Membrane Transport

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Abstract

This thesis is about transport of solutes across a biological membrane, a 'lipid bilayer'. The goal is to create a template of differential equations, which is applicable to many types of molecules and ways of transport. The approach aims to be abstract but aplicable and built up entirely from biophysics and clearly stated, reasonable assumptions.

Free diffusion over the membrane will be considered explicitely, from which Ficks law will be derived. An ion-trap mechanism will also be specifically considered.

Contents

1 Context and composition

We will consider transport through a biological membrane, a lipid bilayer. Such a membrane consists of lipid molecules arranged such that they have their hydrophobic tails put together on the inside of the membrane and on the border of the membrane the hydrophillic heads. We consider this membrane to be in an environment of water, potentially with solutes like ions and glucose. They create a membrane with a width of two molecules, typically 4-6 nm thick.
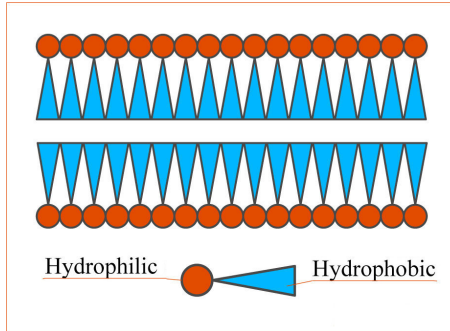
It is important to notice that the lipid bilayer is in a liquid state, so solutes can dissolve into the membrane if the chemical properties of the solute allow it. On both sides of this membrane we have compartments: the interior compartment, which we call 'inside', and the exterior compartment, which we call 'outside'. Together with the membrane itself, we have the three compartments we will consider. For instance, the membrane could represent the plasma-membrane, with the inside being the cytoplasm, or it could be the membrane of the Endoplasmatic Reticulum (ER), in which case the cytoplasm would be the outside and the ER the inside.

The goal is to model and analyse the change in concentration of a certain solute over time given initial concentrations for all the compartments.

1.1 First assumptions

To gain a simple but reasonable abstract model, we shall make some assumptions.

The problem becomes very complex if we were to consider all the particles seperately. This would lead to the use of spacial coordinates and modelling over 10^7 particles. This is too complicated for practical use, so we will assume the different compartments to be 'well-stirred'. This gives rise to the following:

Assumption A1. *Every compartment has a <u>uniform distribution</u> of particles, so a concentration which is constant over the <u>whole space of that compartment</u>. These concentrations are denoted C_O, C_I and C_M for the outside, inside and membrane respectively.*

The total number of particles in the system at a certain time can be calculated from the concentrations. Let V_O, V_I and V_M denote the volumes of the outside, inside and membrane respectively. At a given time the total number of particles is $V_O \cdot C_O + V_I \cdot C_I + V_M \cdot C_M$. We shall not consider what happens to C_O, C_I and C_M other than transport into and out of the membrane. This gives rise to the following:

Assumption A2. *No particles get into or out of the system of three compartments. Only particles moving from one compartment to another will influence the concentrations. In other words, we have a <u>closed system</u>.*

Assumption A2 gives that the total number of particles in the system $V_O \cdot C_O + V_I \cdot C_I + V_M \cdot C_M$ is constant over time.

1.2 Model of transport

For the transport, we have four types of movement. These are described by the following functions of flux of particles:

- The movement from the inside into the membrane, denoted as J_I^-.

- The movement from the outside into the membrane, denoted as J_O^-.

- The movement from the membrane to the inside, denoted as J_I^+.

- The movement from the membrane to the outside, denoted as J_O^+.
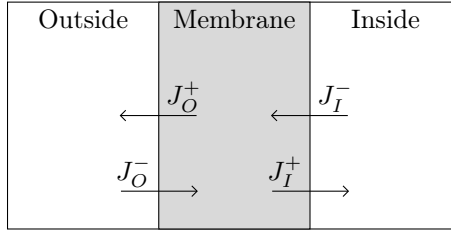
Although these functions are not specified yet, they give rise to a system of differential equations. The net movement of particles into a compartment is given by the total movement into it, minus the total movement out of it. If we divide this by the volume of that compartment, we get the change of concentration over a unit of time. Let t denote the time. We get a system of Ordinary Differential Equations (ODEs):

$$\frac{dC_O}{dt} = \frac{1}{V_O}(J_O^+ - J_O^-)$$
$$\frac{dC_M}{dt} = \frac{1}{V_M}(J_O^- - J_O^+ + J_I^- - J_I^+)$$
$$\frac{dC_I}{dt} = \frac{1}{V_I}(J_I^+ - J_I^-)$$

Notice that (A2) is satisfied:

$$\frac{d}{dt}(V_O C_O + V_I C_I + V_M C_M) = V_O \frac{dC_O}{dt} + V_I \frac{dC_I}{dt} + V_M \frac{dC_M}{dt}$$
$$= J_O^+ - J_O^- + J_I^+ - J_I^- + J_O^- - J_O^+ + J_I^- - J_I^+$$
$$= 0$$

This model is very abstract and can be applied to a large class of transport systems. The specific form of the equations varies with the specific system we are considering. We start out by analysing what happens in simple diffusion.

2 Simple Diffusion

The first model we will analyse is simple diffusion over the membrane. This means that we will consider particles that can dissolve back and forth from the membrane, into the outside compartment or the inside compartment. No particular molecules in the membrane regulate this absorbtion into the membrane and release of particles.

2.1 Biophysics

To further specify and analyse the model, we need to derive functions J. When we consider our system from a microscopic level, biophysical consideration will yield a more detailed description.

Particles in a compartment move around rapidly due to diffusion. They sometimes hit the border of this compartment with another compartment. In the event of a particle hitting the border, the particle either bounces off and stays in the compartment or it dissolves into the other compartment. A particle could e.g. be in the outside compartment and hit the membrane. There is a probability it gets dissolved in the membrane and a complementary probability that it bounces off. So the movement of particles per unit of time going into the membrane from the outside is, due to the law of large numbers, the frequency of hits on the membrane from the outside times the probability of uptake. Hence we get the following functions:

- We have f_{OM}, f_{MO}, f_{IM} and f_{MI}, where f_{ij}, $i, j \in \{O, M, I\}$ is the frequency with which particles hit the border of compartment j, from inside compartment i. These frequencies depend on C_i, the concentration in the compartment in which *the particles reside*.

- We have p_O, p_M and p_I, with p_i, $i \in \{O, M, I\}$ the probability that, in the event of a particle hitting the border of compartment i, the particle dissolves into compartment i. They depend on C_i, the concentration in the compartment the particle *dissolves into*.

The figure below illustrates a situation where a particle (A) moves in the outside compartment and hits the border with the membrane.
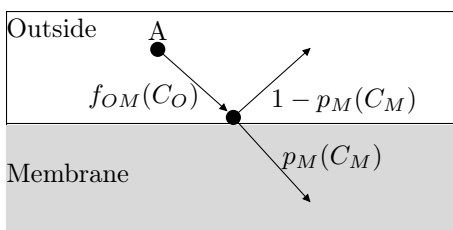
We now have:

$$J_O^- = f_{OM}(C_O) \cdot p_M(C_M)$$
$$J_O^+ = f_{MO}(C_M) \cdot p_O(C_O)$$
$$J_I^- = f_{IM}(C_I) \cdot p_M(C_M)$$
$$J_I^+ = f_{MI}(C_M) \cdot p_I(C_I)$$

2.2 Further assumptions

The frequency with which particles hit the border of a compartment increases with the concentration in that compartment. It is not unreasonable to say that this is a linear relation. Also we shall assume that the frequency function of hits on the border is equal for both borders of the membrane compartment.

Assumption A3. *There exist constants $k_O, k_M, k_I > 0$ such that each function $f_{ij}(C_i)$, $i,j \in \{O,M,I\}$ is <u>linear</u> in C_i and given by*

$$f_{ij}(C_i) = k_i C_i$$

We say the functions p_O, p_M and p_I are analytic, for if the concentration varies only a little, the probability for a solute to dissolve into a compartment varies very little. We say they are also strictly decreasing, because if the amount of particles in a compartment increases, it is less likely a solute hitting the border will dissolve into the membrane. So we assume a certain property of saturation. As these functions represent a probability we will assume they have a positive value smaller or equal to 1.

Assumption A4. p_O, p_M *and* p_I *take values in* $(0,1]$, *are <u>analytic</u> and <u>strictly decreasing</u> functions of C_O, C_M and C_I respectively <u>where</u>*

$$\lim_{C_O \to \infty} p_O(C_O) = 0, \qquad \lim_{C_M \to \infty} p_M(C_M) = 0, \qquad \lim_{C_I \to \infty} p_I(C_I) = 0$$

With these new assumptions, we can rewrite the functions which describe the flux of particles and the system of ODEs.

$$J_O^- = k_O C_O \cdot p_M(C_M)$$
$$J_O^+ = k_M C_M \cdot p_O(C_O)$$
$$J_I^- = k_I C_I \cdot p_M(C_M)$$
$$J_I^+ = k_M C_M \cdot p_I(C_I)$$

$$\frac{dC_O}{dt} = \frac{1}{V_O} \left[k_M C_M p_O(C_O) - k_O C_O p_M(C_M) \right]$$
$$\frac{dC_M}{dt} = \frac{1}{V_M} \left[k_O C_O p_M(C_M) - k_M C_M p_O(C_O) + k_I C_I p_M(C_M) - k_M C_M p_I(C_I) \right]$$
$$\frac{dC_I}{dt} = \frac{1}{V_I} \left[k_M C_M p_I(C_I) - k_I C_I p_M(C_M) \right]$$

2.3 Time-scale Analysis

The membrane is very thin compared to the outside and inside, so V_M is very small compared to V_O and V_I. Therefore, C_M will change rapidly in comparison to the change of C_O and C_I. This means that C_M at any time during the diffusion process will be very close to its equilibrium value induced by C_O and C_I and the system of ODEs.
This relation induces a reasonable assumption to simplify our system of ODEs, setting the equation for $\frac{dC_M}{dt}$ equal to zero and letting it follow C_O and C_I.

Assumption A5. *We have $C_M = C_M^*$, with C_M^* a <u>quasi steady state</u>, which is the function of C_O and C_I, such that $\frac{dC_M^*}{dt} = 0$.*

Further motivation of this approach is given in Section 4.

To further specify the function C_M^* we will work out this relation:

$$\frac{dC_M^*}{dt} = 0$$

$$\frac{1}{V_M} \left[-k_O C_O p_M(C_M^*) + k_M C_M^* p_O(C_O) - k_I C_I p_M(C_M^*) + k_M C_M^* p_I(C_I) \right] = 0$$

$$- p_M(C_M^*)(k_O C_O + k_I C_I) + k_M C_M^* (p_O(C_O) + p_I(C_I)) = 0$$

$$\boxed{\frac{k_M C_M^*}{p_M(C_M^*)} = \frac{k_O C_O + k_I C_I}{p_O(C_O) + p_I(C_I)}} \tag{1}$$

This is an implicit relation, which cannot be further solved for C_M^*, because the functions p_O, p_M and p_I are not specified in detail. However, we have the following:

Lemma 1. *For any given values of $C_O, C_I \geq 0$, there is only one value $C_M^* \geq 0$ for which (1) is satisfied.*

Proof. For these values, (1) gives a unique value of $\frac{k_M C_M^*}{p_M(C_M^*)}$, because the right part of the equation solely depends on C_O and C_I. This value is positive, because k_O, k_I are positive by assumption A3 and therefore $k_O C_O + k_I C_I$ is non-negative. p_O and p_I are positive by assumption A4.

The fraction at the left hand side in (1) only depends on C_M^*. The numerator is an increasing linear function. And by assumption A4, we know that the denominator is decreasing, nonzero and analytic. Therefore the whole fraction must be strictly increasing and analytic in terms of C_M^*. For $C_M^* = 0$, the whole fraction is 0, so the left part of the equation is continuous and strictly increasing from 0. Moreover, as $C_M^* \to \infty$, $\frac{C_M^*}{p_M(C_M^*)} \to \infty$ by assumption A4. The value we must find is non-negative, so there is exactly one value of C_M^* which satisfies (1). $\qquad \square$

Remark. We can thus consider C_M^* as a function of C_O and C_I. Notice that if C_O or C_I increases, the right side of (1) increases. The left side is a strictly increasing function of C_M^*. Thus, to satisfy equation (1), C_M^*, must increase. So C_M^* is strictly increasing as function of C_O and C_I seperately.

2.4 Steady State

Now we have a model with two variables. It is given by the old system of ODEs and the implicit relation (1). We denote:

$$\frac{dC_O}{dt} = F_O(C_O, C_I) = \frac{1}{V_O} \left[k_M C_M^* p_O(C_O) - k_O C_O p_M(C_M^*) \right]$$

$$\frac{dC_I}{dt} = F_I(C_O, C_I) = \frac{1}{V_I} \left[k_M C_M^* p_I(C_I) - k_I C_I p_M(C_M^*) \right]$$

$$\frac{k_M C_M^*}{p_M(C_M^*)} = \frac{k_O C_O + k_I C_I}{p_O(C_O) + p_I(C_I)}$$

By assumption A2 we know

$$\frac{d}{dt}\left(V_O C_O + V_I C_I + V_M C_M^*\right) = 0$$

$$V_O \frac{dC_O}{dt} = -\left(V_I \frac{dC_I}{dt} + V_M \frac{dC_M^*}{dt}\right)$$

By assumption A5 we know $\frac{dC_M^*}{dt} = 0$, so

$$V_O \frac{dC_O}{dt} = -V_I \frac{dC_I}{dt}$$

This means that whenever $\frac{dC_I}{dt} = 0$, it follows that $\frac{dC_O}{dt} = 0$, which gives a steady state.

We denote C_O^* and C_I^* the steady state values for C_O and C_I respectively. We derive:

$$F_I(C_O^*, C_I^*) = 0$$

$$0 = \frac{1}{V_I}\left[-k_I \cdot C_I^* \cdot p_M(C_M^*) + k_M \cdot C_M^* \cdot p_I(C_I^*)\right]$$

$$0 = -k_I \cdot C_I^* + \frac{k_m C_M^*}{p_M(C_M^*)} \cdot p_I(C_I^*)$$

Notice that equation (1) gives us an expression for the fraction above.

$$0 = -k_I \cdot C_I^* + \frac{k_O \cdot C_O^* + k_I \cdot C_I^*}{p_O(C_O^*) + p_I(C_I^*)} \cdot p_I(C_I^*)$$

$$k_I \cdot C_I^* \cdot (p_O(C_O^*) + p_I(C_I^*)) = p_I(C_I^*) \cdot (k_O \cdot C_O^* + k_I \cdot C_I^*)$$

$$k_I \cdot C_I^* \cdot p_O(C_O^*) = p_I(C_I^*) \cdot k_O \cdot C_O$$

$$\boxed{\frac{k_I \cdot C_I^*}{p_I(C_I^*)} = \frac{k_O \cdot C_O^*}{p_O(C_O^*)}} \tag{2}$$

Remark. This is again an implicit expression, not further specified nor solvable, because the functions p_O, p_M and p_I are not further specified. But (2) is essential for our further analysis to prove the following lemmas and theorem.

Lemma 2. *For every value of $C_O^* \geq 0$ there is exactly one value of C_I^* for which (C_O^*, C_I^*) is the steady state of the system. We call the graph of this relation between C_I^* and C_O^* the __I-nullcline__.*
Moreover, in this relation $C_I^* \to \infty$ as $C_O^* \to \infty$.

Proof. (C_O^*, C_I^*) is the steady state of the system if and only if (2) is satisfied. For a given $C_O^* \geq 0$, the right hand side of (2) is fixed. We call this value S. S is non-negative for the numerator is non-negative because $k_O > 0$ and $C_O^* \geq 0$ and the denominator is positive by assumption A4.
We set $f(C_I^*) = \frac{k_I \cdot C_I^*}{p_I(C_I^*)}$, the left hand side of (2). We have $f(0) = 0$. Furthermore, we see that the numerator of f increases linearly as C_I^* increases, the denominator decreases strictly, is analytic and nonzero due to assumption A4. Therefore f is continuous and strictly increasing. Moreover $f(C_I^*) \to \infty$ as $C_I^* \to \infty$, so there is exactly one solution to the equation $f(C_I^*) = S$. Thus for every C_O^* there is exactly one C_I^* such that (2) is satisfied. $\square$

Remark. We see that on the I-nullcline, if C_O^* increases, S increases, so to satisfy (2), C_I^* must also increase. Thus the I-nullcline describes a strictly increasing function C_I^* in terms of C_O^*.

Lemma 3. *The assumption that we have a closed system* (A2) *implies that in the model C_I follows a strictly decreasing function of C_O*

Proof. Assumption A2 gives us that for every $C_O(0), C_I(0)$, the total amount of particles N, given by

$$N := V_O \cdot C_O(0) + V_I \cdot C_I(0) + V_M \cdot C_M^*(C_O(0), C_I(0))$$

is constant. We derive from this, at any time t a relation for C_I in terms of C_O.

$$N = V_O \cdot C_O(t) + V_I \cdot C_I(t) + V_M \cdot C_M^*(C_O(t), C_I(t))$$
$$V_I \cdot C_I(t) = N - V_O \cdot C_O(t) - V_M \cdot C_M^*(C_O(t), C_I(t))$$
$$C_I(t) = \frac{1}{V_I}\left(N - V_O \cdot C_O(t) - V_M \cdot C_M^*(C_O(t), C_I(t))\right) \tag{3}$$

We see that the function implied by (3) is strictly decreasing:
At any time (3) is satisfied.

1. If at a certain time C_O increases, $N - V_O \cdot C_O$ decreases. For fixed C_I, C_M^* also increases, so (3) is no longer satisfied. So C_I must decrease which means that C_M^* increases slightly and (3) is again satisfied.

2. If at a certain time C_O decreases, $N - V_O \cdot C_O$ increases. For fixed C_I, C_M^* also decreases, so (3) is no longer satisfied. So C_I must increase which means that C_M^* decreases slightly and (3) is again satisfied.

$\square$

Under assumptions A1-A5 we can now prove from these lemmas the main result of this section:

Theorem 4. *For every starting values of $C_O(0), C_I(0) \geq 0$, there is exactly one solution for the steady state, (C_O^*, C_I^*).*

Proof. Lemma 2 gives us that all possibilities for steady state, regardless of the starting values is given by the I-nullcline, which is strictly increasing from 0 to ∞. We know that, at any time, (2) is satisfied, which implies a decreasing function for C_I in terms of C_O. If we now start at $C_O = 0$ and increase C_O, the curve imlpied by (2) will be downwards and the curve implied by (2) will go upwards. Therefore there will be exactly one meeting point, because the I-nullcline tends to infinity. To illustrate:

$\square$

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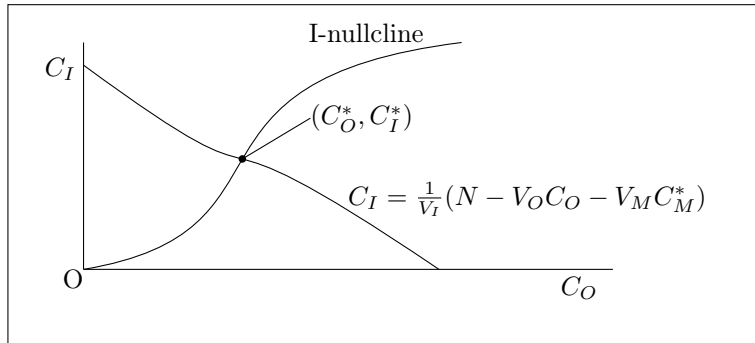
Remark. The derivation that the model without the quasi steady state assumption A5 has a unique steady state is completely similar, for we would set all differential equations equal to zero and could start our reasoning from the fact that $\frac{dC_M}{dt} = 0$, which then satisfies (A5). The rest of the derivation and lemmas is therefore valid to find that also the full model has exactly one steady state.

3 Ficks Law

Ficks law is commonly used (Keener et al) to describe the flux of particles through a biological membrane. Ficks law is stated as follows for the solute X (Blom et al).

Law (Fick).

$$\Phi_X(t) = A\frac{K_X D_X}{l}\left(C_O(t) - C_I(t)\right)$$

In this expression:

- $\Phi_X(t)$ is the flux of X-type particles over the membrane at time t.

- $A\frac{K_X D_X}{l}$ are some constants representing the influence of some biological properties of the solute and membrane. They are considered here only as being constant and positive. In section A.1 they are specified.

So in the form of a differential equation we have:

$$\frac{dC_I}{dt} = \frac{1}{V_I}A\frac{K_X D_X}{l}\left(C_O(t) - C_I(t)\right)$$

We notice the following:

1. It is linear in terms of C_O and C_I.

2. There are no constant terms.

3. The steady state is at $C_O = C_I$

We would like Ficks law to follow from the model we have just derived.

3.1 Taylor expansion

Our model so far is not necessarily linear, so for Ficks law to follow from it, let us consider the first order Taylor expansion of F_I around $(C_O, C_I) = (C_O^0, C_I^0)$. We see:

$$F_I(C_O, C_I) \approx F_I(C_O^0, C_I^0)$$
$$+ \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)(C_O - C_O^0) + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)(C_I - C_I^0)$$

We choose (C_O^0, C_I^0) on the I-nullcline, where $F_I(C_O^0, C_I^0) = 0$.
Lemma 2 tells us that we can consider C_I^0 as an increasing function of C_O^0 on the I-nullcline, denote: $C_I^0(C_O^0)$

Lemma 5. *For any $C_O^0 \geq 0$, $C_I^0(C_O^0)$, we have*

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0(C_O^0)) \cdot C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0(C_O^0))\frac{dC_I^0}{dC_O^0}(C_O^0) \cdot C_O^0 = 0 \qquad (4)$$

Proof. $\forall C_O^0 \geq 0$, using implicit differentiation,

$$0 = F_I(C_O^0, C_I^0(C_O^0))$$

$$0 = \frac{d}{dC_O^0}\left[F_I(C_O^0, C_I^0(C_O^0))\right]$$

$$0 = \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0(C_O^0)) + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0(C_O^0))\frac{dC_I^0}{dC_O^0}(C_O^0)$$

$$0 = \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0(C_O^0)) \cdot C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0(C_O^0))\frac{dC_I^0}{dC_O^0}(C_O^0) \cdot C_O^0$$

$\square$

With these initial values on the I-nullcline, we have for our Taylor expansion, because $F_I(C_O^0, C_I^0) = 0$:

$$F_I(C_O, C_I) \approx \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)(C_O - C_O^0) + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)(C_I - C_I^0)$$

$$= \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I$$

$$- \left(\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I^0\right)$$

We noticed before, that in Ficks law, there are no constant terms. So, for it to follow from our model, the constant terms given above must be zero:

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I^0 = 0 \tag{5}$$

We compare (5) to (4).

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I^0 = 0$$

$$= \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0) \cdot C_O^0 + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)\frac{dC_I^0}{dC_O^0}(C_O^0) \cdot C_O^0$$

So the constant terms are zero if we have the following relation for $C_I^0(C_O^0)$

$$\boxed{\frac{dC_I^0}{dC_O^0}(C_O^0) \cdot C_O^0 = C_I^0(C_O^0)} \tag{6}$$

We can derive what this means by integration:

$$\frac{dC_I^0}{dC_O^0}(C_O^0) \cdot C_O^0 = C_I^0(C_O^0)$$

$$\frac{1}{C_I^0}\frac{dC_I^0}{dC_O^0} = \frac{1}{C_O^0}$$

$$\log(C_I^0) = \log(C_O^0) + c$$

$$\boxed{C_I^0 = aC_O^0} \tag{7}$$

This means that the constant terms are zero if the I-nullcline is linear.
We know that the I-nullcline is described by (2). This equation does not necessarily imply a linear relation. So Ficks law does not follow from our model yet. It is clear that we have to make more assumptions about the model we observe.

3.2 Symmetry in simple diffusion

In simple diffusion over the membrane, we now have different functions $k_O C_O$, $k_I C_I$ and different functions $p_O(C_O)$, $p_I(C_I)$. However, we can assume that on both sides of the membrane, the solute acts in a similar way. If the concentration of particles is equal on both sides, the number of particles that hit the border of the membrane on both sides must be about equal, for the membrane has the same surface area, because the membrane is only two molecules thick. We shall therefore assume $k_O = k_I$. Notice though that this implicitly means that the molecules of the solute are small, for inside the cell, the diffusion rate is smaller for very large molecules because it is crowded with all kinds of solutes and organelles.

Based on the same arguments we can assume that the probability functions p_O and p_I are also equal.

Assumption A6. *We have symmetry on both sides of the membrane. $k_O = k_I$ and for $x \geq 0$, $p_O(x) = p_I(x)$.*
Notation: $k = k_O = k_I$, $p(x) = p_O(x) = p_I(x)$

3.3 Ficks Law

Now we can derive the following:

Lemma 6. *If C_O and C_I are on the I-nullcline, then $C_O = C_I$*

Proof. C_O and C_I are on the I-nullcline. If we substitute the functions we have from (A6) into (2), we get that the following expression is valid.

$$\frac{k \cdot C_O}{p(C_O)} = \frac{k \cdot C_I}{p(C_I)}$$
$$\frac{C_O}{p(C_O)} = \frac{C_I}{p(C_I)} \tag{8}$$

- If $C_O = C_I$, $\frac{C_O}{p(C_O)} = \frac{C_I}{p(C_I)}$.

- If $C_O > C_I$, assumption A4 gives us that $p(C_O) < p(C_I)$, so $\frac{1}{p(C_O)} > \frac{1}{p(C_I)}$. Therefore $\frac{C_O}{p(C_O)} > \frac{C_I}{p(C_I)}$, which contradicts (8). Thus $C_O \not> C_I$.

- If $C_O < C_I$, assumption A4 gives us that $p(C_O) > p(C_I)$, so $\frac{1}{p(C_O)} < \frac{1}{p(C_I)}$. Therefore $\frac{C_O}{p(C_O)} < \frac{C_I}{p(C_I)}$, which contradicts (8). Thus $C_O \not< C_I$.

We must conclude that $C_O = C_I$. $\qquad\qquad\square$

Remark. Lemma 6 gives us that the I-nullcline is represented by $C_I = C_O$, which satisfies (7) for $a = 1$. So the constant terms of the Taylor expansion equal zero.
This means that the Taylor expansion now equals

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I$$

Thus from Lemma 5 and 6 we can now conclude that:

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0) = -\frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)$$

Thus by this derivation we have that the Taylor expansion of our model equals

$$\frac{dC_I}{dt} \approx \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)C_O + \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)C_I$$

$$= \frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)(C_O - C_I) \tag{9}$$

This is Ficks law if $\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)$ is the right value. We know it has to be positive

Lemma 7. *For (C_O^0, C_I^0) on the I-nullcline we have:*

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0) > 0$$

Proof.

$$F_I(C_O, C_I) = \frac{1}{V_I}k_M C_M^*(C_O, C_I)p_I(C_I) - k_I C_I p_M(C_M^*(C_O, C_I))$$

We fix C_I at C_I^0. Now:

- if C_O is slightly increased from C_O^0, C_M^* increases, as it is increasing in C_O and therefore $p_M(C_M^*)$ decreases. This means $F_I(C_O, C_I^*) > 0$

- if C_O is slightly decreased from C_O^0, C_M^* decreases, as it is increasing in C_O and therefore $p_M(C_M^*)$ increases. This means $F_I(C_O, C_I^*) < 0$

We know that $F_I(C_O, C_I)$ consists only of analytical functions of C_O and C_I. It is positive to the right of the I-nullcline, it is negative to the left of the I-nullcline, so in passing it, from left to right, it must be increasing. So

$$\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0) > 0$$

$\square$

Remark. *Thus we now have Ficks law.*
Lemma 7 means that the linearisation around the steady state value is stable.

4 Numerical Analysis

Assumption A5 was a simplification from the full model we had before, with three variables. We would like to compare the full model and the simplified model with only two variables. To do this numerically, we need to have explicit functions p and p_M in order to be able to solve equation (1) for C_M^*. We want them to be decreasing, positive and analytic. We can for instance use

$$p(C) = \frac{e}{1 + C} \qquad\qquad C = C_O \text{ or } C = C_I$$
$$p_M(C_M) = \frac{e_M}{1 + C_M} \qquad\qquad\qquad (10)$$

with $e, e_M \in \mathbb{R}_+$ the initial probabilities at $C = 0$, $C_M^* = 0$ respectively. If we plot these functions, we see:
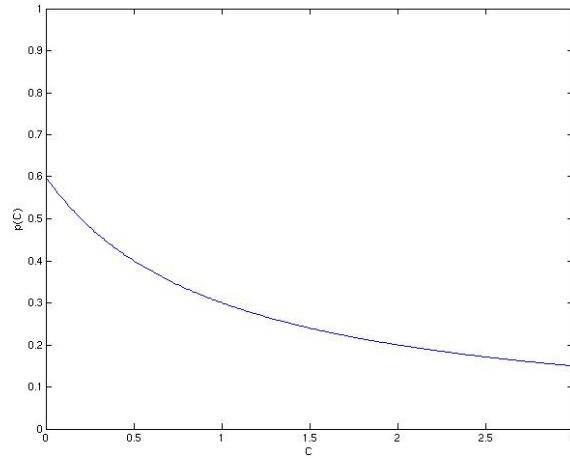
We will use these explicit functions to simulate both the full model and the simplified model with (A5).

4.1 The full model

We can program from this, in XPPaut code, the full three-dimensional model. We choose $e = 0.6$, $e_M = 0.5$, $k = 1$ and $k_M = 0.8$. We set similar to our reasoning $V_M << V_I$, but still computable. The results are graphs given in Section 4.3

```
c0'=(km*cm*(e/(1+c0))-k*C0*(em/(1+cm)))/v0
cm'=(k*c0*(em/(1+cm))+k*ci*(em/(1+cm))-km*cm*((e/(1+c0))+(e/(1+ci))))/vm
ci'=(km*cm*(e/(1+ci))-k*ci*(em/(1+cm)))/vi
par k=1,km=0.8,v0=5,vm=0.1,vi=1
param e=0.6,em=0.5
init c0=1,cm=0,ci=0
@ dt=0.05,total=150,ylo=0,yhi=1,xlo=0,xhi=30,xp=t,yp=ci
done
```

4.2 The simplified model

We substitute these functions into (1).

$$\frac{k_M C_M^*}{p_M(C_M^*)} = \frac{k(C_O + C_I)}{p(C_O) + p(C_I)}$$

$$\frac{(k_M C_M^*)(1 + C_M^*)}{e_M} = \frac{k(C_O + C_I)}{\frac{e}{1+C_O} + \frac{e}{1+C_I}}$$

$$0 = C_M^{*\,2} + C_M^* - \frac{e_M k(C_O + C_I)}{k_M \left(\frac{e}{1+C_O} + \frac{e}{1+C_I} \right)}$$

$$C_M^* = \frac{-1 + \sqrt{1 + 4 \dfrac{e_M k(C_O + C_I)}{k_M \left(\frac{e}{1+C_O} + \frac{e}{1+C_I} \right)}}}{2}$$

$$C_M^* = \frac{1}{2} \left(-1 + \sqrt{1 + \frac{4 e_M k(C_O + C_I)}{k_M \left(\frac{e}{1+C_O} + \frac{e}{1+C_I} \right)}} \right) \tag{11}$$

We can program from (11), in XPPaut code, the simplified two-dimensional model. We choose the same constants as for the full model: $e = 0.6$, $e_M = 0.5$, $k = 1$ and $k_M = 0.8$. $V_M \ll V_I$, but still computable. The results are graphs given in subsection 4.3

```
c0'=(km*cm(c0,ci)*(e/(1+c0))-k*c0*(em/(1+cm(c0,ci))))/v0
ci'=(km*cm(c0,ci)*(e/(1+ci))-k*ci*(em/(1+cm(c0,ci))))/vi
aux cm(x,y)=(1/2)*(-1+sqrt(1+((4*em*k*(x+y))/(km*(e/(1+x)+(e/(1+y)))))))
par k=1,km=0.8,v0=5,vm=0.1,vi=1
param e=0.6,em=0.5
@ dt=0.05,total=150,ylo=0,yhi=1,xlo=0,xhi=30,xp=t,yp=ci
done
```

4.3 The simplified model vs the full model

The full model (solid lines) and **the simplified model** (dotted lines)

C_O vs time
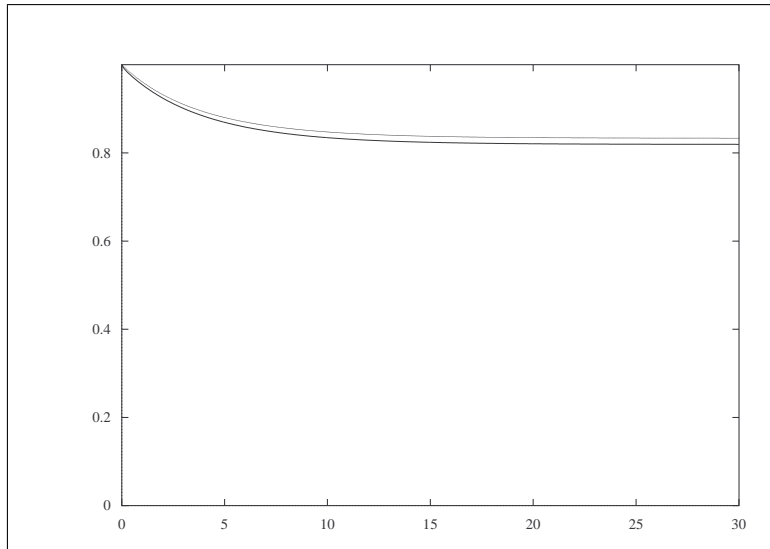
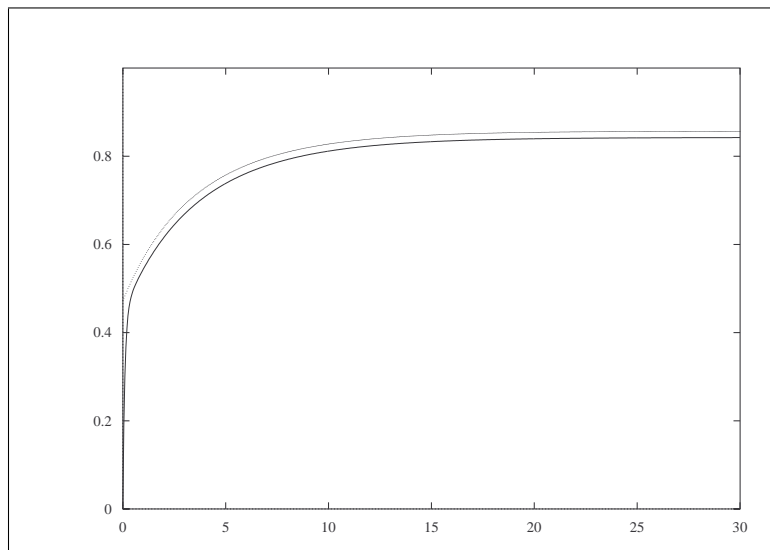C_M (C_M^* for simplified model) vs time

C_I vs time
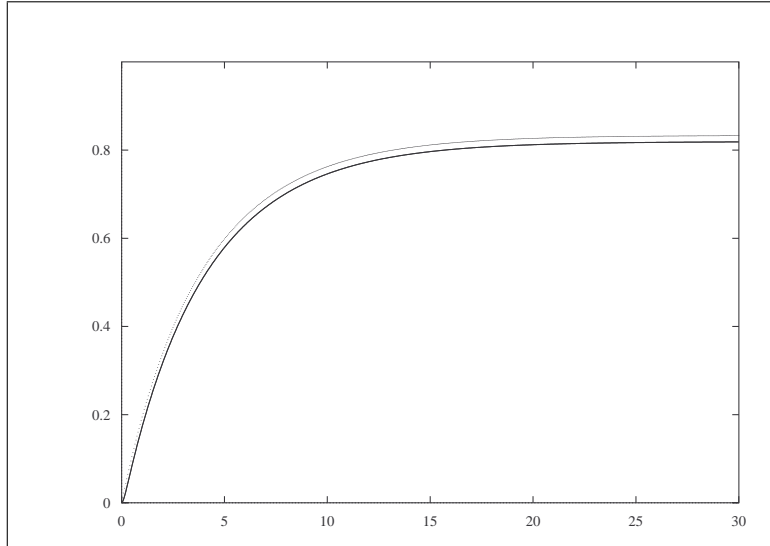
We see that the models are almost completely the same. The equilibrium seems to be somewhat higher in the simplified model.

This is because they have a different total amount of particles, because the initial conditions are different. For the full model, C_M is initially not at the equilibrium value for $(C_O, C_I) = (1, 0)$, but at 0. For the simplified model, C_M^* is, by assumption A5, at the equilibrium value for $(C_O, C_I) = (1, 0)$, which is at $C_M^*(1, 0) = 0.4718$.

So for the total amount of particles we have:

$$\text{Full model} \quad \text{vs} \quad \text{Simplified model}$$
$$V_O \cdot 1 + V_M \cdot 0 + V_I \cdot 0 \qquad V_O \cdot 1 + V_M \cdot 0.4718 + V_I \cdot 0$$
$$1 \cdot V_O \quad < \quad 1 \cdot V_O + 0.4718 \cdot V_M$$

But because V_M is very small, this error is negligible for realistic biological conditions.

5 Transport with buffering in the interior compartment

The buffer mechanism we will consider is based on the model described in Blom et al, Chapter 4:

> Models for uptake and accumulation by diffusion of solutes, such as alkaloids, that associate with protons to charged forms.

In Blom et al, the uptake of alkaloids by plant cells is described. The plant cells placed in an environment with alkaloids at a low concentration. The alkaloids accumulate in the cell. They find that in the cell, the alkaloids rapidly react with free protons and that alkaloids with bound protons can no longer freely diffuse through the membrane, because the cross-membrane potential prevents them from crossing the cellular membrane (Fall et al). This results in a higher concentration of alkaloids in the interior compartment than the outside compartment.

5.1 Buffer Mechanism

We will discus the buffering reaction described in Blom et al. in a more general way using the model we have created.

We have some type of particle X, which can move freely over the membrane. In the interior compartment, we will have some compound B, 'buffer', which cannot diffuse through the membrane. It can bind X in the reaction $(*)$, yielding the compound XB, which cannot cross the membrane either. We have:

$$\boxed{\quad X + B \quad \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \quad XB \quad} \qquad (*)$$
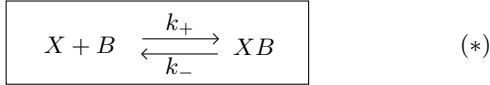
$$J_X^+ = k_- \cdot [XB]$$
$$J_X^- = k_+ \cdot [X] \cdot [B]$$
$$J_B^+ = k_- \cdot [XB] = J_X^+$$
$$J_B^- = k_+ \cdot [X] \cdot [B] = J_X^-$$

where

- J_X^+ and J_B^+, are the total amount of particles X and B respectively formed out of XB per unit of time.

- J_X^- and J_B^-, are the total amount of particles X and B respectively formed into XB per unit of time.

- $[X]$, the concentration in the inside of the membrane of the unbuffered molecule X

- $[XB]$, the concentration in the inside of the membrane of the buffered molecule XB

- $[B]$, the concentration in the inside of the membrane of the Buffer molecule B

- k_+ and k_- reaction rates of the buffering process.

X diffuses freely over the membrane, therefore we can apply the model for simple diffusion described before using assumptions A1, A2, A3, A4 and A6. If we take the

buffering process into account, we have the following model.

$$\frac{dC_O}{dt} = \frac{1}{V_O}\left(-kC_O p_M(C_M) + k_M C_M p(C_O)\right)$$

$$\frac{dC_M}{dt} = \frac{1}{V_M}\left(k[X]p_M(C_M) - k_M C_M p([X]) + kC_O p_M(C_M) - k_M C_M p(C_O)\right)$$

$$\frac{d[X]}{dt} = \frac{1}{V_I}\left(-k[X]p_M(C_M) + k_M C_M p([X])\right) + \frac{1}{V_I}(J_X^+ - J_X^-)$$

$$\frac{d[XB]}{dt} = \frac{1}{V_I}(J_X^- - J_X^+) \qquad\qquad \frac{d[B]}{dt} = \frac{1}{V_I}(J_X^+ - J_X^-)$$

As this is a different model, we cannot use the derivation steps made before in simple diffusion.

5.2   Time scale Analysis

We will assume that the buffering reaction is fast compared to the diffusion over the membrane. This means that we can assume a quasi steady state for this reaction.

Assumption A7. *The buffer reaction is in equilibrium and every change in $[X]$ will be instantaneously corrected to the equilibrium*

This means that $J_X^+ - J_X^- = 0 = J_B^+ - J_B^- \Rightarrow \frac{d[XB]}{dt} = 0 = \frac{d[B]}{dt}$. This defines the quasi steady state values $[XB]^*$ and $[B]^*$ for $[XB]$ and $[B]$ respectively as functions of $[X]$.
We define: $C_I = [X] + [XB]^*$, the total concentration of the compound X.
Now, we have

$$\frac{dC_I}{dt} = \frac{d[X]}{dt} + \frac{d[XB]^*}{dt}$$

$$= \frac{d[X]}{dt} + \frac{1}{V_I}(J_X^- - J_X^+)$$

$$= \frac{d[X]}{dt}$$

As the diffusion reaction over the membrane of X is the only thing changing C_I, we can now consider C_I as the variable, defining $[X] = [X]^*(C_I)$ a quasi steady state. Now $[B]^*$ and $[XB]^*$ are also functions of C_I. Assuming (A5), we can now rewrite the system of ODEs.

$$\frac{dC_O}{dt} = \frac{1}{V_O}\left(-kC_O p_M(C_M^*) + k_M C_M^* p(C_O)\right)$$

$$0 = \frac{1}{V_M}\left(k[X]^* p_M(C_M^*) - k_M C_M^* p([X]^*) + kC_O p_M(C_M^*) - k_M C_M^* p(C_O)\right)$$

$$\frac{dC_I}{dt} = \frac{1}{V_I}\left(-k[X]^* p_M(C_M^*) + k_M C_M^* p([X]^*)\right)$$

5.3   Fixed unbound buffer concentration

In some mechanisms (like the alkaloids binding with protons, Blom et al), we know that the concentration of free buffer molecules is kept constant by another process in the cell, i.e. $[B]^*$ is constant. We denote $\hat{k}_+ = k_+ \cdot [B]^*$, which is constant. We can

now derive the following:

$$-k_+ \cdot [B]^* \cdot [X]^* + k_- \cdot [XB]^* = 0 \qquad\qquad C_I = [X]^* + [XB]^*$$

$$k_- \cdot [XB]^* = \hat{k}_+ \cdot [X]^* \qquad\qquad C_I = [X]^* + \frac{\hat{k}_+}{k_-}[X]^*$$

$$[XB]^* = \frac{\hat{k}_+}{k_-}[X]^* \qquad\qquad C_I = \left(1 + \frac{\hat{k}_+}{k_-}\right)[X]^*$$

$$\boxed{[X]^* = \frac{k_-}{k_- + \hat{k}_+} C_I} \tag{12}$$

(12) is an explicit relation. Let $K = \frac{k_-}{k_- + \hat{k}_+}$, note that $K < 1$. We substitute this into our membrane diffusion model.

$$\frac{dC_O}{dt} = \frac{1}{V_O}\left(-kC_O p_M(C_M^*) + k_M C_M^* p(C_O)\right)$$

$$0 = \frac{1}{V_M}\left(kKC_I p_M(C_M) - k_M C_M^* p(KC_I) + kC_O p_M(C_M^*) - k_M C_M^* p(C_O)\right)$$

$$\frac{dC_I}{dt} = \frac{1}{V_I}\left(-kKC_I p_M(C_M^*) + k_M C_M^* p(KC_I)\right)$$

We substitute into this model the shifted variables $(C_O, \hat{C}_I) = (C_O, K \cdot C_I)$. Which gives

$$\frac{dC_O}{dt} = \frac{1}{V_O}\left(-kC_O p_M(C_M^*) + k_M C_M^* p(C_O)\right)$$

$$0 = \frac{1}{V_M}\left(k\hat{C}_I p_M(C_M) - k_M C_M^* p(\hat{C}_I) + kC_O p_M(C_M^*) - k_M C_M^* p(C_O)\right)$$

$$\frac{d\hat{C}_I}{dt} = \frac{K}{V_I}\left(-k\hat{C}_I p_M(C_M^*) + k_M C_M^* p(\hat{C}_I)\right)$$

Now we have exactly the same model as in subsection 2.4. The system is shifted, so when we have a certain value $\hat{C}_I$, C_I is larger $\left(\frac{1}{K}\hat{C}_I\right)$, but because the shift is linear, it retains all properties we used. So the same results are valid.

The results are as follows: This system has a unique steady state where $C_O = \hat{C}_I$ and the first order Taylor expansion is:

$$F_I \approx \frac{\partial F_I}{\partial C_O}(C_O^0, \hat{C}_I^0)(C_O - \hat{C}_I)$$

This means that we have an equilibrium at $C_I = \frac{1}{K}C_O$ and as $0 < K < 1$ this means accumulation. The value $\frac{1}{K}$ is called the accumulation ratio. And for the Taylor expansion we have the following.

$$\frac{dC_I}{dt} \approx \frac{\partial F_I}{\partial C_I}(C_O^0, C_I^0)(C_O - K \cdot C_I) \tag{13}$$

A Suggestions for further research

A.1 Physical constants in Ficks law

Ficks law gave the differential equation:

$$\frac{dC_I}{dt} = \frac{1}{V_I} A \frac{K_X D_X}{l} \left(C_O(t) - C_I(t) \right)$$

In this differential equation the constant terms are given by biological properties.

- V_I is the volume of the inside.
- A is the area of the membrane.
- K_X is the partition coefficient of solute X in the membrane.
- D_X is the diffusion coefficient of solute X in the membrane.
- l is the thickness of the membrane.

Ficks law was derived in Section 3 for $\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0) = \frac{1}{V_I} A \frac{K_X D_X}{l}$. It can be researched if the properties that influence F_I and thereby $\frac{\partial F_I}{\partial C_O}(C_O^0, C_I^0)$ also influence $\frac{1}{V_I} A \frac{K_X D_X}{l}$ and in what manner. This gives more insight into the way these constants vary for different types of sulutes and cells.

A.2 Fixed total buffer concentration

Section 5.2 ends with an buffered system, where the concentrations $[B]^*$, $[X]^*$ and $[XB]^*$ are not yet specified, but only set at their quasi steady state values depending on C_I. We then discussed the specific form where $[B]^*$ was constant.

In this Section we consider a system in which there is a fixed *total* amount of buffer molecules in the interior. One may think of B as a buffer protein. The total amount is kept fixed by constitutive expression of the corresponding gene, counteracting continuous degeneration of this protein in the cytoplasm.

So we have some constant M for which at any time $M = [B]^* + [XB]^*$. Then the complete system would be different, for we would have:

$$
\begin{aligned}
M = [B]^* + [XB]^* \quad &\rightarrow \quad [B]^* = M - [XB]^* \\
C_I = [X]^* + [XB]^* \quad &\rightarrow \quad \boxed{[XB]^* = C_I - [X]^*} \\
&\qquad \boxed{[B]^* = M - C_I + [X]^*} \\
(A7): J_X^+ - J_X^- = 0 \quad &\rightarrow \quad k_-[XB]^* - k_+[X]^*[B]^* = 0 \\
&\qquad k_-(C_I - [X]^*) - k_+[X]^*(M - C_I + [X]^*) = 0 \\
&\qquad ([X]^*)^2 + \left(\frac{k_-}{k_+} + M - C_I \right)[X]^* - \frac{k_-}{k_+} C_I = 0 \\
\text{We set } K_{eq} = \frac{k_-}{k_+} \quad &\rightarrow \\
[X]^* \quad = \quad & \frac{1}{2}\left(\sqrt{(K_{eq} + M - C_I)^2 + 4K_{eq}C_I} - (K_{eq} + M - C_I) \right)
\end{aligned}
$$

$$\boxed{[X]^*(C_I) = \frac{1}{2}(K_{eq} + M - C_I)\left(-1 + \sqrt{1 + \frac{4K_{eq}C_I}{(K_{eq} + M - C_I)^2}} \right)}$$

So the quasi steady state $[X]^*$ is not a simple linear function of C_I. One could simplify further for the right conditions. For $\dfrac{4K_{eq}C_I}{(K_{eq} + M - C_I)^2} << 1$:

$$[X]^*(C_I) \approx \frac{1}{2}(K_{eq} + M - C_I)\left(\frac{1}{2}\frac{4K_{eq}C_I}{(K_{eq} + M - C_I)^2}\right)$$
$$= \frac{K_{eq}C_I}{K_{eq} + M - C_I}$$

So even when the expression is simplified in such a way, we still have an expression for $[X]^*$, which is not linear. This gives an entirely different model. This means we cannot use the derivations in section 2.4.

It could be researched what the behaviour of this model is.

B   Index of notation

B	Buffer compound.
C_I	The concentration in the inside.
C_I^0	The specific C_I from which we derive a Taylor expansion.
C_I^*	C_I in steady state.
$C_I^0(C_O^0)$	A function C_I^0 of C_O^0 which represents the I-nullcline.
C_M	The concentration in the membrane.
C_M^*	The concentration in the membrane in quasi steady state as a function of C_O and C_I.
C_O	The concentration in the outside.
C_O^0	The specific C_O from which we derive a Taylor expansion.
C_O^*	C_O in steady state.
$\hat{C}_I$	$= K \cdot C_I$
e	Starting value of p.
e_M	Starting value of p_M.
ER	Endoplasmatic Reticulum.
f_{IM}	The amount of particles hitting the border of the membrane from the inside per unit of time.
f_{MI}	The amount of particles hitting the border of the inside from the membrane per unit of time.
f_{OM}	The amount of particles hitting the border of the membrane from the outside per unit of time.
f_{MO}	The amount of particles hitting the border of the outside from the membrane per unit of time.
F_I	The function of C_O and C_I that gives $\frac{dC_I}{dt}$.
F_O	The function of C_O and C_I that gives $\frac{dC_O}{dt}$.
I-nullcline	Given by $F_I = 0$.
J_I^-	The movement from the inside into the membrane.
J_I^+	The movement from the membrane into the inside.
J_O^-	The movement from the outside into the membrane.
J_O^+	The movement from the membrane into the outside.
J_X^+	The amount of X forming out of XB per unit of time.
J_X^-	The amount of X forming into XB per unit of time.
J_B^+	The amount of B forming out of XB per unit of time.
J_B^-	The amount of B forming onto XB per unit of time.
k_O	The slope of f_{OM} after assuming A3.
k_M	The slope of f_{MO} and f_{MI} after assuming A3.
k_I	The slope of f_{IM} after assuming A3.
k	Notation for $k = k_I = k_O$ after assuming (A6).
k_+	Reaction rate for binding of B.
k_-	Reaction rate for unbinding of XB.
$\hat{k}_+$	$= k_+ \cdot [B]$ for constant $[B]$.
K	The part of X that is free when the buffering reaction is in equilibrium.
M	$= [B] + [XB]$.
N	The total amount of particles in the system.

p_I	The probability that a particle dissolves into the inside given that this particle just hit the border of the inside, as a function of C_I.
p_M	The probability that a particle dissolves into the membrane given that this particle just hit the border of the membrane, as a function of C_M.
p_O	The probability that a particle dissolves into the outside given that this particle just hit the border of the outside, as a function of C_O.
p	Notation for $p = p_I = p_O$ after assuming (A6).
t	time.
V_O	The volume of the outside.
V_M	The volume of the membrane.
V_I	The volume of the inside.
X	The compound we consider.
XB	The compound X with bound buffer compound.
$[B]$	Concentration of B in the inside.
$[B]^*$	$[B]$ in quasi steady state as a function of C_I.
$[X]$	Concentration of X in the inside.
$[X]^*$	$[X]$ in quasi steady state as a function of C_I.
$[XB]$	Concentration of XB in the inside.
$[XB]^*$	$[XB]$ in quasi steady state as a function of C_I.

C   Bibliography

- Blom, T.J.M., Val, L., Moerkamp, L., Iren, F. van and Libbenga, K.R. (1991). Transport and accumulation of alkaloids in plant cells, *PhD-Thesis*, Leiden University.

- Keener, J. and Sneyd, J. (2004). Mathematical Physiology, Springer.

- Fall, C.P., Marland, E.S., Wagner, J.M. and Tyson, J.J. (2005). Computational Cell Biology, *Interdisciplinary Applied Mathemetics*, Springer.