ESTIMATION OF THE RATE CONSTANTS IN THE MICHAELIS-MENTEN EQUATIONS WITHOUT RESTRICTIONS AS TO TIME SCALE

G.M. WILLEMS
Mathematical Centre, Amsterdam

and

H.C. HEMKER
Laboratory for Pathological Chemistry II, University Hospital, Leiden, The Netherlands

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The usual way of estimating the reaction constants of an enzymatic reaction is by graphical analysis of experimental data in a Lineweaver-Burk plot or related graphs [1]. The validity of this method depends upon the a priori assumption that steady-state conditions prevail.

Analogously pre-steady state kinetics make a priori presumption that we are dealing with a pre-steady state.

Both types of approach rely upon the fact that enzymatic reactions develop in two widely different time scales; the pre-steady state in fractions of seconds, and the steady state in minutes or hours.

This difference in time scale is the justification of the usual techniques for solution of the differential equations that govern the process as they essentially are finite perturbation methods [2].

In the course of an investigation on the reaction kinetics of blood coagulation the need was felt to estimate the parameters in an experimental situation where we did not know in what time domain of the reaction we were operating, nor even if indeed a sufficiently sharp distinction of time domains was feasible.

We thus set out to find a method which would enable us to estimate the parameters in an enzymatic reaction without knowing beforehand that it would behave according to the steady state assumptions. We considered the classical reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C$$

$$C \xrightarrow{k_{+2}} E + P$$

The known parameters are the enzyme and the substrate concentration at zero time (E_0 and S_0, respectively). The parameters asked are k_{+1}, k_{+2} and k_{-1}.

This leads to the differential equations:

$$\frac{dS}{dt} = -k_{+1} \cdot E \cdot S$$

and

$$\frac{dC}{dt} = k_{+1} \cdot E \cdot S \cdot - (k_{-1} + k_{+2}) C$$

with the starting conditions $E = E_0$, $S = S_0$. These are more easily written in the dimensionless form

$$\frac{ds}{dt} = -E_0 k_{+1}((1-c)S + K_e c/S_0) \qquad (1.1)$$

$$\frac{dc}{dt} = k_{+1} S_0 (K_m c/S_0 + (1-c)S)$$

where $s = S/S_0$, $c = C/E_0$, $K_e = k_{-1}/k_{+1}$ and $K_m = (k_{-1} + k_{+2})/k_{+1}$.

The approach considered here is based on the comparison of the numerically computed solution $sol(par, t) = (s(par, t), c(par, t))$ of the initial value problem (1.1) as function of the parameters *par* with all the observations $obs(t_i)$ of s or p. By an iterative procedure a sequence of parameter vectors par_n is contructed such that the observations $obs(t_i)$ are increasingly better fitted by $sol(par_n, t_i)$.

Estimation of the parameters through minimization of Res(par)

We accept every value *par* of the parameter vector such that $sol(par, t)$ is very close to $obs(t_i)$ as a good estimate of par_0; "very close" being small compared to the experimental error. As a measure for the distance between $obs(t_i)$, $t_i \in T$, and $sol(par, t)$ we introduce the function

$$Res(par) = \Sigma_{t_i \in T} \| obs(t_i) - sol(par, t_i) \|^2, \quad (1.2)$$

where $\| \cdot \|$ is defined by

$$\| (x_1, x_2) \|^2 = x_1^2 + x_2^2,$$

or

$$\| (x_1) \|^2 = x_1^2$$

The parameter vector par_0 is now estimated by the vector par_{est} for which $res(par)$ attains its minimal value.

Not disposing of an analytic expression of the complicated function $Res(par)$ of the argument *par*, it is necessary to use an iterative procedure for the minimization of $Res(par)$. We have chosen a method which is fundamentally a pseudo Newton scheme for the determination of a zero of the function $(\partial/\partial\, par)\, Res(par)$. When par_n is known, par_{n+1} is determined by minimization of

$$\overline{Res}(par) = \Sigma_{t_i \in T} \| obs(t_i) - \overline{sol}(par, t) \|^2, \quad (1.3)$$

where $\overline{sol}(par)$ is obtained by linearization of $sol(par)$ in the point par_n, i.e.

$$sol(par, t_i) = sol(par_n, t) + \frac{\partial}{\partial\, par} sol(par_n, t)(par - par_n)$$

As the function $\overline{Res}(par)$ is a quadratic expression in *par*, the minimization is easily accomplished using the classical methods of numerical algebra.

As a first estimate the values can be used obtained by the classical graphic methods, or from an estimate of the values of dS/dt computed by fitting a polynomial of low degree to the observations. From a theoretical analysis of the iterative procedure it follows that in the case when there exists a value *par* such that $obs(t_i) - sol(par, t_i) = 0$, $t_i \in T$, that the process is quadratically convergent for start values sufficiently near the minimizing parameters *par*. When there is no such parameter then the process is linear convergent with convergence factor proportional to $(Res(par_s))^{1/2}$, where par_s is the minimizing value of *par*, for start values sufficiently near par_s.

Computation of sol(par, t_i) and $(\partial/\partial\, par)\, sol(par, t_i)$

For the use of the above mentioned iteration scheme it is necessary to have at one's disposal the values of $sol(par_n, t_i)$ and $(\partial/\partial\, par)\, sol(par_n, t_i)$. As $sol(par, t)$ and $(\partial/\partial\, par)\, sol(par, t)$ are solutions of the pair coupled initial value problems

$$\frac{d}{dt} sol(par, t) = f(par, sol\,(par, t)), \quad (1.4)$$

$$\frac{d}{dt} \frac{\partial}{\partial\, par} sol(par, t) = \frac{\partial}{\partial\, par} f(par, sol(par, t)) + \frac{\partial}{\partial\, sol} f(par, sol(par, t)) \frac{\partial}{\partial\, par} sol(par, t),$$

$$\frac{\partial}{\partial\, par} sol(par, o) = 0, \quad sol(par, o) = (1, 0).$$

Where $f(par, t)$ is the righthand side of equation (1.1); they can be computed by numerical integration of these initial value problems. Since in this special case of the Michaelis–Menten equation the differential equations are usually stiff, it is of some importance to use an appropriate method of numerical integration* We have chosen the exponential fitted Taylor method, developed at the Mathematical Centre, Amsterdam, for the solutions of these kind of stiff equations [3].

* Especially because there is not high accuracy required, as the observations are disturbed with a measuring error of about 5%, we can save a lot of computing time by using an appropriate integration method.

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