
plot(P,timeP)
hold on
scatter(P,timeP)
set(gca, 'Xscale', 'log','XTick', P, 'XTickLabel', P)
hold off
title('Sensitivity Analysis Expanded Model for P Values Alves')
```

```
xlabel('Partition Coefficient')
ylabel('Time Required to Reach Half a Concentration of Bound Receptors')
grid on
axis([7600 8800 0 M.T_max], 'square')

%partition coefficient values and the corresponding required times to reach half a concentration of bound receptors
Q = [0.8 8 80 800 8000 8*10^4];
timeQ = [125; 65; 59; 59; 59; 59];
%scatter plot and trendline
plot(Q,timeQ)
hold on
scatter(Q,timeQ)
set(gca, 'Xscale', 'log','XTick', Q, 'XTickLabel', Q)
hold off
title('Sensitivity Analysis Expanded Model for P with factors 10')
xlabel('Partition Coefficient')
ylabel('Time Required to Reach Half a Concentration of Bound Receptors')
xticks([0.8 8 80 800 8*10^3 8*10^4])
xticklabels({'0.8', '8', '80','800', '8000','8*10^4'})
yticks(0:100:M.T_max)
grid on
axis([0 80000 0 M.T_max], 'square')
```

Code Sensitivity Analysis Dimensionfull System Expanded Model

```
%multiplication factors adsorption and desorption rates and corresponding required times to reach half a concentration of bound receptors
Q = [0.01 0.1 1 10 100 1000 1*10^4];
timeQ1 = [1.45; 0.6; 0.5; 0.5; 0.5; 0.5; 0.5];
%scatter plot and trendline
plot(Q,timeQ1)
hold on
scatter(Q,timeQ1)
set(gca, 'Xscale', 'log','XTick', Q, 'XTickLabel', Q)
hold off
title('Sensitivity Analysis Expanded Model with Dimension')
xlabel('Multiplication Factor Parameters')
ylabel('Time Required to Reach Half Concentration Bound Receptors (minutes)')
xticks([0 0.01 0.1 1 10 100 1*10^3 1*10^4])
xticklabels({'0','0.01','0.1', '1', '10','100', '1000','1*10^4'})
grid on
axis([0 10000 0 5],'square')
```

Code System pH Model

```
%dimensionfull pH model
dcdt = @(t,c) [((M.P_diff)/(10^9*M.V_cell))*(-c(1)+(1/M.P)*c(2));
    (M.W*(M.P_diff)/(10^9*M.V_cell))*(c(1)-(1/M.P)*c(2))];

%solution dimensionless system
eul = vpa(exp(sym(1)),25); %Euler's number
z_1 = @(t) 1/(1+M.P)+(M.P/(1+M.P))*eul.^(-(1+1/M.P)*t);
z_2 = @(t) M.P/(1+M.P)+(-M.P/(1+M.P))*eul.^(-(1+1/M.P)*t);
```

```

%plotting the ode of the dimensionless pH Model
syms z(t)
dzdt = diff(z,1) == -z*(1+1/M.P) + 1/M.P;
cond = z(0) ==1; %initial condition pH Model
sol = dsolve(dzdt, cond);
fplot(sol,[0,8]);
hold on
fplot(1-sol,[0,8]);
title('Transport through Compartments (pH Model)')
xlabel('Time t')
ylabel('Concentration Drugs in Compartments z_i')

grid on;
axis([0 8 0 1], 'square')
```

Code Sensitivity Analysis pH Model

```
%partition coefficient values and corresponding required times to reach
    half a concentration of bound receptors
P= [10 100 1000 1e4 1e5];
timeP = [10; 10; 18; 20; 24];
%scatter plot and trendline
plot(P,timeP)
hold on
scatter(P,timeP)
set(gca, 'Xscale', 'log','XTick', P, 'XTickLabel', P)
hold off
title('Sensitivity Analysis pH Model')
xlabel('Partition Coefficient')
ylabel('Time Required to Reach Equilibrium')
xticks([10 100 1000 1*10^4 1*10^5])
xticklabels({'10', '100', '1000','1*10^4','1*10^5'})
grid on
axis([10 100000 0 25], 'square')
```

Code Dimensionless System Expanded pH Model

```
%dimensionless Expanded pH Model
dudt =  @(t,u) [(-u(1)+(1/M.P)*u(2));
    (u(1)-(1/M.P)*u(2)-(M.G_cn+M.G_cm)*u(2)+M.G_nc*(1-u(1)-u(2)-u(3)-u(4))+
       M.G_mc*u(3));
    (M.G_cm*u(2)-M.G_mc*u(3));
    ((1-M.C_t*u(4)/M.r_nt)*(1-u(1)-u(2)-u(3)-u(4))-M.H_off*u(4))];

%plotting the ode
[t,ua] = ode15s(dudt,[0 100],[1 0 0 0]);
plot(t,ua(:,1))
hold on
plot(t,ua(:,2))
plot(t,(1-ua(:,1)-ua(:,2)-ua(:,3)-ua(:,4)))
plot(t,ua(:,3))
plot(t,(1-M.C_t*ua(:,4)/M.r_nt))
plot(t,ua(:,4))
hold off
```

```
title('Transport through Compartments (Expanded pH Model)')
legend('u_1 (Outside Cell)', 'u_2 (Cytoplasm)','u_3 (Nucleus)', 'u_4 (
    Mitochondria)', 'u_5 (Free Receptors)', 'u_6 (Bound Receptors)' )
xlabel('Time t')
ylabel('Concentration Drugs in Compartments u_i')
grid on
axis([0 100 0 1], 'square')
```

B Detailed Calculations

Calculation Particular Solution β

Now, we will do extensive calculations to determine the particular solution β of the Basic Model as we did for section 3.3. In order to calculate this particular solution β, we must solve $\beta = -Df(x_*)^{-1}\left(0,0,J_{c,im}\right)^\top$.
So first we will find the inverse matrix of the Jacobian $Df(x_*)$. Note that we never divide by zero since all parameters are strictly positive.

$$\left(\begin{array}{ccc|ccc} -1 & P_o & 0 & 1 & 0 & 0 \\ 1 & -(P_o+I_1) & I_2 & 0 & 1 & 0 \\ -J_{c,im} & I_1-J_{c,im} & -(I_2+J_{im,c}+J_{c,im}) & 0 & 0 & 1 \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 1 & -(P_o+I_1) & I_2 & 0 & 1 & 0 \\ -J_{c,im} & I_1-J_{c,im} & -(I_2+J_{im,c}+J_{c,im}) & 0 & 0 & 1 \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & -I_1 & I_2 & 1 & 1 & 0 \\ -J_{c,im} & I_1-J_{c,im} & -(I_2+J_{im,c}+J_{c,im}) & 0 & 0 & 1 \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & -I_1 & I_2 & 1 & 1 & 0 \\ -J_{c,im} & -J_{c,im} & -(J_{im,c}+J_{c,im}) & 1 & 1 & 1 \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & -I_1 & I_2 & 1 & 1 & 0 \\ 1 & 1 & P_c+1 & \frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 1 & 1 & P_c+1 & \frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & P_o+1 & P_c+1 & 1+\frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} & \frac{1}{J_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & -P_o & 0 & -1 & 0 & 0 \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & P_o & P_c+1+\frac{I_2}{I_1} & 1+\frac{1}{J_{c,im}}-\frac{1}{I_1} & \frac{1}{J_{c,im}}-\frac{1}{I_1} & \frac{1}{J_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & P_o & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1J_{c,im}+I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & 1 & \frac{I_1(P_c+1)+I_2}{P_oI_1} & \frac{I_1J_{c,im}+I_1-J_{c,im}}{P_oI_1J_{c,im}} & \frac{I_1-J_{c,im}}{P_oI_1J_{c,im}} & \frac{1}{P_oJ_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & 0 & \frac{I_1(P_c+1)+I_2(P_o+1)}{P_oI_1} & \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{P_oI_1J_{c,im}} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{P_oI_1J_{c,im}} & \frac{1}{P_oJ_{c,im}} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \\ 0 & 1 & -\frac{I_2}{I_1} & \frac{1}{I_1} & \frac{1}{I_1} & 0 \\ 0 & 0 & 1 & \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & \frac{I_1(P_c+1)+I_2}{I_1} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{I_1-J_{c,im}}{I_1J_{c,im}} & \frac{1}{J_{c,im}} \\ 0 & 1 & 0 & \frac{1}{I_1}+\frac{I_2(I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{1}{I_1}+\frac{I_2(I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ 0 & 0 & 1 & \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \end{array}\right)$$

$$\rightsquigarrow \left(\begin{array}{ccc|ccc} 1 & 0 & 0 & \frac{I_1J_{c,im}(P_c+1)+I_2(P_o-J_{c,im}+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ 0 & 1 & 0 & \frac{1}{I_1}+\frac{I_2(I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{1}{I_1}+\frac{I_2(I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ 0 & 0 & 1 & \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \end{array}\right)$$

So

$$Df(x_*)^{-1} = \left(\begin{array}{ccc} \frac{I_1J_{c,im}(P_c+1)+I_2(P_o-J_{c,im}+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ \frac{1}{I_1}+\frac{I_2(I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{1}{I_1}+\frac{I_2(I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \end{array}\right)$$

Hence

$$Df(x_*)^{-1}\begin{pmatrix} 0 \\ 0 \\ J_{c,im} \end{pmatrix} = \left(\begin{array}{ccc} \frac{I_1J_{c,im}(P_c+1)+I_2(P_o-J_{c,im}+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2+P_oJ_{c,im}(P_c+1)}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{P_oI_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ \frac{1}{I_1}+\frac{I_2(I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{1}{I_1}+\frac{I_2(I_1-J_{c,im}-P_oJ_{c,im})}{I_1J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_2}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \\ \frac{I_1J_{c,im}+I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1-J_{c,im}-P_oJ_{c,im}}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} & \frac{I_1}{J_{c,im}(I_1(P_c+1)+I_2(P_o+1))} \end{array}\right)\begin{pmatrix} 0 \\ 0 \\ J_{c,im} \end{pmatrix}$$

Thus

$$\beta = \begin{pmatrix} \frac{P_oI_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{I_2}{I_1(P_c+1)+I_2(P_o+1)} \\ \frac{I_1}{I_1(P_c+1)+I_2(P_o+1)} \end{pmatrix}$$

First and Second Derivative Tests

In this part of the Appendix we will perform first and second derivative tests to substantiate the results from the solution of the Basic Model from section 3.3. The first derivative of the solution of the Basic Model is

$$x'(t) = e^{-8001t}\begin{pmatrix}0\\0\\-0.496\end{pmatrix} + e^{-t}\begin{pmatrix}-1.020\\1.035\\0.015\end{pmatrix} + e^{-0.029t}\begin{pmatrix}0\\-0.0148\\0.0148\end{pmatrix}$$

Hence, we have for the first equation the derivative $x_1'(t) = -1.020e^{-t}$. The derivative is negative for all t. Consequently, x_1 is a monotonically decreasing function with neither a minimum nor a maximum. In figure 6 we see that x_1 indeed shows this behavior.

For the second equation we derive $x_2'(t) = 1.035e^{-t} - 0.0148e^{-0.029t}$. Then the solution for $x_2'(t) = 0$ gives $t = 4.3$. According to the first derivative test we should find a minimum or maximum for this time point. Note that the derivative is positive for $t < 4.3$ and negative for $t > 4.3$. In figure 6 we see that x_2 indeed shows this behavior.

For the third equation we derive $x_3'(t) = -0.496e^{-8001t} + 0.015e^{-t} + 0.0148e^{-0.029t}$. The derivative is positive for all t. Consequently, x_3 is a monotonically increasing function with neither a minimum nor a maximum. In figure 6 we see that x_3 indeed shows this behavior.

For the fourth equation we first must find the solution. This is $1 - x_1(t) - x_2(t) - x_3(t)$, where x_1, x_2, x_3 are the solutions corresponding to the first three equations. Then $x_4(t) = -6.2 \cdot 10^{-5} - 6.2 \cdot 10^{-5}e^{-8001t}$ is the solution to the fourth equation. Thus $x_4'(t) = 0.5e^{-8001t}$ is the first derivative of x_4. The derivative is positive, with values close to zero, for all t. Consequently, x_4 increases slowly and neither has a minimum nor a maximum. In figure 6 we see that x_4 indeed shows this behavior.

The second derivative of

$$x''(t) = e^{-8001t}\begin{pmatrix}0\\0\\3968\end{pmatrix} + e^{-t}\begin{pmatrix}1.020\\-1.035\\-0.015\end{pmatrix} + e^{-0.029t}\begin{pmatrix}0\\4.3 \cdot 10^{-4}\\-4.3 \cdot 10^{-4}\end{pmatrix}$$

Because x_2 is the only graph that has either a minimum or a maximum, we only need to apply the second derivative test for this equation. We find $x_2''(t) = -1.035e^{-t} + 4.3 \cdot 10^{-4}e^{-0.029t}$ which is less than zero for $t = 4.3$. Hence, we can conclude from the second derivative test that x_2 must have a maximum at this time point. We indeed see that x_2 has a maximum at this time point in figure 6.

Solution of pH Model

Now, we determine the solution of the dimensionless pH Model from section 5.1 by the method of Braun [2]. From the Jacobian of the dimensionless system of the pH Model, we have determined that the eigenvalues are $\lambda_1 = 0$ and $\lambda_2 = -(1 + \frac{1}{P})$. Note that P is strictly positive, so we never divide by zero.

$\lambda_1 = 0$: We seek a nonzero vector v_1 such that

$$Df(x_*)v_1 = \begin{pmatrix}-1 & \frac{1}{P}\\ 1 & -\frac{1}{P}\end{pmatrix}\begin{pmatrix}v_{11}\\v_{12}\end{pmatrix} = \begin{pmatrix}0\\0\end{pmatrix}$$

This implies that $-v_{11} + \frac{1}{P}v_{12} = 0$ and $v_{11} - \frac{1}{P}v_{12} = 0$. Solving for v_{11} in terms of v_{12} gives $v_{11} = \frac{1}{P}v_{12}$. Hence, each vector $v_1 = a_1\begin{pmatrix}\frac{1}{P}\\1\end{pmatrix}$ is an eigenvector of $Df(x_*)$ with eigenvalue zero. Consequently,

$$z^1(t) = a_1\begin{pmatrix}\frac{1}{P}\\1\end{pmatrix}$$

is a solution of the differential equation for any constant a_1.

$\lambda_2 = -(1 + \frac{1}{P})$: We seek a nonzero vector v_2 such that

$$(Df(x_*) + (1 + \tfrac{1}{P}))v_2 = \begin{pmatrix}\frac{1}{P} & \frac{1}{P}\\ 1 & 1\end{pmatrix}\begin{pmatrix}v_{21}\\v_{22}\end{pmatrix} = \begin{pmatrix}0\\0\end{pmatrix}$$

This implies that $\frac{1}{P}v_{21} + \frac{1}{P}v_{22} = 0$ and $v_{21} + v_{21} = 0$. Solving for v_{21} in terms of v_{22} gives $v_{21} = -v_{22}$. Hence, each vector $v_2 = a_2 \begin{pmatrix} -1 \\ 1 \end{pmatrix}$ is an eigenvector of $Df(x_*)$ with eigenvalue $-(1 + \frac{1}{P})$. Consequently,

$$z^2(t) = 2 \begin{pmatrix} -1 \\ 1 \end{pmatrix}$$

is a second solution of the differential equation for any constant a_2.

Since $Df(x_*)$ has distinct eigenvalues, these solutions must be linearly independent. Therefore, every solution $z(t)$ must be of the form

$$z(t) = a_1 \begin{pmatrix} \frac{1}{P} \\ 1 \end{pmatrix} + a_2 e^{-(1+\frac{1}{P})t} \begin{pmatrix} -1 \\ 1 \end{pmatrix}$$

The initial condition of this system is $z(0) = \begin{pmatrix} 1 \\ 0 \end{pmatrix}$. Therefore constants a_1 and a_2 can be determined by solving

$$\begin{pmatrix} 1 \\ 0 \end{pmatrix} = a_1 z^1(0) + a_2 z^2(0) = \begin{pmatrix} \frac{a_1}{P} \\ a_1 \end{pmatrix} + \begin{pmatrix} -a_2 \\ a_2 \end{pmatrix}$$

Thus $\frac{a_1}{P} - a_2 = 1$ and $a_1 + a_2 = 0$. The solution to these two equations is $a_1 = \frac{P}{1+P}$ and $a_2 = -\frac{P}{1+P}$.

Consequently, the solution to the dimensionless system of the pH model is

$$z(t) = \begin{pmatrix} \frac{1}{1+P} \\ \frac{P}{1+p} \end{pmatrix} + e^{-(1+\frac{1}{P})t} \begin{pmatrix} \frac{P}{1+P} \\ -\frac{P}{1+P} \end{pmatrix}$$