Complexity-reduction by first-order approximation of non-linear kinetics

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Abstract: Ecological, toxicological, and pharmacological research is often concerned with the answer to the question of how a substance is processed within a biological system. The exact knowledge of the corresponding kinetic pattern forms the basis for a useful answer. In order to identify non-linear kinetics, a first-order approximation method is proposed for complexity-reduction. A simulation study is presented to investigate the error of the approximation in case of a simple Michaelis-Menten kinetic process. The proposed method shows to give useful results which allow to characterize the underlying kinetic pattern. Furthermore it could be shown that in simulating kinetic processes the applied numerical methods may perform with considerable numerical instabilities.

Key Words: Compartmental model, Dynamic process, First-order approximation, Michaelis-Menten kinetic, Non-linear kinetic, Numerical integration

1. Introduction

A major part of ecological, toxicological, and pharmacological research is concerned with the answer to the question of how a substance is processed within a biological system. With qualitatively increasing technology, however, the required quality of the answers has been increased in the last years as well, and will continue to increase.

Where risk assessment formerly has been based on incidences and animal results in combination with haphazardly chosen safety factors, it becomes more and more mechanistically based now. In carcinogenesis, for instance, models are considered, where on a molecular basis DNA-damage by adducts after exposition to a substance and DNA-repair mechanisms form concurrent dynamic systems, preceding a potential carcinogenic cascade (Hoel *et al.* 1983). The development of a tumor then depends on the repair potential of the species in contrast to the DNA-damage potential of the substance under view. The relevant mechanistic characteristics are investigated and modeled for the target species in order to get access to more scientifically based threshold-values and low-dose extrapolations.

In the development process of new drugs, the detailed knowledge of their dynamic and kinetic characteristics in different patient populations becomes economically more and more urgent. New drugs will have to be optimally „designed“ regarding efficacy and adverse side effects in the individual patient.

The above mentioned problems are naturally concerned with highly complex answers and appropriate statistical tools have to be applied, respectively, to be established.

Characterizing processes of transport or transformation within dynamic systems in terms of kinetics is not a trivial task. This problem was exemplified by a re-analysis of *in vivo* kinetic data from Golka et al. (1989) using a two-compartment model and nonlinear regression. The development and application of further statistical methods for this purpose offer a means to account for the common discrepancies found in the different analyses of toxicokinetic models (Becka et al.(1992), Csanády and Filser (1993), and Becka (1993)).

2. Compartment models and kinetic pattern

The flow of material in dynamic systems, as for example in biological units can be modeled by compartments. The term „compartment” here usually means a possibly fictitious area within the system detached from others by possibly fictitious boundaries where the material under view is distributed homogeneously.

Using compartment-models, main interest is given to the amount of material being exchanged between the compartments in the course of time resulting in special material profiles for each compartment. There are many cases where the amount of material can not be observed in all of the areas so that observations are often only available from a subset of compartments.

To investigate dynamic systems it is usually necessary to disturb the natural steady state of the units, which is usually done by input of material in one or more of the compartments under view. The input then may be continuous, however constant over time, or a bolus.

A typical characteristic in the context of investigating kinetic patterns is that all information have to be derived from observing dynamic processes over time. In contrast to the time-dependency of an observed process, the kinetic pattern describes the law behind or, in other words, the velocity of the process. Therefore, the first obligatory step is to extract the velocity characteristics out of the observed course.

A closer look at the underlying data structure is necessary to reveal that the statistical modeling process indeed is no trivial task. The interesting question is how a process works at different concentrations of a substrate, whereas the observable data consist of the dynamic reaction of this process to a single disturbance. To answer the first question then it is necessary to analyze multiple different disturbances.

In practice, it is often assumed that all kinetics of a dynamic system are of first-order which means that the velocities of the participating processes are linear functions of the amount of corresponding substrates. This assumption allows for a quite easy mathematical handling with easy to obtain results. However, almost all biochemical processes are catalyzed or influenced by enzymes or proteins where the velocity of a processes depends on the amount of substrate in a non-linear way, the number of binding sites, the affinity, and so on. In this case the acceleration of the process, i.e. the change of the velocity in connection with the amount of substrate becomes of interest. Referring to a first-order process, the acceleration is a constant. However, disturbing a special process in changing the amount of substrate in one compartment may result in a high acceleration at low concentration of substrate until an optimal condition is reached, followed by a decrease of acceleration with further increasing concentration. Such characteristics may be due to occupied binding sites and queuing symptoms.

The previously described situation reflects the well known saturable Michaelis-Menten-type kinetics.

At low concentration of substrate, there also may be a phase of low acceleration due to few binding sites and a low chance of complex-binding. This situation then reflects sigmoidal-type kinetics.

3. Statistical modeling and reduction of complexity

Let us investigate a dynamic system where one partial process consists of the amount of material being transferred from one special compartment i to another compartment j in the course of time. The combination of all the participating processes results in a material profile for each compartment.

In order to investigate the dynamic characteristics, we will disturb the natural steady state of this system by input of material in one or more compartments. Denoting $\tilde{a}$ the amount of input which may be continuous, however, constant over time, the transport reactions of the dynamic system to this input are used to analyze the inherent processes.

Denoting $Y_i(t)$ the amount of material in compartment i at time t, the transport from compartment i to compartment j in the course of time is usually described by the differential equation

$$\frac{dY_i(t)}{dt} = f_{ij}\left(\omega_{ij}, Y_i(t)\right)$$

where f_{ij} denotes the functional description of a so-called kinetic process and ω_{ij} is a path-dependent vector of unknown parameters. Furthermore, it is usually assumed that this kinetic is a function of the amount of material $Y_i(t)$ present in compartment i at time t.

If the dynamic system is modeled by, say, n compartments, it will be assumed that the corresponding compartment-model is kind of a regular one in so far that

1.) there is no compartment in the system where the concentration-time course simply reflects those of another compartment in a linear way, in terms: there are no compartments $i, j \in \{1,2,\ldots,n\}$ with $i \neq j$ and $Y_i(t) = a + b \cdot Y_j(t)$ for all $t \geq 0$ and some constants a and b,

2.) each compartment in the system can receive material after some time, in terms: $Y_i(t,\tilde{a}) > 0$ for all $i \in \{1,2,\ldots,n\}$ and time-lack τ with $0 < \tau \leq t$, and

3.) the dynamic system monotonous responds to input of material, in terms: $\tilde{a} < \tilde{a}*$ implies $Y_i\left(t,\tilde{a}\right) \leq Y_i\left(t,\tilde{a}*\right)$ for all $i \in \{1,2,\ldots,n\}$ and $t \geq 0$.

Regarding one special compartment i, it will be additionally assumed that the change in the amount of material $Y_i(t)$ at time t is due to the sum of the related kinetic processes, i.e.

$$\frac{dY_i(t)}{dt} = -\sum_{\substack{j=1\\i\neq j}}^{n} f_{ij}\left(\omega_{ij}, Y_i(t)\right) + \sum_{\substack{j=1\\i\neq j}}^{n} f_{ji}\left(\omega_{ji}, Y_j(t)\right) .$$

In practice, it is often assumed that all kinetics of a dynamic compartment-model are of first-order which implies $f_{ij}\left(\omega_{ij}, Y_i(t)\right) = k_{ij} \cdot Y_i(t)$ and

$$\frac{dY_i(t)}{dt} = -\sum_{\substack{j=1\\i\neq j}}^{n} k_{ij} \cdot Y_i(t) + \sum_{\substack{j=1\\i\neq j}}^{n} k_{ji} \cdot Y_j(t)$$

with constant rate parameters k_{ij} for $i, j \in \{1,2,\ldots,n\}$ with $i \neq j$. Usually the model then yields a system of linear differential equations which can be solved numerically or analytically for the corresponding material profiles $Y_i(t)$, $i = 1,2,\ldots,n$.

There are many cases where the amount of material can not be observed in all of the compartments so that observations are only available from a subset of compartments. If these observations still allow to identify all of the rate parameters, the rate constants may be estimated by least squares using the solution of the system of linear differential equations, however, accounting for heterogeneous and auto-correlated errors.

If the kinetics of one or more of the partial processes from compartment i to compartment j are not known exactly, it will be necessary to investigate different input values $\tilde{a}_1 < \tilde{a}_2 < \cdots < \tilde{a}_m$ to produce m different profiles $Y_{i,l}(t)$, $l = 1,2,\ldots,m$, for characterizing the kinetics as functions of the amount of material. The input into the system through one single compartment may be continuous, however constant over time, or a bolus, as in the above notation. The aim then is to characterize the corresponding kinetic relationships $f_{ij}\left(\omega_{ij}, Y_i\right)$, $i \neq j$, first. Note that the required characterization does not depend on t , which is related to the duration of the special experiment. To eliminate the influence of experimental time it is useful to look for a time-independent characterization of $Y_{i,l}(t)$. If only those kinetics are taken into account where

$$f_{ij}{}^*\left(\omega_{ij}, Y_{i,l}(t)\right) := \frac{f_{ij}\left(\omega_{ij}, Y_{i,l}(t)\right)}{Y_{i,l}(t)}$$

is monotone and continuous as a function of the concentration Y for $i \neq j$ and $t \geq \tau$, the concentration time-course $Y_{i,l}(t)$ may be characterized by the maximum value $Y_{i,\max}$ which may be defined as the value at the beginning or the end of the experiment, if the local maximum does not exist. This can be done because of the monotone relationships, where $\tilde{a}_1 < \tilde{a}_2$ implies

$$Y_i(t, \tilde{a}_1) \le Y_i(t, \tilde{a}_2)$$

and, consequently,

$$Y_{i,\max}(\tilde{a}_1) \le Y_{i,\max}(\tilde{a}_2)$$

yielding

$$f_{ij} * \left(\omega_{ij}, Y_{i,\max}(\tilde{a}_1)\right) \le f_{ij} * \left(\omega_{ij}, Y_{i,\max}(\tilde{a}_2)\right) \ .$$

Approximating, therefore,

$$f_{ij} * \left(\omega_{ij}, Y_{i,l}(t)\right) \ \text{ by } \ f_{ij} * \left(\omega_{ij}, Y_{i,\max}(\tilde{a}_l)\right) =: f_{ij} * (\tilde{a}_l)$$

a system with first-order kinetics can be used in a first step, where

$$\frac{dY_i(t)}{dt} = -\sum_{\substack{j=1 \\ i \neq j}}^{n} f_{ij} * (\tilde{a}_l) \cdot Y_i(t) + \sum_{\substack{j=1 \\ i \neq j}}^{n} f_{ji} * (\tilde{a}_l) \cdot Y_j(t)$$

for each experiment l , $l = 1,2,\ldots,m$. If the observed material profiles allow for identifying the rate parameters of this first-order system, the so defined $f_{ij} * (\tilde{a}_l)$ will be estimated by a simple least squares technique accounting for heterogeneous errors, yielding estimates $\hat{f}_{ij} * (\tilde{a}_l)$ for $i, j = 1,2,\ldots,n$, $i \neq j$ and each experiment $l \in \{1,2,\ldots,m\}$. This linear approximation characteristically results in a systematic auto-correlated error-structure. If the maximum concentrations $Y_{i,\max}(\tilde{a}_l)$ can not be observed directly for $i = 1,2,\ldots,n$, they will be replaced by estimates $\hat{Y}_{i,\max}(\tilde{a}_l)$ calculated by using the estimated material profiles of the first-order system.

In an explorative manner, the following step is to analyze the relationship between the estimates $\hat{f}_{ij} * (\tilde{a}_l)$ and the corresponding maximum amounts of material $Y_{i,\max}(\tilde{a}_l)$ for $l = 1,2,\ldots,m$, and for all unknown kinetic processes $f_{ij}\left(\omega_{ij}, Y_i\right)$, $i, j \in \{1,2,\ldots,n\}$, $i \neq j$, which may require appropriate standardization of the estimated rate parameters first. In the case of a true first-order kinetic process with $f_{ij}\left(\omega_{ij}, Y_{i,l}(t)\right) = k_{ij} \cdot Y_{i,l}(t)$ this yields

$f_{ij}*\left(\omega_{ij}, Y_{i,l}(t)\right) = k_{ij}$ so that the $\hat{f}_{ij}*(\tilde{a}_l)$ should not depend on $Y_{i,\max}(\tilde{a}_l)$, instead being constant except some inter- and intra-experimental variability. This hypothesis can be tested using, for example, a nonparametric test of independence of $\hat{f}_{ij}*(\tilde{a}_l)$ and $Y_{i,\max}(\tilde{a}_l)$ for $l = 1, 2, \ldots, m$. If there is a certain structure of dependence, the corresponding shape may be analyzed according to

$$E\left[\hat{f}_{ij}*(\tilde{a}_l)\right] = \frac{f_{ij}\left(\omega_{ij}, Y_{i,\max}(\tilde{a}_l)\right)}{Y_{i,\max}(\tilde{a}_l)} \quad , \quad l = 1, 2, \ldots, m \ . \qquad (1)$$

Depending on the functional description of the kinetic process f_{ij} and using the maximum amounts of material $Y_{i,\max}(\tilde{a}_l)$, the estimates $\hat{f}_{ij}*(\tilde{a}_l)$ in model (1) may not be assumed to be unbiased.

Based on a two-compartmental model and assuming first-order kinetics, this approach using the acceleration information was applied to investigate the kinetic processes of propylene inhaled by Sprague-Dawley rats (Becka and Urfer, 1996).

4. First-order approximation of a Michaelis-Menten kinetic process - a simulation study

4.1 Systematic error and quality of a first-order approximation

To characterize the systematic error and the quality of a first-order approximation, a one-dimensional dynamic Michaelis-Menten type process was simulated (Figure 1) and analyzed assuming a first-order kinetic (Figure 2).

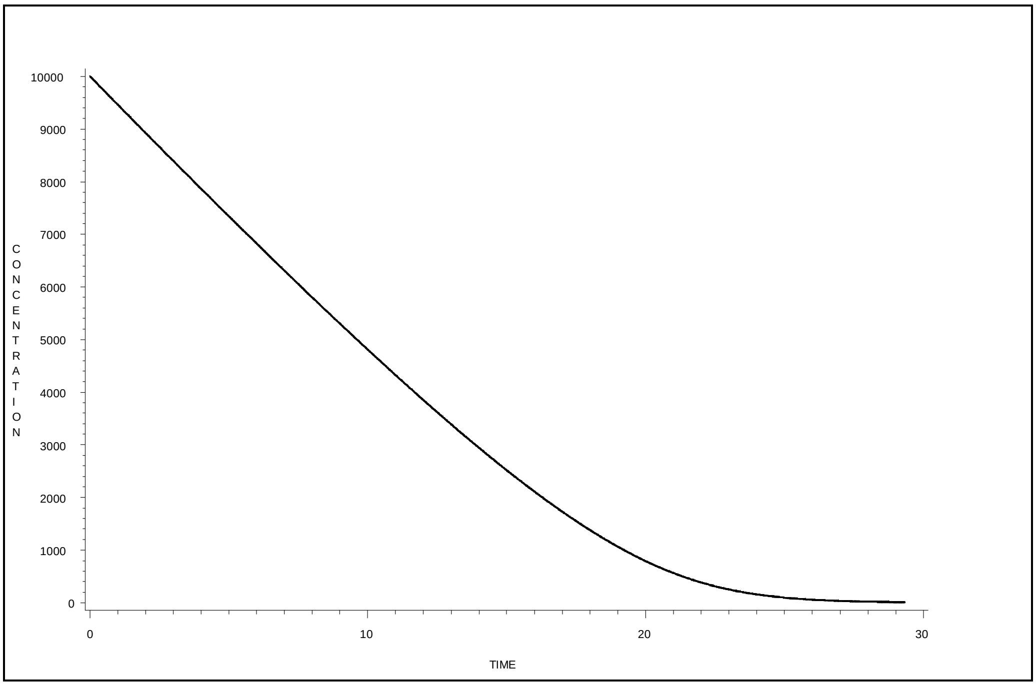

Figure 1: Concentration-time curve based on a Michaelis-Menten kinetic with v_{max}=600 units/time unit, k_m=1100 units, and an initial concentration of 10000 units.

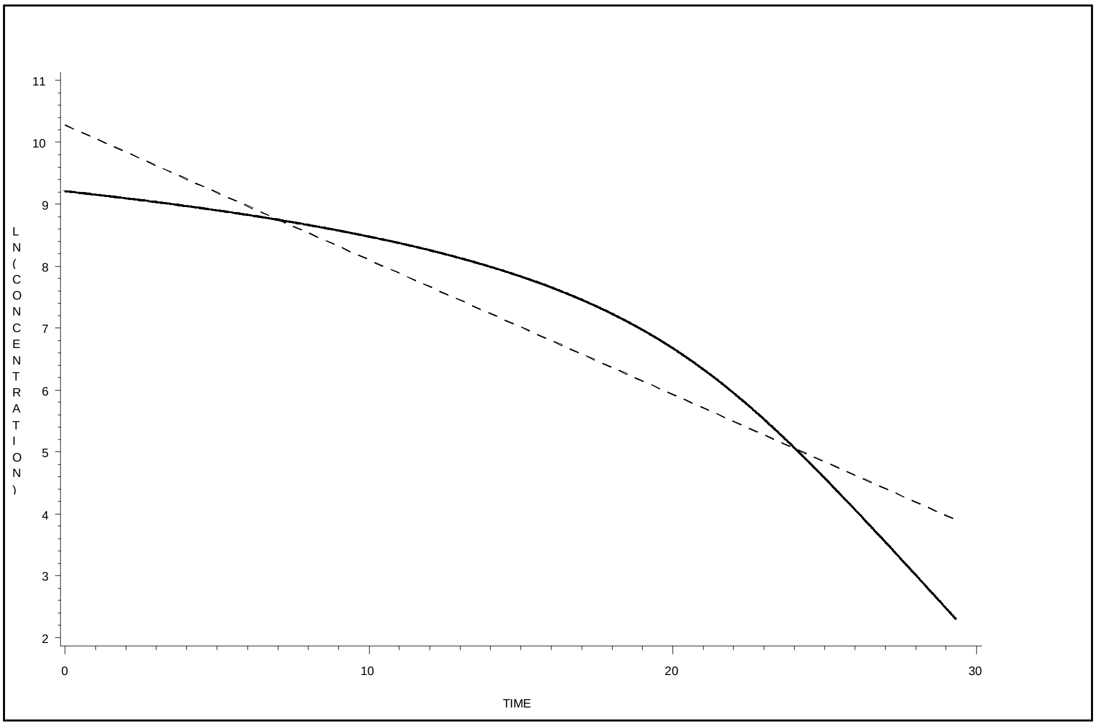

Figure 2: Concentration-time curve of Figure 1 on a logarithmic scale (solid line) with the first-order approximation (dotted line).

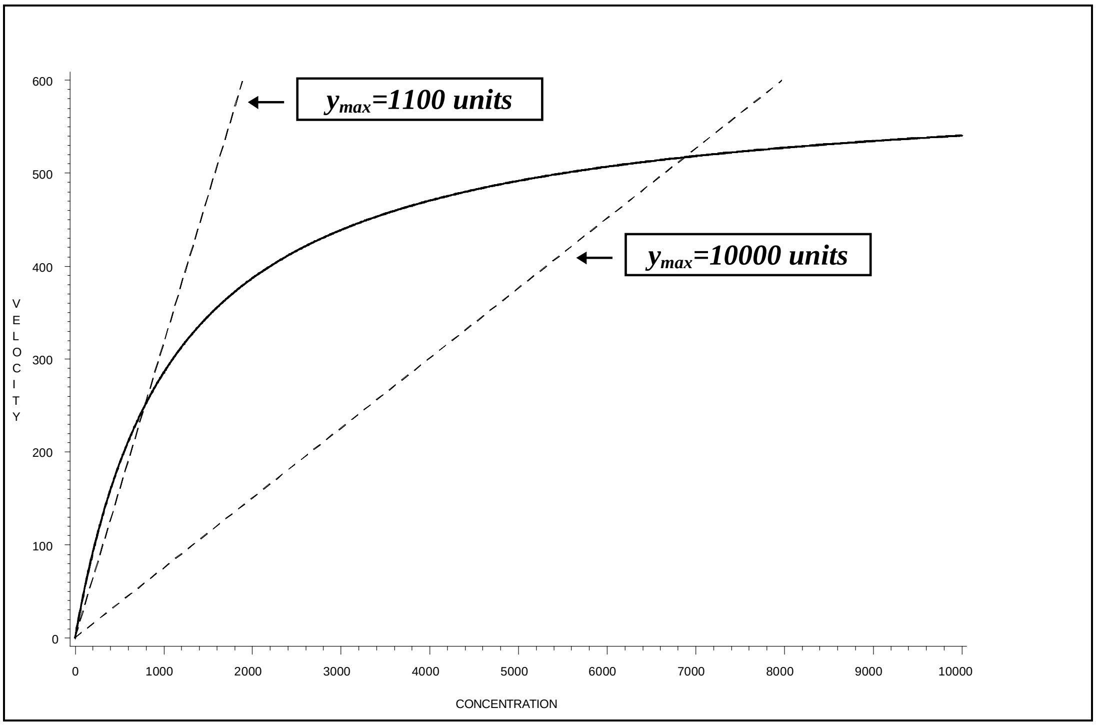

Figure 3: Michaelis-Menten kinetic with v_{max}=600 units/time unit, k_m=1100 units (solid line), and exact first-order approximations based on maximum concentrations of 1100 and 10000 units (dashed lines).

Figure 3 shows a Michaelis-Menten kinetic and an exact linear approximation, i.e. a least-squares approximations based on discrete points of the Michaelis-Menten curve itself up to 1100 units, respectively, 10000 units concentration. Since the linear approximation of the observed logarithmized concentration-time curve (Figure 2) is based on completely different data, the resulting approximation must not necessarily be the same.

Since the velocity of a Michaelis-Menten process is described by

$$f(y) = \frac{v_{\max} \cdot y}{(k_m + y)}$$

we have the acceleration

$$\frac{df(y)}{dy} = \frac{v_{\max} \cdot k_m}{(k_m + y)^2} = \dot{f}(y).$$

If we are asking for the concentration at which the acceleration of the process is c we get

$$\dot{f}(y) = c \Leftrightarrow \frac{v_{\max} \cdot k_m}{(k_m + y)^2} = c \Leftrightarrow y = \sqrt{\frac{v_{\max} \cdot k_m}{c}} - k_m$$

Since we have $0 < \dot{f}(y) \leq \frac{v_{\max}}{k_m}$ we may choose the constant c so that $c = \vartheta \cdot \frac{v_{\max}}{k_m}$ with $0 < \vartheta \leq 1$. We then get

$$y = \sqrt{\frac{v_{\max} \cdot k_m}{c}} - k_m = \sqrt{\frac{v_{\max} \cdot k_m}{\vartheta \cdot \frac{v_{\max}}{k_m}}} - k_m = \sqrt{\frac{k_m^2}{\vartheta}} - k_m = \left(\sqrt{\frac{1}{\vartheta}} - 1\right) \cdot k_m.$$

In order to characterize the curvature of the kinetic we may ask at which concentration the actual acceleration of the process is 1/4 of the maximum acceleration in the origin, i.e. $\frac{v_{\max}}{k_m}$.

If there is a strong curvature the corresponding concentration would be much smaller than if the kinetic would be quite flat.

According to the formulas derived before, the point at which the actual acceleration of the process is 1/4 of the maximum acceleration in the origin is given with concentration k_m independent from the maximum velocity of the process $v_{\max}$.

We may consider how much the initial concentration of the process deviates from this point in terms of y_0/k_m. From Figure 3 we may expect that the quality of the approximation increases as y_0/k_m decreases.

Another remarkable feature of the first-order approximation is the resulting systematic error-pattern as depicted in Figure 4.

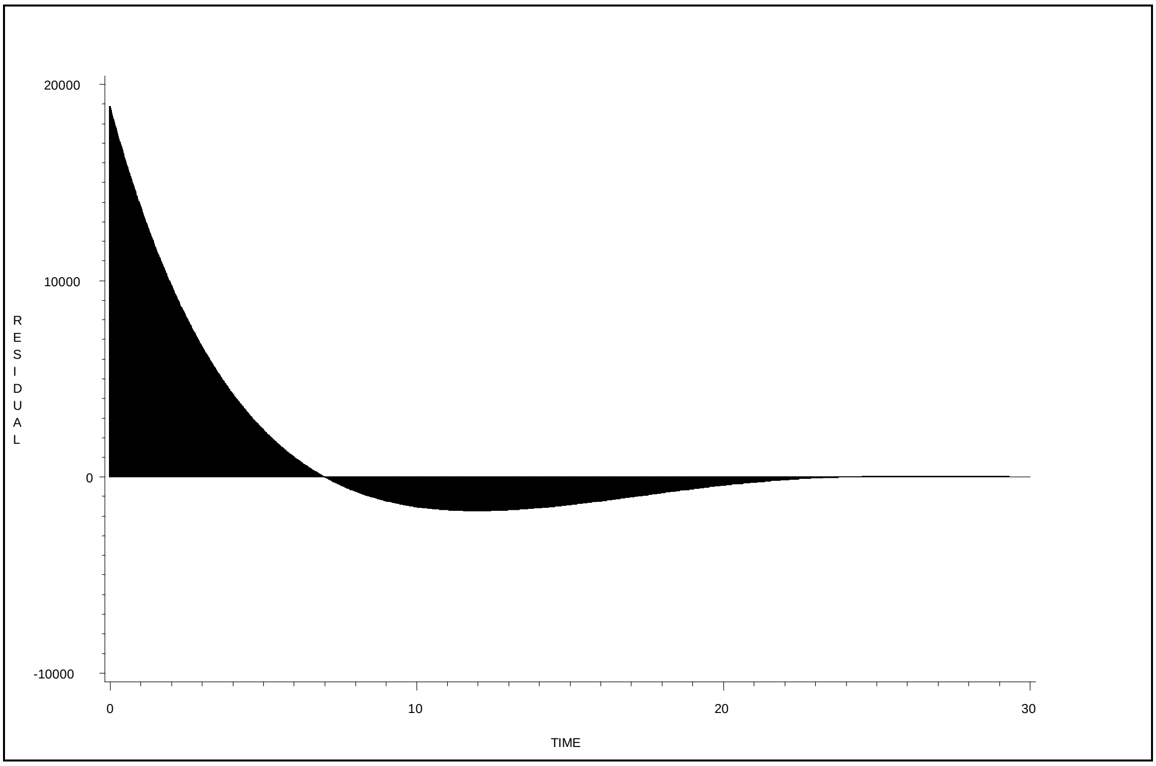

Figure 4: Residuals of first-order approximation on original scale (estimated minus observed).

4.2 Numerical Solution of the Differential Equation and Processing of the Data

All calculations were performed using SAS, version 6.12 TS level 0020, on an Intel 200 MHz MMX workstation under Window NT, version 4.0.

Given an initial concentration of y_0 units the chosen Michaelis-Menten kinetic process defined by

$$\frac{dy(t)}{dt} = -\frac{v_{\max} \cdot y(t)}{k_m + y(t)}$$

was integrated using Adams-Bashforth's 4-step method with controlled step-width starting with an initial step of 10^{-9} and keeping the local error of discretization between 10^{-8} and 10^{-6}, respectively, with different constant step-widths in the range of 10^{-2} up to 10^{-5}. For calculating the starting values, the classical Runge-Kutta method of order 4 and step-width 10^{-9} was applied. The differential equation was integrated unless 99.9% of the initial amount had been eliminated. From the resulting concentration-time curve 20 equidistant data-points were selected and used to fit the first-order approximation with knowledge of the actual initial concentration.

11015 parameter-combinations were investigated, where the maximum velocity v_{max} and the Michaelis-Menten constant k_m ranged from 1 to 1000 units per time unit, respectively units. For each parameter-combination the initial concentrations 10, 100, 1000, and 10000 units were considered. Only those runs where analyzed further where the numerical integration took more than 1000 steps so that in total 41035 runs were investigated.

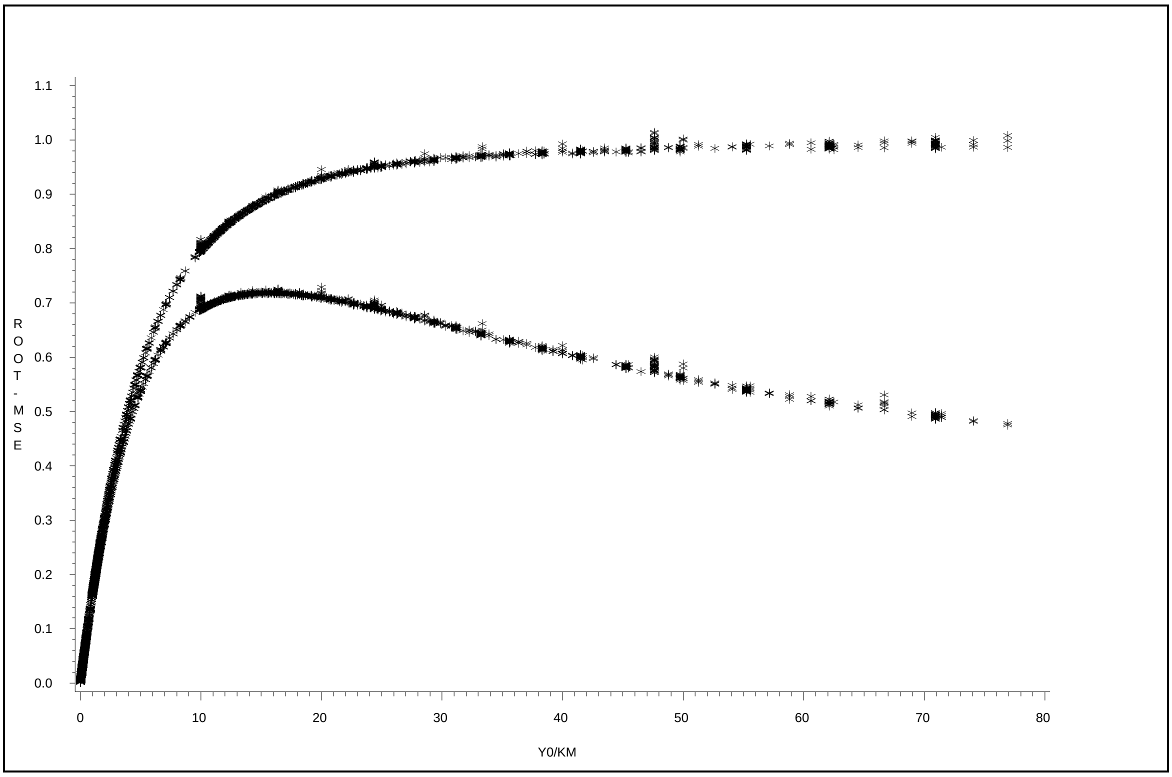

Figure 5: Relation of root-MSE and y_0/k_m (95.8% of all runs with controlled step-width).

All simulations revealed considerable numerical instabilities in the tails, i.e. yielded higher or lower concentration values in the tails than actually would be present. This deficiency became obvious in the subsequent data-processing. Figure 5 shows the root mean squared error of the linear approximation (logarithmized concentration scale) in relation to the ratio y_0/k_m up to a value of 100. The two curves actually reveal two different instability processes. With smaller constant step-widths this deficiency could not be corrected and the pattern remained the same, however, the number of obviously different instability processes increased. Also applying the classical Runge-Kutta method of order 4 could not improve this instability characteristics.

Step-Width	V_{max}	***Duration of Process (Time Units)***	***No. of Integration Steps***	***Root-MSE of First-Order Approximation***	***Estimated Velocity Constant***	***Estimated Initial Concentration***
Controlled	41	27.92	479	0.990	-0.167	1461.3
10^{-2}	41	27.91	2794	0.979	-0.165	1422.9
10^{-3}	41	27.90	28000	0.977	-0.165	1416.6
10^{-4}	41	27.90	280000	0.977	-0.165	1416.4
Controlled	61	18.76	483	0.571	-0.193	773.2
10^{-2}	61	18.76	1879	0.980	-0.246	1425.8
10^{-3}	61	18.75	19000	0.977	-0.245	1417.7
10^{-4}	61	18.76	190000	0.977	-0.245	1416.5

Table 1: Numerical instability in integrating a Michaelis-Menten equation with k_m=21 units and initial concentration of 1000 units applying step-width control.

4.3 Results

The analyzed data were considered to be non-normally distributed with systematic error-structure defined by the instability processes of the numerical integration. The estimated (negative) first-order velocity constants $\hat{f}*(y_0)$ were compared with $-v_{\max}/(k_m+y_o)$ (see for formula 1 of section 3) using their differences

$$\mathrm{D} = \hat{f}*(y_0)+\frac{v_{\max}}{k_m-y_0}$$

and their ratios

$$R=\frac{\hat{f}*(y_0)}{-\dfrac{v_{\max}}{k_m-y_0}}=-\frac{\hat{f}*(y_0)\cdot(k_m-y_0)}{v_{\max}}.$$

Since the Michaelis-Menten kinetic is strict monotonous increasing in y and because we are using all concentration data $\leq y_0$ for calculations we have

$$\hat{f}*(y_0) \leq -\frac{v_{\max}}{k_m - y_0} \quad .$$

The results are given in Tables 2 and 3.

y_0	***STEP-WIDTH***	***N***	***MEAN***	***SD***	***MIN***	***Q_{25}***	***MEDIAN***	***Q_{75}***	***MAX***
10	***0.00001***	1250	-0.352	1.981	-20.312	-0.0200	-0.0029	-0.0009	-0.0003
10	***0.0001***	3600	-0.175	1.020	-15.539	-0.0215	-0.0040	-0.0013	-0.0000
10	***0.001***	300	-0.016	0.092	-1.195	-0.0020	-0.0004	-0.0001	-0.0000
10	***0.01***	1804	-0.006	0.010	-0.280	-0.0070	-0.0045	-0.0021	-0.0000
100	***0.00001***	1250	-0.356	1.418	-14.286	-0.0946	-0.0188	-0.0062	-0.0022
100	***0.0001***	3600	-0.320	0.986	-10.931	-0.1359	-0.0292	-0.0096	-0.0002
100	***0.001***	300	-0.030	0.089	-0.841	-0.0125	-0.0027	-0.0010	-0.0001
100	***0.01***	1849	-0.047	0.041	-0.418	-0.0615	-0.0398	-0.0188	-0.0000
100	***controlled***	3324	-0.309	0.752	-7.345	-0.2158	-0.0717	-0.0122	-0.0001
1000	***0.00001***	1250	-0.016	0.011	-0.057	-0.0221	-0.0124	-0.0076	-0.0038
1000	***0.0001***	3600	-0.084	0.170	-1.221	-0.0759	-0.0310	-0.0098	-0.0004
1000	***0.001***	300	-0.006	0.012	-0.091	-0.0062	-0.0026	-0.0011	-0.0003
1000	***0.01***	2305	-0.189	0.118	-0.482	-0.2827	-0.1860	-0.0892	-0.0001
1000	***controlled***	3142	-0.383	0.452	-2.533	-0.5469	-0.2419	-0.0250	-0.0004
10000	***0.00001***	1250	-0.000	0.000	-0.001	-0.0004	-0.0003	-0.0003	-0.0002
10000	***0.0001***	3600	-0.001	0.001	-0.003	-0.0017	-0.0008	-0.0003	-0.0000
10000	***0.001***	300	-0.000	0.000	-0.000	-0.0002	-0.0001	-0.0000	-0.0000
10000	***0.01***	5150	-0.151	0.089	-0.316	-0.2268	-0.1504	-0.0741	-0.0000
10000	***controlled***	2861	-0.103	0.093	-0.315	-0.1841	-0.0869	-0.0062	-0.0002
	Overall	41035	-0.167	0.664	-20.312	-0.1437	-0.0252	-0.0029	-0.0000

Table 2: Difference D between estimated first-order velocity constants $\hat{f}*(y_0)$ **and** $-v_{\max}/(k_m + y_o)$**.**

In 1166 cases (2.8%) the absolute difference $|D|$ showed to be ≥1 and in 7735 cases (18.9%) the absolute difference was ≥0.2.

y_0	STEP-WIDTH	N	MEAN	SD	MIN	Q_{25}	MEDIAN	Q_{75}	MAX
10	***0.00001***	1250	1.028	0.096	1.001	1.0014	1.0032	1.0112	1.7966
10	***0.0001***	3600	1.038	0.097	1.001	1.0050	1.0098	1.0270	1.7969
10	***0.001***	300	1.038	0.097	1.002	1.0049	1.0096	1.0269	1.7968
10	***0.01***	1804	1.020	0.077	1.004	1.0102	1.0125	1.0180	4.0840
100	***0.00001***	1250	1.121	0.348	1.006	1.0116	1.0241	1.0705	4.0817
100	***0.0001***	3600	1.264	0.489	1.006	1.0440	1.0836	1.2262	4.0831
100	***0.001***	300	1.266	0.496	1.015	1.0428	1.0816	1.2230	4.0830
100	***0.01***	1849	1.176	0.194	1.034	1.1011	1.1248	1.1759	4.0202
100	***controlled***	3324	1.264	0.237	1.088	1.1169	1.1694	1.3041	2.4648
1000	***0.00001***	1250	1.046	0.021	1.019	1.0289	1.0407	1.0598	1.1100
1000	***0.0001***	3600	1.368	0.509	1.020	1.1165	1.2126	1.3857	4.2748
1000	***0.001***	300	1.318	0.411	1.051	1.1196	1.1956	1.3418	4.1085
1000	***0.01***	2305	2.287	0.609	1.133	1.8975	2.0888	2.4890	4.2837
1000	***controlled***	3142	2.473	0.588	1.780	1.9826	2.2854	2.8356	4.0921
10000	***0.00001***	1250	1.008	0.001	1.006	1.0074	1.0079	1.0085	1.0100
10000	***0.0001***	3600	1.041	0.021	1.007	1.0233	1.0408	1.0592	1.0861
10000	***0.001***	300	1.038	0.016	1.017	1.0195	1.0379	1.0553	1.0646
10000	***0.01***	5150	4.096	0.449	1.043	4.1001	4.1942	4.2567	4.2886
10000	***controlled***	2861	3.922	0.320	3.057	3.7230	4.0830	4.2301	4.2858
	Overall	41035	1.881	1.203	1.001	1.0320	1.1430	2.4102	4.2886

Table 3: Ratio R of estimated first-order velocity constants $\hat{f}^*(y_0)$ **and** $-v_{\max}/(k_m + y_o)$.

In 18511 cases (45.1%) the relative difference *R* showed to be ≥20%. Of these 7295 (17.8%) had an absolute difference of ≥0.2 and 1139 (2.8%) an absolute difference of ≥1.

5. Discussion and outlook

Due to the observed numerical instabilities, simulations of kinetic processes using numerical integration have to be applied very carefully with regard to used procedures and the kinetic processes under view. Simulating complete concentration-time courses using numerical methods seem to be no useful tool for analysis purposes since the simulated concentration-time curve may not necessarily represent the actual course very well.

Analyzing complete concentration-time courses for a single kinetic process as presented in this simulation mainly refer to re-analyses of historical data, where kinetic experiments were performed for many reasons and where the data were analyzed more or less under the assumption of first-order kinetics.

In this situation the presented approach showed to work quite well, although for the simulations the underlying error-distribution (i.e. the numerical instability) was not exactly identified. Only about 3% of the 41035 simulations yielded critical results where a „non-critical" result was defined as an absolute deviation of <1 from the expected value in addition to a relative deviation of <20%. It may be assumed that under approximate normally distributed errors the presented procedure would yield even better results. Analyzing a single non-linear kinetic process, the described approach is based on easy to perform calculations.

In experiments purely designed to identify single kinetic processes, concentration-time courses would not be observed until the system returns to its steady-state. Since the concentration changes over time the velocity of the process will also change, if the underlying kinetics are not of first-order. The most important dynamic characteristics concerned with the disturbance of the system may, therefore, be seen in a short time window after the system was disturbed.

However, analyzing the inherent kinetic processes of dynamic systems simultaneously will always require to investigate complete concentration-time courses in order to reach maximum velocities in all involved compartments. How the proposed procedure works in this situation remains to be elucidated.

Studies and experiments designed for kinetic *in vivo* assessments are usually based on collections of individual subjects. One or more interesting response variables are measured at several selected times leading to so-called time-dependent response curves. The selected individual subjects are assumed to be a representative sample of a population of individuals, all of whose responses are related to time in a basically similar way but with some variation from individual to individual. It is important to consider the measurement process on the individual response curves and also to take into account the variation of these individual response curves around a mean population curve.

The measured data structure is determined by the number k of individuals sampled from the population and the number n_i of observations made on individual i ($i=1,2, \dots ,k$) . The observed response of the i-th individual at time t_{ij} will be denoted by y_{ij} ($i=1,2, \dots ,k$; $j=1,2,\dots ,n_i$) .

We assume that the relationship between the response y_{ij} and the time of measurement t_{ij} takes the form

$$y_{ij} = f(\theta_i, t_{ij}) + e_{ij} \quad , \quad i = 1,2,\dots,k \quad ; \quad j = 1,2,\dots,n_i \quad ,$$

where f describes the expected value of the response at the different times t_{ij} for a given parameter vector θ_i of individual i and e_{ij} is a zero-mean measurement error assumed independent from observation to observation with constant variance.

For a sample of individuals from the same population the same form of functional relationship f is expected. The parameter vector θ_i which determines the precise shape of the curve for each individual is assumed to vary across the population in the manner of a random sample.

Several statistical methods have been proposed for the inference of kinetic parameters in models that combine individual and population components, however, there is only sparse literature regarding the identification of the underlying functional relationship itself.

Whatever procedure is followed up to now, a substantial amount of diagnostic and cross validating checking has to be performed to investigate the appropriateness of the choice of response curves, error variance forms, distributional assumptions, and approximations invoked in the inference procedure. The presented approach using a first-order approximation may help to reduce the complexity for this purpose.

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