MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume 10, Number 5&6, October & December 2013

doi:10.3934/mbe.2013.10.1541

pp. 1541–1560

MICHAELIS-MENTEN KINETICS, THE OPERATOR-REPRESSOR SYSTEM, AND LEAST SQUARES APPROACHES

Karl Peter Hadeler

Mathematics, University of Tübingen
Auf der Morgenstelle 10, 72076 Tübingen, Germany

Dedicated to Carlos Castillo-Chavez on the Occasion of his 60th Birthday

Abstract. The Michaelis-Menten (MM) function is a fractional linear function depending on two positive parameters. These can be estimated by non-linear or linear least squares methods. The non-linear methods, based directly on the defect of the MM function, can fail and not produce any minimizer. The linear methods always produce a unique minimizer which, however, may not be positive. Here we give sufficient conditions on the data such that the nonlinear problem has at least one positive minimizer and also conditions for the minimizer of the linear problem to be positive.

We discuss in detail the models and equilibrium relations of a classical operator-repressor system, and we extend our approach to the MM problem with leakage and to reversible MM kinetics. The arrangement of the sufficient conditions exhibits the important role of data that have a concavity property (chemically feasible data).

1. **Introduction.** Michaelis-Menten kinetics, 100 years after the original paper by Michaelis and Menten [5], and fundamental research by Briggs and Haldane [1], Segel and Slemrod [7], and many others, still poses interesting mathematical problems in singular perturbation theory and quasi-steady state approximation, in particular in the reversible case, see [6]. There are many other mathematical problems in enzyme kinetics, for example operator-repressor kinetics, [8] [4]. As the operator-repressor system is one example where least squares methods have been applied, we include an extended analysis of the kinetics and the equilibrium relations of that system and discuss the approximation given by Yagil & Yagil [8].

Our main goal is parameter identification. The Michaelis-Menten function depends on two positive parameters that can be fitted to data by a least squares approach. In general the least squares problem is not well-posed as the infimum may be assumed at zero or infinity. In [2] sufficient conditions for the existence of a minimizer have been given by studying the behavior of the goal function at the boundary of a two-dimensional domain, i.e., for parameter values near 0 and ∞ and subsequent use of the Chebyshev sum inequality. Already there it turned out that a concavity property of the data plays a role.

2010 *Mathematics Subject Classification.* Primary: 92C45, 93E24; Secondary: 34C12, 34D15.
Key words and phrases. Parameter identification, nonlinear least squares, enzyme kinetics, operator repressor kinetics.

Here we study the same problem and we apply two analytical tricks that had escaped our attention five years ago. The first trick: keep one parameter fixed and minimize the goal function with respect to the other. Then minimize the resulting function of one variable. We recover the results from [2] and rearrange them in such a way that the logical interdependence becomes obvious. The proofs get much shorter. The second trick: Rearrange the inequalities in such a way that the role of the concavity property (and hence the chemical or physical meaning of the required inequalities) becomes evident. It turns out that the Chebyshev sum inequality is not needed any more.

The novel approach can be extended to other functions that occur in enzyme kinetics. The first trick can be used to eliminate all parameters that occur linearly and to reduce the dimension of the least squares problem. The second trick of rearranging the data in ascending order leads to useful expressions for the derivative of the goal function and the formulas for optimal parameters.

In section 2 we review the reversible Michaelis-Menten (for short MM) kinetics using the results of [6], and in section 3 we extend the discussion of the operator-repressor system of [8] and [4]. In section 4 we define chemically feasible data. In section 5 we give conditions for the estimated parameters in a linear function to be positive. In section 6 we present the least squares approximation for the non-reversible MM, and in section 7 for decaying data. In section 8 we discuss problems with three parameters in general, and in sections 9, 10 least squares approximations for MM with leakage and reversible MM, respectively. Finally, in section 11 we study linear least squares approximations for the MM function. Most proofs are deferred to section 12. The paper closes with a discussion in section 13.

2. Michaelis-Menten kinetics.

We present a short derivation of the MM kinetics. An enzyme E binds to a substrate S to form a complex C, the complex splits into the enzyme and the final product P. Both reactions may be reversible,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P$$

These reactions are described by the following four differential equations,

$$\begin{aligned} \dot{s} &= -k_1 es + k_{-1}c \\ \dot{c} &= k_1 es - k_{-1}c - k_2 c + k_{-2}ep \\ \dot{p} &= k_2 c - k_{-2}ep \\ \dot{e} &= -k_1 es + k_{-1}c + k_2 c - k_{-2}ep. \end{aligned} \tag{2.1}$$

The total amount of enzyme e_0 and the total amount of substrate s_0 provide two invariants of motion

$$\begin{aligned} e + c &= e_0 \\ s + c + p &= s_0. \end{aligned} \tag{2.2}$$

These can be used to reduce the system to two equations for the enzyme and the substrate,

$$\begin{aligned} \dot{s} &= -k_1(e_0 - c)s + k_{-1}c \\ \dot{c} &= k_1(e_0 - c)s - k_{-1}c - k_2 c + k_{-2}(e_0 - c)(s_0 - s - c). \end{aligned} \tag{2.3}$$

We have a two-dimensional system. The positive quadrant $\mathbb{R}^2_+$ is positively invariant. The set $\mathcal{S} = \{(s, e) : 0 \le c \le e_0,\, s \ge 0,\, s + c \le s_0\}$ (the set of chemically meaningful solutions: a triangle or a convex quadrangle, depending on the parameters) is positively invariant and attracts all trajectories in $\mathbb{R}^2_+$. In $\mathbb{R}^2_+$ there is a single stationary point and no periodic orbit. The stationary point is a stable node and attracts all solutions in $\mathbb{R}^2_+$.

Chemists are interested in the short time behavior of the solution starting at $s = s_0 > 0$, $c = 0$ as it runs towards the stationary point. The MM idea is to describe this behavior by a scalar differential equation for the variable s alone.

The MM approach is based on the quasi steady state assumption $\dot{c} = 0$. Then the second equation in (2.3) becomes

$$0 = k_1(e_0 - c)s - k_{-1}c - k_2c + k_{-2}(e_0 - c)(s_0 - s - c). \tag{2.4}$$

In the classical case the second reaction is non-reversible, $k_{-2} = 0$. Then the stationary point is $(0, 0)$. In this case the equation (2.4) is linear in c. We can solve for c,

$$c = \frac{e_0k_1s}{k_1s + k_{-1} + k_2} \tag{2.5}$$

and insert this expression into the first equation of the system (2.3),

$$\dot{s} = -\frac{e_0k_2s}{s + \frac{k_{-1}+k_2}{k_1}}. \tag{2.6}$$

We replace $s, \dot{s}$ as

$$x = s, \quad y = -\frac{\dot{s}}{e_0}. \tag{2.7}$$

Then the equation (2.6) assumes the form of an MM function

$$y = \frac{ax}{b + x} \tag{2.8}$$

with

$$a = k_2, \quad b = \frac{k_{-1} + k_2}{k_1}. \tag{2.9}$$

In the reversible case (2.4) is a quadratic equation and the same procedure would lead to nasty expressions with square roots. If we write the $\dot{c}$ equation in (2.3) as

$$c = \frac{e_0(k_1s + k_2(s_0 - s)) - k_{-2}(e_0 - c)c - \dot{c}}{k_1s + k_{-1} + k_2 + k_{-2}(s_0 - s)} \tag{2.10}$$

and insert this expression into the $\dot{s}$ equation, then we get

$$\dot{s} = -\frac{e_0[k_1k_2s - k_{-1}k_{-2}(s_0 - s)] + (k_1s + k_{-1})(\dot{c} + k_{-2}(e_0 - c)c)}{k_1s + k_{-1} + k_2 + k_{-2}(s_0 - s)}. \tag{2.11}$$

In [6] it has been proved that in a quasi steady state approach not only $\dot{c}$ is small but also the term $k_{-2}c$ is small. Hence (2.11) can be reduced to

$$\dot{s} = -\frac{e_0[k_1k_2s - k_{-1}k_{-2}(s_0 - s)]}{k_1s + k_{-1} + k_2 + k_{-2}(s_0 - s)}. \tag{2.12}$$

We define three positive parameters

$$a = \frac{k_1k_2}{k_1 - k_{-2}} + \frac{k_{-1}}{k_1 - k_{-2}}k_{-2} \tag{2.13}$$

$$b = \frac{k_{-1} + k_2}{k_1 - k_{-2}} + \frac{1}{k_1 - k_{-2}}k_{-2}s_0 \tag{2.14}$$

$$c = \frac{k_{-1}}{k_1 - k_{-2}}k_{-2}s_0. \tag{2.15}$$

Again we use the notation (2.7). Then the equation (2.12) has the form

$$y = \frac{ax - c}{b + x}. \tag{2.16}$$

The parameter c has nothing to do with the variable c in the differential equations.

We shall also consider the function that describes MM kinetics with "leakage",

$$y = \frac{ax}{b + x} - c \tag{2.17}$$

with positive parameters a, b, c.

3. **Operator-repressor dynamics.**

The reaction (following the Jacob-Monod approach to inducible systems)

$$R + nE \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C, \qquad R + O \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} D \tag{3.1}$$

describes an operator O and an effector E "competing" for a repressor R to form complexes $C = E_n R$ (of one unit of R and n units of E; so $E_n R$ is the standard notation, as in H_2O for water), and $D = OR$. A classical reference for this system is [8]. The goal of the paper [8] is to find "a simple relation connecting the rate of enzyme synthesis with effector concentration". Indeed the authors found a linear approximative relation between some proportions that can be used to determine reaction constants by least squares fitting. However, this relation has been derived under a certain smallness assumption that may be not justified. Here we derive the exact (nonlinear) relation from the kinetic equations and show that the approximation is valid under a different smallness assumption.

The authors [8] discuss whether a system with a very small number of molecules (up to four operators per cell) can be described by equilibrium equations (or differential equations in the present case). Their answer is affirmative in case the experiment is performed with a large number of cells.

3.1. **The approach of Yagil & Yagil.**

We follow [8], with $K_i = k_{-i}/k_i$. The total amount of operator is

$$[O_t] = [O] + [OR] \tag{3.2}$$

and the total amount of repressor is

$$[R_t] = [R] + [E_n R] \quad (+[OR]) \tag{3.3}$$

where $[OR]$ is considered negligible as compared to $[E_n R]$ and $[R]$. This is an approximation, the term $+[OR]$ is omitted. Here is the critical step in the derivation of [8]: Is $[OR]$ small against $[R]$ and $[E_n R]$?

We have two equilibrium equations

$$K_1 = \frac{[R][E]^n}{[E_n R]}, \quad K_2 = \frac{[O][R]}{[OR]}. \tag{3.4}$$

We multiply equation (3.3) by K_1 and replace the expression $K_1[E_n R]$ from the first equation in (3.4),

$$K_1[R_t] = K_1[R] + [R][E]^n$$

and hence

$$[R] = \frac{K_1[R_t]}{K_1 + [E]^n}. \tag{3.5}$$

We consider the proportion of free operator $[O]/[O_t]$ and of bound operator $[OR]/[O_t]$. We form the quotient, use the second equation in (3.4), and then (3.5),

$$\frac{[O]}{[OR]} = \frac{[O]K_2}{[O][R]} = \frac{K_2}{[R]} = K_2 \frac{K_1 + [E]^n}{K_1[R_t]} = \frac{K_2}{[R_t]} + \frac{K_2}{K_1[R_t]}[E]^n. \tag{3.6}$$

Thus, we have a relation between the quotient and the effector,

$$\frac{[O]}{[OR]} = \frac{K_2}{[R_t]} + \frac{K_2}{K_1[R_t]}[E]^n. \tag{3.7}$$

The authors [8] take logarithms,

$$\log\left(\frac{[O]}{[OR]} - \frac{K_2}{[R_t]}\right) = \log\frac{K_2}{[R_t]} - \log K_1 + n\log[E] \tag{3.8}$$

and use this formula for a least squares approach. The goal is to check the validity of the model and to find n and K_1 under the condition that $K_2/[R_t]$ is known. Notice the difference: the functions in section 2 describe some time course and the present function describes some relation at equilibrium. The authors [8] have applied the formula to a large set of experimental data (including data on the lac operon) and have found that in many cases plotting the left hand side of (3.8) against $\log[E]$ produced a straight line with small deviations, whereby typically the value of n turned out to be about two.

3.2. **The complete dynamics.** We formulate the system of kinetic equations for the reaction (3.1), investigate their dynamic behavior, and find the only stationary point. We write $e = [E]$, $r = [R]$, $c = [E_nR]$, $d = [OR]$, $o = [O]$. The reactions are described by five differential equations

$$\begin{aligned}
\dot{e} &= -nk_1e^nr + nk_{-1}c \\
\dot{r} &= -k_1e^nr + k_{-1}c - k_2or + k_{-2}d \\
\dot{c} &= k_1e^nr - k_{-1}c \\
\dot{d} &= k_2or - k_{-2}d \\
\dot{o} &= -k_2or + k_{-2}d.
\end{aligned} \tag{3.9}$$

There are three invariants of motion for the three constitutive species r, e, o,

$$\begin{aligned}
r + c + d &= r_0 \\
ne + c &= e_0 \\
o + d &= o_0.
\end{aligned} \tag{3.10}$$

The invariants can be used to eliminate all variables except e and o from (3.9). We are left with a two-dimensional system for the effector e and the operator o,

$$\begin{aligned}
\dot{e} &= -nk_1r_0e^n + (nk_1e^n + nk_{-1})(e_0 - ne) + nk_1e^n(o_0 - o) \\
\dot{o} &= -k_2r_0o + (k_2o + k_{-2})(o_0 - o) + k_2o(e_0 - ne).
\end{aligned} \tag{3.11}$$

The nonlinearity depends on all chemical constants and the total effector e_0, the total operator o_0, and the total repressor r_0.

The system (3.11) is a two-dimensional competitive system. From the general theory of cooperative and competitive systems (see [3]) we know that every bounded trajectory goes to equilibrium. The rectangle $\{(e, o) : 0 < e < e_0,\ 0 < o < o_0\}$ is positively invariant and attracts all trajectories from $\mathbb{R}^2_+$. In the rectangle there is a single stationary point (for a proof see subsection 3.5). Hence this point is globally attracting.

3.3. **The exact formula.** At any stationary point of (3.11) we have the two equations

$$\begin{aligned} 0 &= -k_1 r_0 e^n + (k_1 e^n + k_{-1})(e_0 - ne) + k_1 e^n (o_0 - o) \\ 0 &= -k_2 r_0 o + (k_2 o + k_{-2})(o_0 - o) + k_2 o (e_0 - ne). \end{aligned} \tag{3.12}$$

As in the approach of Yagil & Yagil we want a chemically meaningful relation between e and o at the equilibrium without knowing the total amount e_0 of effector. We can form *arbitrary* combinations of the two equations in (3.12) to get single equations for these unknowns but there is only one that is independent of the total amount of effector e_0. We solve in both equations of (3.12) for $e_0 - ne$ and equate the expressions,

$$\frac{k_1 r_0 e^n - k_1 e^n (o_0 - o)}{k_1 e^n + k_{-1}} = \frac{k_2 r_0 o - (k_2 o + k_{-2})(o_0 - o)}{k_2 o}. \tag{3.13}$$

In this way we obtain a relation at equilibrium between o and e^n that depends only on the total amount of operator o_0, the reaction constants, and the total amount of repressor r_0. The relation (3.13) holds for any amount e_0 of total effector. The equation (3.13) can be reformulated as follows,

$$\frac{o}{o_0 - o} = \frac{1}{r_0 - (o_0 - o)} \frac{k_{-2}}{k_2} \left(1 + \frac{k_1}{k_{-1}} e^n\right). \tag{3.14}$$

On the left hand side we have the quotient $[O]/[OR]$. The right hand side depends on $[OR] = o_0 - o$. If we assume that $o_0 - o$ is small against r_0 then we can neglect the term $o_0 - o$ and get

$$\frac{o}{o_0 - o} = \frac{1}{r_0} \frac{k_{-2}}{k_2} \left(1 + \frac{k_1}{k_{-1}} e^n\right) \tag{3.15}$$

which is (3.7). To render this approximation meaningful we should estimate the error in terms of some small parameter. We choose o_0 as a parameter.

In (3.13) we solve for e^n and get

$$e^n = F(o) \equiv \frac{k_{-1}}{k_1} \frac{k_2}{k_{-2}} \frac{r_0 o - (\frac{k_{-2}}{k_2} + o)(o_0 - o)}{o_0 - o}. \tag{3.16}$$

The right hand side is negative for small values of $o/(o_0 - o)$. The behavior of the function F and its inverse is described in the following proposition.

Proposition 3.1. *The amount of free effector e can be expressed as an explicit function of the amount of free operator as in (3.16). There is a value $\bar{o} \in (0, o_0)$ such that $F(o) < 0$ for $0 < o < \bar{o}$ and $F(o) > 0$ for $\bar{o} < o < o_0$. The function $F : (\bar{o}, o_0) \to (0, \infty)$ is onto and strictly increasing, hence invertible.*

The inverse function $G : (0, \infty) \to (\bar{o}, o_0)$ is given by

$$o = G(e^n) \equiv \frac{1}{2} \left[\sqrt{(r_0 + Q(e^n) - o_0)^2 + 4Q(e^n) o_0} - (r_0 + Q(e^n) - o_0)\right] \tag{3.17}$$

where

$$Q(e^n) = \frac{k_{-2}}{k_2}(1 + \frac{k_1}{k_{-1}} e^n). \tag{3.18}$$

Proof. The function F is a rational function, the degree of the numerator is 2, the degree of the denominator is 1. There is one pole, at $o = o_0$. Since the numerator is negative for $o = 0$, there is one negative zero $\underline{o}$, and one positive zero $\bar{o}$. Since the numerator is positive for $o = o_0$, we have $0 < \bar{o} < o_0$. The derivative F' has the denominator $(o_0 - o)^2$, the numerator is a quadratic polynomial. Since the function F goes to $+\infty$ for $o \to -\infty$ and to $-\infty$ for $o \to +\infty$, it has a minimum at some point in $(\underline{o}, \bar{o})$ and a maximum in (o_0, ∞). The derivative F' has no other zeros and we see, without further calculation, that the function F is strictly increasing in $(\bar{o}, o_0)$.

The equation $F(o) = e^n$ is equivalent with the equation

$$\frac{k_{-2}}{k_2}\left(1 + \frac{k_1}{k_{-1}} e^n\right) = \frac{r_0 - o_0(1 - \frac{o}{o_0})}{1 - \frac{o}{o_0}} \frac{o}{o_0}. \tag{3.19}$$

If we put

$$x = \frac{o}{o_0}, \quad \epsilon = o_0, \tag{3.20}$$

then (3.19) becomes

$$r_0 x - \epsilon x(1 - x) = Q(e^n)(1 - x). \tag{3.21}$$

Now compute the larger root of this quadratic equation and replace x, ϵ from (3.20). □

The expression (3.17) is complicated and the expression for $o/(o_0 - o)$ is even more complicated. Furthermore, the latter depends explicitly on o_0 which usually cannot be measured. The formula (3.7) of Yagil & Yagil is an approximation that is independent of o_0. We want to find out in what sense it is an approximation.

3.4. **Discussion of the approximation.** We show the following proposition.

Proposition 3.2. *The formula (3.7), equivalently (3.15), of Yagil & Yagil is an approximation for small amounts of total operator. It gives the proportion $o/(o_0 - o)$ in the limit where the total amount o_0 goes to zero. The next term in the expansion in powers of o_0 is given in*

$$\frac{o}{o_0 - o} = \frac{1}{r_0} \frac{k_{-2}}{k_2}\left(1 + \frac{k_1}{k_{-1}} e^n\right)\left(1 + \frac{1}{r_0 + \frac{k_{-2}}{k_2}(1 + \frac{k_1}{k_{-1}} e^n)} o_0 + \cdots\right). \tag{3.22}$$

The formula (3.15) is an approximation for small o_0. Although o_0 is small, the proportion o/o_0 need not be small.

Proof. In equation (3.21) we put $\epsilon = 0$ and solve for x. The first term of the expansion is $Q/(r_0 + Q)$ with $Q = Q(e^n)$. In (3.21) differentiate with respect to ϵ,

$$r_0 x_\epsilon - x(1 - x) - \epsilon x_\epsilon (1 - x) + \epsilon x x_\epsilon = -Q x_\epsilon, \tag{3.23}$$

put $\epsilon = 0$ and solve for x_ϵ. The second term of the expansion is $Q r_0/(r_0 + Q)^3$. From the expansion

$$x = \frac{Q}{r_0 + Q} + \frac{Q r_0}{(r_0 + Q)^3}\epsilon + \cdots \tag{3.24}$$

find

$$\frac{x}{1 - x} = \frac{Q}{r_0} + \frac{Q}{r_0(Q + r_0)} o_0 + \cdots, \tag{3.25}$$

replace $Q = Q(e^n)$ and x as before. □

We see that [8] got the right result with a somewhat weak argument. We are not allowed to assume that $d = [OR]$ is small without making assumptions on other quantities. However, if we assume that $o_0 = [O_t]$ is small against $r_0 = [R_t]$ then also $o = [O]$ and $d = [OR]$ become small and the formula (3.15) is justified. Mathematically, the formula (3.22) is an improvement of (3.15). However, it can only be used if o_0 is known.

In [4] the formula (3.15) is used to describe gene regulatory dynamics (with different notation, $K_i = k_i/k_{-i}$, the formulas look slightly different).

3.5. **Uniqueness of the stationary state.** We show that the system (3.11) has a unique stationary state. From (3.16) and the second equation in (3.12) we have two equations for the stationary state (these together are equivalent to the equations (3.12)),

$$e = \left[\frac{k_{-1}}{k_1} \frac{k_2}{k_{-2}} \frac{r_0 o - (\frac{k_{-2}}{k_2} + o)(o_0 - o)}{o_0 - o} \right]^{1/n} \tag{3.26}$$

$$e = \frac{1}{no} \left[e_0 o - \left[r_0 o - (\frac{k_{-2}}{k_2} + o) \right] \right]. \tag{3.27}$$

The square bracket in (3.26) is negative for small $o > 0$ and positive and increasing in $(\bar{o}, o_0)$ where $\bar{o}$ is the positive solution of

$$(\frac{k_{-2}}{k_2} + o)(o_0 - o) = r_0 o. \tag{3.28}$$

The function (3.27) is large and positive for small $o > 0$ and is decreasing in $(0, o_0)$. If it has a positive zero o then

$$(\frac{k_{-2}}{k_2} + o)(o_0 - o) = (r_0 - e_0) o \tag{3.29}$$

and hence this zero is in $(\bar{o}, \infty)$. Therefore the graphs of (3.26) and (3.27) have a unique intersection in $(0, o_0)$.

4. **Chemically feasible data.** The Michaelis-Menten function (2.8) depends on two positive parameters a, b that can be fitted to data. The function is positive, increasing and concave with respect to zero, i.e., $y(x)/x$ is a decreasing function. Data to be fitted by a MM function should reflect these properties as follows. The x_i are ordered as

$$0 < x_1 \leq x_2 \leq \cdots \leq x_n, \quad x_1 < x_n. \tag{4.1}$$

The y_i are non-decreasing,

$$0 < y_1 \leq y_2 \leq \cdots \leq y_n. \tag{4.2}$$

The quotients are non-increasing,

$$\frac{y_1}{x_1} \geq \frac{y_2}{x_2} \geq \cdots \geq \frac{y_n}{x_n}. \tag{4.3}$$

We require that there is at least one pair such that

$$i > k, \quad x_i > x_k, \quad y_i > y_k, \tag{4.4}$$

and at least one pair such that

$$i > k, \quad x_i > x_k, \quad \frac{y_i}{x_i} < \frac{y_k}{x_k}. \tag{4.5}$$

We call such data chemically feasible. In the next sections we show various sufficient conditions for positivity and existence of minimizers. These conditions are satisfied for chemically feasible data. Hence the notion of chemically feasible data appears quite natural in connection with the MM problem.

In the following there are many formulas that involve sums. These formulas become clumsy unless we use a simplified notation. Our subscripts run always from 1 to n but may be restricted by some inequalities. We use

$$\sum_i \quad \sum_{i,k} \quad \sum_{i>k} \quad \text{for} \quad \sum_{i=1}^n \quad \sum_{i,k=1}^n \quad \sum_{\substack{i,k=1\\ i>k}}^n .$$

5. **Fitting a linear function.** Here we show that the notion of chemically feasible data is useful even in the case of a linear function as it yields a criterion for positivity of the estimated parameters.

We estimate the parameters of the function $y = ax+b$ by a least squares method, i.e., we minimize the expression

$$F = \sum_i p_i(ax_i + b - y_i)^2 \tag{5.1}$$

over $a, b \in \mathbb{R}$. Here the p_i are given positive weights. We assume (4.1).

Proposition 5.1. *The optimal parameters are*

$$\begin{aligned} a &= \frac{\sum_{i>k} p_i p_k (x_i - x_k)(y_i - y_k)}{\sum_{i>k} p_i p_k (x_i - x_k)^2} \\ b &= \frac{\sum_{i>k} p_i p_k x_i x_k (x_i - x_k)(\frac{y_k}{x_k} - \frac{y_i}{x_i})}{\sum_{i>k} p_i p_k (x_i - x_k)^2}. \end{aligned} \tag{5.2}$$

Corollary 5.1. *Suppose that the data are chemically feasible. Then the estimated parameters (5.2) are positive.*

It is surprising that the conditions that appear relevant for the MM problem (see the next section) also play a role in fitting a linear function. Of course the conditions are not necessary for a, b to be positive.

6. **Least squares: Michaelis-Menten.** Here we consider the least-squares problem for the MM function (2.8). Suppose we have data (x_i, y_i), with (4.1) and $y_i > 0$ for $i = 1, \ldots, n$. We want to fit a MM curve (2.8) to these data with a least squares approach, i.e., we want to minimize the expression

$$F = \sum_i p_i \left(\frac{ax_i}{b + x_i} - y_i\right)^2 \tag{6.1}$$

in the range $a, b > 0$, where the $p_i > 0$ are some given weights.

In [2] the function F has been minimized on the two-dimensional set of parameters $a, b > 0$. Here we fix the parameter b and take the minimum over a. This step is easily done as the function F is quadratic in a. There is a unique minimizer $\hat{a}(b)$ (which turns out to be positive),

$$\hat{a}(b) = \frac{\sum_i p_i \frac{x_i y_i}{b+x_i}}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2}}. \tag{6.2}$$

We introduce this value into (6.1) and get

$$F = \sum_i p_i y_i^2 - \phi(b) \tag{6.3}$$

where

$$\phi(b) = \frac{(\sum_i p_i \frac{x_i y_i}{b+x_i})^2}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2}}. \tag{6.4}$$

Minimizing F amounts to maximizing $\phi(b)$ on $0 < b < \infty$. Our problem is that the function $\phi(b)$ may assume its supremum at $b = 0$ or for $b \to \infty$ such that no feasible maximizer exists. The next theorem gives two sets of sufficient conditions for the existence of a minimizer of the function F.

Theorem 6.1. *Suppose any of the two sets of two inequalities is satisfied:*
i)

$$\frac{\sum_i p_i y_i}{\sum_i p_i} > \frac{\sum_i p_i \frac{y_i}{x_i}}{\sum_i p_i \frac{1}{x_i}} \quad \text{and} \quad \frac{(\sum_i p_i x_i y_i)^2}{\sum_i p_i x_i^2} \le \frac{(\sum_i p_i y_i)^2}{\sum_i p_i} \tag{6.5}$$

ii)

$$\frac{(\sum_i p_i x_i y_i)^2}{\sum_i p_i x_i^2} \ge \frac{(\sum_i p_i y_i)^2}{\sum_i p_i} \quad \text{and} \quad \frac{\sum_i p_i x_i^2 y_i}{\sum_i p_i x_i^3} < \frac{\sum_i p_i x_i y_i}{\sum_i p_i x_i^2}. \tag{6.6}$$

Then the function F has at least one minimizer with $a, b > 0$.

An immediate consequence is the following theorem.

Theorem 6.2. *Suppose the following two inequalities are satisfied*

$$\frac{\sum_i p_i y_i}{\sum_i p_i} > \frac{\sum_i p_i \frac{y_i}{x_i}}{\sum_i p_i \frac{1}{x_i}} \quad \text{and} \quad \frac{\sum_i p_i x_i^2 y_i}{\sum_i p_i x_i^3} < \frac{\sum_i p_i x_i y_i}{\sum_i p_i x_i^2}. \tag{6.7}$$

Then the function F has at least one minimizer with $a, b > 0$.

Theorem 6.1 has been shown in [2]. Theorem 6.2 follows from Theorem 6.1. But Theorem 6.1 is the stronger result. One can construct examples for $n = 3$ such that (6.5) or (6.6) is satisfied but not (6.7). On the other hand, Theorem 6.2 covers the case of chemically feasible data, as will be shown below.

If there are only two data points, $n = 2$, then the least squares problem becomes an interpolation problem. The exact values for a and b are

$$b = \frac{y_2 - y_1}{\frac{y_1}{x_1} - \frac{y_2}{x_2}}, \quad a = \frac{\frac{1}{x_1} - \frac{1}{x_2}}{\frac{y_1}{x_1} - \frac{y_2}{x_2}} y_1 y_2. \tag{6.8}$$

The values a, b are positive if and only if the data are chemically feasible.

In [2] it has been shown, using the Chebyshev sum inequalities, that chemically feasible data satisfy (6.7). Here we give a much more transparent proof based on the following proposition.

Proposition 6.1. *The stationary points of the function $\phi(b)$ are the zeros of the function*

$$\sum_{i>k} p_i p_k \frac{x_i^2 x_k^2 (x_i - x_k)}{(b + x_i)^3 (b + x_k)^3} \left[y_i - y_k - b \left(\frac{y_k}{x_k} - \frac{y_i}{x_i} \right) \right]. \tag{6.9}$$

If the function (6.9) changes from positive to negative at some value b then this b is a local maximum of the function ϕ.

Any zero b of the function (6.9) satisfies

$$b = \frac{\sum_{i>k} q_{ik}(y_i - y_k)}{\sum_{i>k} q_{ik} \left(\frac{y_k}{x_k} - \frac{y_i}{x_i} \right)}, \quad \text{with} \quad q_{ik} = p_i p_k \frac{x_i^2 x_k^2 (x_i - x_k)}{(b + x_i)^3 (b + x_k)^3}. \tag{6.10}$$

Evidently, terms with $x_i = x_k$ drop out. This fact does not imply that the optimal b is independent of such data, since the weights q_{ik} depend on the unknown b. From the properties of generalized arithmetic means it follows: If all inequalities (4.3) are strict then any minimizer satisfies the inequality

$$\min_{i>k} \frac{y_i - y_k}{\frac{y_k}{x_k} - \frac{y_i}{x_i}} \le b \le \max_{i>k} \frac{y_i - y_k}{\frac{y_k}{x_k} - \frac{y_i}{x_i}}. \tag{6.11}$$

Thus, in the case of chemically feasible data, we can restrict the search for b to a bounded interval (6.11) depending only on the given data.

Proposition 6.1 allows us to reformulate the condition iii) of theorem 6.1.

Theorem 6.3. *Suppose that the inequalities*

$$\sum_{i>k} p_i p_k \left(\frac{1}{x_k} - \frac{1}{x_i} \right) (y_i - y_k) > 0 \tag{6.12}$$

$$\sum_{i>k} p_i p_k x_i^2 x_k^2 (x_i - x_k) \left(\frac{y_k}{x_k} - \frac{y_i}{x_i} \right) > 0 \tag{6.13}$$

are satisfied. Then the least squares problem has a solution.

This theorem allows immediate application to chemically feasible data. Indeed, for such data each term of the sum, apart from a positive factor, is either zero or a linear function that decreases from positive to negative values.

Corollary 6.1. *Suppose the data are chemically feasible. Then the least squares problem (6.1) has a solution.*

It seems nearly impossible to find a useful criterion for uniqueness of the minimizer of F. However, once the inequalities (4.3) are strict, it is easy to find the maximum of $\phi(b)$ in the bounded interval (6.11) for b by some numerical method.

7. Least squares: Decaying function.

Here we extend our findings to the function

$$y = \frac{a}{b + x}. \tag{7.1}$$

We minimize

$$F = \sum_i p_i \left(\frac{a}{b + x_i} - y_i\right)^2. \tag{7.2}$$

For the MM function we had found that the conditions for chemically feasible data describe in discrete terms the behavior of the expected function. Can we find a similar set of inequalities for the present problem? As compared to (2.8) the present function (7.1) has opposite properties: the function y is decreasing and the function $y(x)x$ is increasing. Hence we expect that we should require the following inequalities for the data

$$\begin{aligned} & y_1 \ge y_2 \ge \cdots \ge y_n > 0 \\ & x_1 y_1 \le x_2 y_2 \le \cdots \le x_n y_n \end{aligned} \tag{7.3}$$

with the additional property that there is a pair $x_i > x_k$ such that $y_i < y_k$ and also a pair $x_i > x_k$ such that $x_i y_i < x_k y_k$. We call such data feasible for the problem (7.1). If there are only two data points, $n = 2$, then the least squares problem becomes an interpolation problem and the exact values for a, b are

$$b = \frac{x_2 y_2 - x_1 y_1}{y_1 - y_2}, \quad a = \frac{y_1 y_2 (x_2 - x_1)}{y_1 - y_2}. \tag{7.4}$$

If the inequalities (7.3) are strictly satisfied, then a, b are positive.

We keep b fixed and determine the optimal a, given b. After similar steps as in section 6 we get an expression for the optimal a,

$$\hat{a}(b) = \frac{\sum_i p_i \frac{y_i}{b+x_i}}{\sum_i p_i \frac{1}{(b+x_i)^2}} \tag{7.5}$$

and then we maximize the functional

$$\phi(b) = \frac{(\sum_i p_i \frac{y_i}{b+x_i})^2}{\sum_i p_i \frac{1}{(b+x_i)^2}}. \tag{7.6}$$

The first result is an analogue of proposition 6.1.

Proposition 7.1. *The stationary points of the function $\phi(b)$ are the zeros of the function*

$$\sum_{i>k} p_i p_k \frac{x_i - x_k}{(b+x_i)^3(b+x_k)^3} \left[x_i y_i - x_k y_k - b(y_k - y_i)\right]. \tag{7.7}$$

If the function (7.7) changes from positive to negative at some b, then this b is a local maximum of $\phi(b)$.

An immediate consequence is the following result.

Theorem 7.1. *Suppose the following inequalities are satisfied:*

$$\sum_{i>k} p_i p_k \frac{x_i - x_k}{x_i^3 x_k^3} (x_i y_i - x_k y_k) > 0 \tag{7.8}$$

$$\sum_{i>k} p_i p_k (x_i - x_k)(y_k - y_i) > 0. \tag{7.9}$$

Then the functional F has a minimum.

Again we have a result for feasible data.

Corollary 7.1. *Let the data be feasible in the sense of (7.3). Then the function F has a minimum.*

8. **Three-parameter problems.** In the next two sections we treat problems related to the general fractional linear function $(a_{11}x + a_{12})/(a_{12}x + a_{22})$. Although this formula depends on four parameters, it really represents a three-parameter family of functions: The function does not change if numerator and denominator are multiplied with the same factor. However, the family of functions cannot be represented as a smooth three-parameter family. This mathematical difficulty shows up also in the least squares problem: it makes a difference which three parameters are subject to variation. We are interested in two special cases,

$$y = \frac{ax}{b+x} - c, \tag{8.1}$$

and

$$y = \frac{\alpha x - \gamma}{\beta + x}. \tag{8.2}$$

Of course, for $b, \beta > 0$ these formulas are equivalent if we identify parameters

$$\beta = b, \quad \alpha = a - c, \quad \gamma = bc. \tag{8.3}$$

Hence the two least squares problems

$$F = \sum_i p_i \left(\frac{a x_i}{b + x_i} - c - y_i \right)^2 \tag{8.4}$$

$$F = \sum_i p_i \left(\frac{\alpha x_i - \gamma}{\beta + x_i} - y_i \right)^2 \tag{8.5}$$

for $\beta, b > 0$, $\alpha, \gamma, a, c \in \mathbb{R}$ are equivalent. If one of the problems has a minimizer then (8.3) yields a minimizer for the other.

However, if we fix $\beta = b > 0$ and minimize over $a, c \in \mathbb{R}$ or $\alpha, \gamma \in \mathbb{R}$, respectively, then we get different functions of one parameter $\beta = b$. This fact becomes very clear if we look at the two minimization problems for constant $\beta = b > 0$ in a more abstract manner. In (8.4) we minimize over all linear combinations of the functions $x/(b + x)$ and -1 while in (8.5) we minimize over all linear combinations of $x/(\beta + x)$ and $-1/(\beta + x)$. There is no reason why the minimizers should be the same function.

The problem (8.4) occurs in what is called MM with leakage (2.17) while (8.5) shows up in connection with the reversible MM kinetics (2.16).

9. **Least squares: MM with leakage.** The MM function with leakage (2.17) depends on three positive parameters a, b, c. The least squares function is given in (8.4). For positive a, b the function (2.17) is increasing. Therefore we assume that the data have the properties stated in (4.1) and (4.2). Since the function F is linear in a and c, we can fix b and then find the minimum of F over a, c, given b.

Proposition 9.1. *The minimization problem over a, b, c for the function F is equivalent to the maximization problem for the function $\phi(b)$ over b, where*

$$\phi(b) = \frac{\left(\sum_{i>k} p_i p_k \frac{(x_i - x_k)(y_i - y_k)}{(b + x_i)(b + x_k)} \right)^2}{\sum_{i>k} p_i p_k \frac{(x_i - x_k)^2}{(b + x_i)^2 (b + x_k)^2}}. \tag{9.1}$$

Once an optimal b has been found, then the corresponding value of a is

$$\hat{a}(b) = \frac{\sum_{i>k} p_i p_k \frac{(x_i - x_k)(y_i - y_k)}{(b + x_i)(b + x_k)}}{\sum_{i>k} p_i p_k \frac{(x_i - x_k)^2}{(b + x_i)^2 (b + x_k)^2}} \tag{9.2}$$

and finally the value for c is

$$\hat{c}(b) = \hat{a}(b) \sum_i p_i \frac{x_i}{b + x_i} - \sum_i p_i y_i. \tag{9.3}$$

Clearly the value $\hat{a}(b)$ is positive. However, the value $\hat{c}(b)$ need not be positive. We do not have an *a priori* condition on the data that would ensure positivity of $\hat{c}(b)$. Next we give a sufficient condition for the existence of a maximizer.

Theorem 9.1. *Suppose that the data satisfy the inequalities*

$$\begin{aligned} \frac{\sum_{i>k} p_i p_k (x_i^2 - x_k^2)(y_i - y_k)}{\sum_{i>k} p_i p_k (x_i^2 - x_k^2)(x_i - x_k)} &< \frac{\sum_{i>k} p_i p_k (x_i - x_k)(y_i - y_k)}{\sum_{i>k} p_i p_k (x_i - x_k)(x_i - x_k)} \\ \frac{\sum_{i>k} p_i p_k (\frac{1}{x_k^2} - \frac{1}{x_i^2})(y_i - y_k)}{\sum_{i>k} p_i p_k (\frac{1}{x_k^2} - \frac{1}{x_i^2})(\frac{1}{x_k} - \frac{1}{x_i})} &< \frac{\sum_{i>k} p_i p_k (\frac{1}{x_k} - \frac{1}{x_i})(y_i - y_k)}{\sum_{i>k} p_i p_k (\frac{1}{x_k} - \frac{1}{x_i})(\frac{1}{x_k} - \frac{1}{x_i})}. \end{aligned} \tag{9.4}$$

Then the functional $\phi(b)$ in (9.1) has a maximizer.

10. **Least squares: Reversible MM.** For the reversible MM function (2.16), compare also (8.5), the goal function is

$$F = \sum_i p_i \left(\frac{ax_i - c}{b + x_i} - y_i \right)^2 . \tag{10.1}$$

For fixed $b > 0$ the minimizer is $\hat{a}(b)$, $\hat{c}(b)$ where

$$\hat{a}(b) = \frac{\sum_{i>k} p_i p_k \frac{x_i - x_k}{(b+x_i)^2(b+x_k)^2}[b(y_i - y_k) + x_i y_i - x_k y_k]}{\sum_{i>k} p_i p_k \frac{(x_i - x_k)^2}{(b+x_i)^2(b+x_k)^2}} \tag{10.2}$$

$$\hat{c}(b) = \frac{\sum_{i>k} p_i p_k \frac{x_i x_k (x_i - x_k)}{(b+x_i)^2(b+x_k)^2}[b(\frac{y_i}{x_i} - \frac{y_k}{x_k}) + y_i - y_k]}{\sum_{i>k} p_i p_k \frac{(x_i - x_k)^2}{(b+x_i)^2(b+x_k)^2}}. \tag{10.3}$$

Proposition 10.1. *The minimization problem over a, b, c for the function F is equivalent to the maximization problem for the function $\phi(b)$ over b, where*

$$\phi(b) =$$
$$\frac{\sum_i \frac{p_i}{(b+x_i)^2} (\sum_i \frac{p_i x_i y_i}{b+x_i})^2 + 2 \sum_i \frac{p_i x_i}{(b+x_i)^2} \sum_i \frac{p_i x_i y_i}{b+x_i} \sum_i \frac{p_i y_i}{b+x_i} + \sum_i \frac{p_i x_i^2}{(b+x_i)^2} (\sum_i \frac{p_i y_i}{b+x_i})^2}{\sum_i \frac{p_i x_i^2}{(b+x_i)^2} \sum_i \frac{p_i}{(b+x_i)^2} - (\sum_i \frac{p_i x_i}{(b+x_i)^2})^2}. \tag{10.4}$$

Suppose b is a maximizer. If the y_i are increasing as in (4.2) then $\hat{a}(b) > 0$. We want a meaningful condition on the data such that also $\hat{c}(b) > 0$. The function

$$\frac{b+x}{x} y(x) = \frac{b+x}{x} \frac{ax - c}{b+x} = a - \frac{c}{x}$$

is increasing. Hence we require that the data satisfy, with this particular b,

$$i > k \Rightarrow \frac{b+x_i}{x_i} y_i > \frac{b+x_k}{x_k} y_k. \tag{10.5}$$

Corollary 10.1. *Let b be a maximizer of ϕ. Suppose that the data satisfy (4.1) and (10.5). Then $\hat{a}(b) > 0$, $\hat{c}(b) > 0$.*

11. **Linear least squares again.** As has been observed in [2], the parameters can also be estimated from linear least squares problems. But then the estimated parameters need not be positive. However, as it turns out, in some cases the conditions for positivity of the solution to the linear problem are very similar to the conditions of existence for the nonlinear problem. In particular for the standard MM, for chemically feasible data, all solutions exist and are meaningful. The standard MM function (2.8) leads to

$$G = \sum_i p_i (a x_i - b y_i - x_i y_i)^2. \tag{11.1}$$

Proposition 11.1. *The solution of the least squares problem (11.1) is*

$$\hat{a} = \frac{\sum_{i>k} p_i p_k x_i x_k y_i y_k (x_i - x_k) \left(\frac{y_k}{x_k} - \frac{y_i}{x_i} \right)}{\sum_{i>k} p_i p_k x_i^2 x_k^2 \left(\frac{y_k}{x_k} - \frac{y_i}{x_i} \right)^2} \tag{11.2}$$

$$\hat{b} = \frac{\sum_{i>k} p_i p_k x_i^2 x_k^2 (y_i - y_k)\left(\frac{y_k}{x_k} - \frac{y_i}{x_i}\right)}{\sum_{i>k} p_i p_k x_i^2 x_k^2 \left(\frac{y_k}{x_k} - \frac{y_i}{x_i}\right)^2}. \tag{11.3}$$

If the data are chemically feasible in the sense of section 4 then $\hat{a}, \hat{b} > 0$.

The function (7.1) leads to

$$G = \sum_i p_i (a - b y_i - x_i y_i)^2. \tag{11.4}$$

Proposition 11.2. *The solution to the least squares problem (11.4) is*

$$\hat{a} = \frac{\sum_{i>k} p_i p_k y_i y_k (x_i - x_k)(y_k - y_i)}{\sum_{i>k} p_i p_k (y_k - y_i)^2} \tag{11.5}$$

$$\hat{b} = \frac{\sum_{i>k} p_i p_k (y_k - y_i)(x_i y_i - x_k y_k)}{\sum_{i>k} p_i p_k (y_k - y_i)^2}. \tag{11.6}$$

If the data are chemically feasible in the sense of (7.3) then $\hat{a}, \hat{b} > 0$.

The reversible MM function (2.16) leads to

$$G = \sum_i p_i (a x_i - b y_i - c - x_i y_i)^2. \tag{11.7}$$

Although the optimal parameters can be easily expressed in terms of determinants using Cramer's rule, there seems to be no way to get conditions on positivity.

12. **Proofs.** The proof of proposition 5.1 is straightforward. The proof of theorem 6.1 is based on the intermediate value theorem, applied to the derivative $\phi'(b)$. We determine $\phi(0)$, the limit $\phi(\infty)$, then $\phi'(0)$ and a quantity ψ that has the same sign as $\phi'(b)$ for all large b. Then the following situations ensure the existence of a maximizer of ϕ (and hence a minimizer of F).
i) $\phi'(0) > 0$ and $\phi(\infty) \le \phi(0)$.
ii) $\phi(\infty) \ge \phi(0)$ and $\psi < 0$.
iii) $\phi'(0) > 0$ and $\psi < 0$.

We determine the required quantities. We find immediately

$$\phi(0) = \frac{(\sum_i p_i y_i)^2}{\sum_i p_i}. \tag{12.1}$$

We write $\phi(b)$ differently,

$$\phi(b) = \frac{(\sum_i p_i \frac{b x_i y_i}{b + x_i})^2}{\sum_i p_i \frac{b^2 x_i^2}{(b + x_i)^2}}, \tag{12.2}$$

and we see that

$$\phi(\infty) = \lim_{b\to\infty} \phi(b) = \frac{(\sum_i p_i x_i y_i)^2}{\sum_i p_i x_i^2}. \tag{12.3}$$

We determine the derivative

$$\phi'(b) =$$

$$\frac{2\sum_i p_i \frac{x_i y_i}{b + x_i}}{(\sum_i p_i \frac{x_i^2}{(b + x_i)^2})^2} \left[\sum_i p_i \frac{x_i y_i}{b + x_i} \sum_i p_i \frac{x_i^2}{(b + x_i)^3} - \sum_i p_i \frac{x_i y_i}{(b + x_i)^2} \sum_i p_i \frac{x_i^2}{(b + x_i)^2} \right] \tag{12.4}$$

and find

$$\phi'(0) = \frac{2\sum_i p_i y_i}{(\sum_i p_i)^2}\left[\sum_i p_i y_i \sum_i p_i \frac{1}{x_i} - \sum_i p_i \frac{y_i}{x_i}\sum_i p_i\right]. \tag{12.5}$$

Next we investigate the sign of $\phi'(b)$ for large b. Consider the square bracket in (12.4) and multiply by b^4,

$$b^4\left[\sum_i p_i \frac{x_i y_i}{b+x_i}\sum_i p_i \frac{x_i^2}{(b+x_i)^3} - \sum_i p_i \frac{x_i y_i}{(b+x_i)^2}\sum_i p_i \frac{x_i^2}{(b+x_i)^2}\right]. \tag{12.6}$$

Put $b = 1/\epsilon$,

$$\sum_i p_i \frac{x_i y_i}{1+\epsilon x_i}\sum_i p_i \frac{x_i^2}{(1+\epsilon x_i)^3} - \sum_i p_i \frac{x_i y_i}{(1+\epsilon x_i)^2}\sum_i p_i \frac{x_i^2}{(1+\epsilon x_i)^2} \tag{12.7}$$

and expand,

$$\sum_i p_i x_i y_i (1-\epsilon x_i)\sum_i p_i x_i^2(1-3\epsilon x_i) - \sum_i p_i x_i y_i(1-2\epsilon x_i)\sum_i p_i x_i^2(1-2\epsilon x_i). \tag{12.8}$$

The terms without a factor ϵ cancel. The factor of ϵ is

$$\psi = \sum_i p_i x_i^2 y_i \sum_i p_i x_i^2 - \sum_i p_i x_i y_i \sum_i p_i x_i^3. \tag{12.9}$$

Now we have all tools for the proofs.

Proof of theorem 6.1. Check the cases i), ii), iii) above.

Proof of theorem 6.2. Suppose (6.7) holds and (6.5) does not hold. Since the first equality is the same in (6.7) and (6.5), the second inequality in (6.5) does not hold. Hence the converse strict inequality holds and hence the first inequality of (6.6). The second inequality of (6.6) holds because it is the same as the second inequality of (6.7).

Proof of proposition 6.1. The square bracket in (12.4) can be written as

$$\sum_{i,k} p_i p_k \left[\frac{x_i x_k^2 y_i}{(b+x_i)(b+x_k)^3} - \frac{x_i y_i x_k^2}{(b+x_i)^2(b+x_k)^2}\right]. \tag{12.10}$$

Rearrange,

$$\sum_{i,k} p_i p_k \frac{x_i y_i x_k^2}{(b+x_i)(b+x_k)^2}\left(\frac{1}{b+x_k} - \frac{1}{b+x_i}\right) \tag{12.11}$$

$$\sum_{i,k} p_i p_k \frac{x_i y_i x_k^2}{(b+x_i)^2(b+x_k)^3}(x_i - x_k) \tag{12.12}$$

$$\sum_{i>k} p_i p_k \frac{x_i y_i x_k^2}{(b+x_i)^2(b+x_k)^3}(x_i - x_k) + \sum_{i<k} p_i p_k \frac{x_i y_i x_k^2}{(b+x_i)^2(b+x_k)^3}(x_i - x_k) \tag{12.13}$$

$$\sum_{i>k} p_i p_k \frac{x_i y_i x_k^2}{(b+x_i)^2(b+x_k)^3}(x_i - x_k) - \sum_{i>k} p_i p_k \frac{x_k y_k x_i^2}{(b+x_k)^2(b+x_i)^3}(x_i - x_k) \tag{12.14}$$

$$\sum_{i>k} p_i p_k \frac{x_i x_k (x_i - x_k)}{(b+x_i)^2(b+x_k)^2}\left(\frac{y_i x_k}{b+x_k} - \frac{y_k x_i}{b+x_i}\right) \tag{12.15}$$

$$\sum_{i>k} p_i p_k \frac{x_i x_k (x_i - x_k)}{(b+x_i)^3(b+x_k)^3}[b(y_i x_k - y_k x_i) + y_i x_k x_i - y_k x_i x_k] \tag{12.16}$$

$$\sum_{i>k} p_i p_k \frac{x_i x_k (x_i - x_k)}{(b+x_i)^3 (b+x_k)^3} \left[b x_i x_k \left(\frac{y_i}{x_i} - \frac{y_k}{x_k} \right) - x_i x_k (y_k - y_i) \right]. \tag{12.17}$$

Proof of proposition 7.1: We find

$$\phi'(b) =$$
$$\frac{2 \sum_i p_i \frac{y_i}{b+x_i}}{(\sum_i p_i \frac{1}{(b+x_i)^2})^2} \left[\sum_i p_i \frac{y_i}{b+x_i} \sum_i p_i \frac{1}{(b+x_i)^3} - \sum_i p_i \frac{y_i x_i}{(b+x_i)^2} \sum_i p_i \frac{1}{(b+x_i)^2} \right]. \tag{12.18}$$

As in the proof of proposition 6.1 the square bracket can be transformed to the expression (7.7).

Proof of proposition 9.1: First find the minimum of F over c, given b, a. The minimum is obtained for

$$c = \sum_i p_i y_i - a \sum_i p_i \frac{x_i}{b+x_i} \tag{12.19}$$

as

$$\begin{aligned} F \quad = \quad & a^2 \left(\sum_i p_i \frac{x_i^2}{(b+x_i)^2} - \left(\sum_i p_i \frac{x_i}{b+x_i} \right)^2 \right) \\ & -2a \left(\sum_i p_i \frac{x_i y_i}{b+x_i} - \sum_i p_i \frac{x_i}{b+x_i} \sum_i p_i y_i \right) \\ & + \sum_i p_i y_i^2 - \left(\sum_i p_i y_i \right)^2 . \end{aligned} \tag{12.20}$$

Hence the optimal a, given b, is

$$a = \frac{\sum_i p_i \frac{x_i y_i}{b+x_i} - \sum_i p_i \frac{x_i}{b+x_i} \sum_i p_i y_i}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2} - \left(\sum_i p_i \frac{x_i}{b+x_i} \right)^2}. \tag{12.21}$$

Hence the minimum is

$$F = \sum_i p_i y_i^2 - \left(\sum_i p_i y_i \right)^2 - \phi(b) \tag{12.22}$$

where

$$\phi(b) = \frac{\left(\sum_i p_i \frac{x_i y_i}{b+x_i} - \sum_i p_i \frac{x_i}{b+x_i} \sum_i p_i y_i \right)^2}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2} - \left(\sum_i p_i \frac{x_i}{b+x_i} \right)^2}. \tag{12.23}$$

Here the denominator can be simplified (compare the proof of proposition 6.1) to

$$b^2 \sum_{i>k} p_i p_k \frac{(x_i - x_k)^2}{(b+x_i)^2 (b+x_k)^2} \tag{12.24}$$

and the expression in brackets in the numerator becomes

$$b \sum_{i>k} p_i p_k \frac{(x_i - x_k)(y_i - y_k)}{(b+x_i)(b+x_k)}. \tag{12.25}$$

Proof of theorem 9.1: The derivative is

$$\phi'(b) = \frac{2\sum_{i>k} p_i p_k \frac{(x_i-x_k)(y_i-y_k)}{(b+x_i)(b+x_k)}}{\left(\sum_{i>k} p_i p_k \frac{(x_i-x_k)^2}{(b+x_i)^2(b+x_k)^2}\right)^2} \times$$
$$\times\Big[\sum_{i>k} p_i p_k \frac{(x_i-x_k)(y_i-y_k)}{(b+x_i)(b+x_k)} \sum_{i>k} p_i p_k \frac{(x_i-x_k)^2}{(b+x_i)^3(b+x_k)^3}(2b-x_i-x_k)$$
$$-\sum_{i>k} p_i p_k \frac{(x_i-x_k)(y_i-y_k)}{(b+x_i)^2(b+x_k)^2}(2b-x_i-x_k) \sum_{i>k} p_i p_k \frac{(x_i-x_k)^2}{(b+x_i)^2(b+x_k)^2}\Big].$$

The next steps are multiplying the square bracket by b^7, putting $b = 1/\epsilon$ and expanding. These steps yield the expression

$$\sum_{i>k} p_i p_k (x_i-x_k)(y_i-y_k)(1-\epsilon(x_i+x_k)) \sum_{i>k} p_i p_k (x_i-x_k)^2 (2-\epsilon 5(x_i+x_k))$$
$$-\sum_{i>k} p_i p_k (x_i-x_k)(y_i-y_k)(2-\epsilon 3(x_i+x_k)) \sum_{i>k} p_i p_k (x_i-x_k)^2 (1-\epsilon 2(x_i+x_k)).$$

The terms linear in ϵ give the desired value.

Proof of proposition 10.1: To understand the structure of the problem we look at the general situation for two functions f, g (notice the $-$ sign before c),

$$F = \sum_{i=1}^{n} p_i \left(af_i - cg_i - y_i\right)^2. \tag{12.26}$$

The minimizer is

$$a = \frac{\sum_i p_i g_i^2 \sum_i p_i f_i y_i - \sum_i p_i f_i g_i \sum_i p_i g_i y_i}{\sum_i p_i f_i^2 \sum_i g_i^2 - (\sum_i p_i f_i g_i)^2} \tag{12.27}$$

$$c = -\frac{\sum_i p_i f_i^2 \sum_i p_i g_i y_i - \sum_i p_i f_i g_i \sum_i p_i f_i y_i}{\sum_i p_i f_i^2 \sum_i p_i g_i^2 - (\sum_i p_i f_i g_i)^2}, \tag{12.28}$$

and the minimum is

$$\sum_i p_i y_i^2$$
$$-\frac{\sum_i p_i g_i^2 (\sum_i p_i f_i y_i)^2 + 2\sum_i p_i f_i g_i \sum_i p_i f_i y_i \sum_i p_i g_i y_i + \sum_i p_i f_i^2 (\sum_i p_i g_i y_i)^2}{\sum_i p_i f_i^2 \sum_i p_i g_i^2 - (\sum_i p_i f_i g_i)^2}. \tag{12.29}$$

Specialize to the functions $x/(\beta+x)$, $-1/(\beta+x)$ to find

$$\hat{a}(b) = \frac{\sum_i p_i \frac{1}{(b+x_i)^2} \sum_i p_i \frac{x_i y_i}{b+x_i} - \sum_i p_i \frac{x_i}{(b+x_i)^2} \sum_i p_i \frac{y_i}{(b+x_i)^2}}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2} \sum_i p_i \frac{1}{(b+x_i)^2} - (\sum_i p_i \frac{x_i}{(b+x_i)^2})^2} \tag{12.30}$$

$$\hat{c}(b) = -\frac{\sum_i p_i \frac{x_i^2}{(b+x_i)^2} \sum_i p_i \frac{y_i}{b+x_i} - \sum_i p_i \frac{x_i}{(b+x_i)^2} \sum_i p_i \frac{x_i y_i}{b+x_i}}{\sum_i p_i \frac{x_i^2}{(b+x_i)^2} \sum_i p_i \frac{1}{(b+x_i)^2} - (\sum_i p_i \frac{x_i}{(b+x_i)^2})^2} \tag{12.31}$$

and the expression (10.4) for $\phi(b)$. Finally rearrange the terms in the numerator and denominator in (12.30), (12.31) to obtain (10.2), (10.3).

Proof of proposition 11.1: The optimal a, b satisfy the equations

$$\begin{aligned} a\sum_i p_i x_i^2 - b\sum_i p_i x_i y_i &= \sum_i p_i x_i^2 y_i \\ -a\sum_i p_i x_i y_i + b\sum_i p_i y_i^2 &= -\sum_i p_i x_i y_i^2 \end{aligned} \tag{12.32}$$

and hence

$$\begin{aligned} a &= \frac{\sum_i p_i x_i y_i^2 \sum_i p_i x_i y_i - \sum_i p_i x_i^2 y_i \sum_i p_i y_i^2}{\sum_i p_i x_i^2 \sum_i p_i y_i^2 - (\sum_i p_i x_i y_i)^2} \\ b &= \frac{\sum_i p_i x_i^2 \sum_i p_i x_i y_i^2 - \sum_i p_i x_i y_i \sum_i p_i x_i^2 y_i}{\sum_i p_i x_i^2 \sum_i p_i y_i^2 - (\sum_i p_i x_i y_i)^2}. \end{aligned} \tag{12.33}$$

Then reorder terms to arrive at (11.2)(11.3).

The proof of proposition 11.2 is almost the same as that of proposition 11.1.

13. Discussion.

We have studied several examples where essential features of a complex dynamics in higher dimension can be approximately described by a scalar problem. In the classical case of the non-reversible Michaelis-Menten kinetics an ordinary differential equation describes the time course of the substrate. In the reversible case similar results hold as has been recently shown in [6]. We review the equations from [8] [4] for an operator-repressor system, we interpret the dynamic equations as a competitive system, we find the exact connection between concentrations of effector and operator and we discuss in what sense the approximation given in [8] is valid.

In all these problems the essential features are represented in a simple fractional linear function depending on two or three positive parameters. Our goal is to show how these parameters can be estimated from data by a (weighted) least squares approach. We distinguish between "nonlinear" (in the denominator) and "linear" parameters in the fraction. Given the nonlinear parameters (typically only one) we solve the least squares problem with respect to the linear parameters, thus reducing the original least squares problem to finding the maximum of a function on the real line. We provide sufficient criteria for such maximum to exist and therefore also for the original least squares problem. It turns out that the least squares problem has a solution if the data reflect some natural qualitative properties of the expected function such as monotonicity and concavity, in particular, in the case of the non-reversible MM kinetics, if the data have a property that we call "chemically feasible".

Acknowledgments. The author thanks Michael Mackey for the suggestion to study the operator-repressor dynamics.

REFERENCES

[1] G. E. Briggs and J. B. S. Haldane, *A note on the kinematics of enzyme action,* Biochem. J., **19** (1925), 338–339.

[2] K. P. Hadeler and D. Jukić, K. Sabo, *Least squares problems for Michaelis-Menten kinetics,* Math. Meth. Appl. Sci., **30** (2007), 1231–1241.

[3] M. W. Hirsch and H. L. Smith, *Monotone dynamical systems,* in "Handbook of Differential Equations, Vol. 2," Elsevier B. V., Amsterdam, (2006), 239–357

[4] M. C. Mackey, M. Tyran-Kaminska and R. Yvinec, *Molecular distributions in gene regulatory dynamics,* J. Theoretical Biology, **274** (2011), 84–96.

[5] L. Michaelis and M. L. Menten, *Die kinetik der invertinwirkung,* Biochem. Z., **49** (1913), 333–369.

[6] L. Noethen and S. Walcher, *Tikhonov's theorem and quasi-steady state,* Discrete Contin. Dyn. Syst. Ser. B, **16** (2011), 945–961.

[7] L. A. Segel and M. Slemrod, *The quasi-steady-state assumption: A case study in perturbation,* Thermochim. Acta, **31** (1989), 446–477.

[8] Gad Yail and Ezra Yagil, *On the relation between effector concentration and the rate of induced enzyme synthesis,* Biophysical Journal, **11** (1971), 11–27.

Received January 29, 2013; Accepted March 15, 2013.

E-mail address: hadeler@uni-tuebingen.de