Extending the Convolution Method:
A General Integral Formalism for Chemical Kinetics.
Application to Enzymatic Reactions

Mário N. Berberan-Santos

Centro de Química-Física Molecular and IN - Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade Técnica de Lisboa, 1049-001 Lisboa, Portugal, e-mail: berberan@ist.utl.pt

ABSTRACT

The standard approach in chemical and photochemical kinetics is to proceed from the kinetic scheme to the corresponding system of first-order differential equations, and then to integrate it. When the species of interest decay via unimolecular or pseudo-unimolecular steps, the evolution equations can be directly written in integral form by application of the convolution method (CM). The CM as presently developed is not applicable when the species of interest decay by one or more bimolecular processes. In this work the CM is extended by developing a completely general integral formulation. This approach allows obtaining approximate solutions to any desired degree of accuracy. Several examples of application are presented, including Henri-Michaelis-Menten enzyme kinetics.

1. INTRODUCTION

The standard approach in formal chemical and photochemical kinetics is to proceed from the kinetic scheme to the corresponding system of first-order differential equations, and then to integrate this system, analytically or numerically, in order to obtain the concentrations of selected species as a function of time, which is the main goal [1,2]. Analytical integration is of course much preferable, but when there are bimolecular elementary steps it is seldom possible without recourse to simplifications such as the quasi-steady-state and pre-equilibrium approximations.

When the species of interest decay solely via unimolecular or pseudo-unimolecular steps, it is nevertheless possible to sidestep the system of differential equations. In fact, the evolution equations can be directly written in integral form by application of the convolution method (CM) [3-6]. The equations are in general coupled, but the explicit solutions can be obtained in most cases by repeated substitution or by use of Laplace transforms. This very useful approach (see [7-10] for specific applications) will only be briefly recalled here.

The CM as presently developed is unfortunately not applicable if at least one of the species of interest decays by one or more bimolecular processes [6]. It is the purpose of this work to extend the CM by developing a completely general integral formulation of chemical and photochemical kinetics.

2. THEORY

The central quantity in the CM is the survival probability of a given species X_i (i = 1, 2, ..) after delta (instantaneous) production [3-6]:

$$X_{i\delta}(t) = \exp(-k_i t) \tag{1}$$

where for simplicity X_i stands also for the concentration of species X_i. The survival probability function is assumed to be the same, whatever the time at which production takes place. This means in particular that the decay of species X_i is not dependent on the concentration of other species, hence it must be unimolecular (or pseudo-unimolecular at most). The response to other types of stimulii, be they reactants or other production modes (e.g. light absorption that generates an excited state) is written as

$$X_i(t) = P_i \otimes X_{i\delta}(t) = P_i \otimes \exp(-k_i t) \tag{2}$$

where $\otimes$ stands for the convolution between two functions, $f \otimes g = \int_0^t f(u)g(t-u)du$. This results from an assumption of linearity [3-6].

We now generalize Eq. (1), and in particular to cases where X_i decays by bimolecular processes. If X_i is generated at time t_0, then the respective survival probability is in general

$$X_{i\delta}(t \mid t_0) = \exp\left(-\int_{t_0}^t k_i(u)\,du\right) \tag{3}$$

where $k_i(t)$ is an appropriate time-dependent rate coefficient, whose form will be detailed in Sect. 3. This time dependence may arise from a concentration dependence, if the respective disappearance step is bimolecular, with $k_i(t) = k_{ij}X_j(t)$, but also from the temperature dependence of a unimolecular rate constant, if the reaction is not isothermal. In both cases the delta response is neither independent of the production time nor of the subsequent system's evolution (unlike the cases previously considered in the so-called transient kinetics [3,4] where the same time-dependent rate coefficient is valid for impulses occurring at different times). The time evolution of a species is linked

to the rest of the system not only by the production, but also by the disappearance rate. Instead of Eq. (2), the response to a general (non-delta) production is now given by

$$X_i(t) = \int_0^t P_i(v) X_{i\delta}(t \mid v) dv \tag{4}$$

This is the main result of the present work, and represents a generalization of the CM. An assumption of linearity is still implicit, which is consistent with the usual differential equation formalism. Indeed, differentiation of Eq. (4) with respect to time gives

$$\frac{dX_i}{dt} = P_i(t) - k_i(t) X_i(t) \tag{5}$$

This result demonstrates the strict equivalence of the differential and integral kinetic formalisms.

In the CM, the long-time limit of X_i, $X_{i\infty}$, is given by [4]

$$X_{i\infty} = \lim_{t \to \infty} P_i \otimes X_{i\delta}(t) = P_i(\infty) \int_0^\infty X_{i\delta}(u) \, du \tag{6}$$

And assumes a simple form if $X_{i\delta}(t)$ is given by Eq. (2).

The more general result in integral kinetics is now

$$X_{i\infty} = P_i(\infty) \int_{t_0}^\infty X_{i\delta}^\infty(u \mid t_0) \, du \tag{7}$$

where $X_{i\delta}^\infty(u \mid t_0)$ stands for the limiting form of $X_{i\delta}(u \mid t_0)$ when t_0 is very large. Eq. (7) is valid only when $P_i(t)$ attains a constant value for $t \to \infty$.

3. APPLICATIONS

3.1 Irreversible reactions

3.1.1 Elementary bimolecular reaction

The simplest possible nontrivial case is the elementary bimolecular reaction

$$X_1 + X_2 \xrightarrow{\ k\ } X_3$$
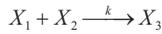
Scheme 1

whose analytical solution is

$$X_2(t) = \frac{X_{10} - X_{20}}{X_{10} \exp[k(X_{10} - X_{20})t] - X_{20}} X_{20} \tag{8}$$

An identical equation exists for X_1.

If X_2 is generated at time t_0, then its survival probability reads

$$X_{2\delta}(t \mid t_0) = \exp\left(-k \int_{t_0}^{t} X_1(u)\, du\right) \tag{9}$$

On the other hand, the production term of X_2 is

$$P_2(t) = X_{20}\, \delta(t) \tag{10}$$

and therefore, using Eq. (4), the time evolution of X_2 is simply

$$X_2(t) = X_{20} \exp\left(-k \int_{0}^{t} X_1(u)\, du\right) \tag{11}$$

An analogous equation exists for X_1,

$$X_1(t) = X_{10} \exp\left(-k \int_{0}^{t} X_2(u)\, du\right) \tag{12}$$

and insertion of Eq. (12) into Eq. (11) gives

$$X_2(t) = X_{20} \exp\left[-k\,X_{10} \int_0^t \exp\left(-k\int_0^u X_2(v)\,dv\right) du\right] \qquad (13)$$

which is an integral equation in X_2 only. This equation can be solved by the repeated insertion of the l.h.s. into the r.h.s., starting from the zero-order approximation, $X_2^{(0)}(t) = X_{20}$. The first-order approximation is

$$X_2^{(1)}(t) = X_{20} \exp\left[-\frac{X_{10}}{X_{20}}\left(1 - e^{-k\,X_{20}\,t}\right)\right] \qquad (14)$$

and is accurate only for short times. A similar equation applies to X_1. The second-order approximation follows from substitution of Eq. (14) into Eq. (13),

$$X_2^{(2)}(t) = X_{20} \exp\left[-k\,X_{10} \int_0^t \exp\left(-e^{-\frac{X_{10}}{X_{20}}}\left[Ei\left(\frac{X_{10}}{X_{20}}\right) - Ei\left(\frac{X_{10}}{X_{20}}e^{-k\,X_{20}\,u}\right)\right]\right) du\right] \qquad (15)$$

It is a good approximation for all times if $X_{10}/X_{20} < 0.8$ or if $X_{10}/X_{20} > 1.3$. Of course, this approximate equation offers no advantage whatsoever over the exact solution, Eq. (8).

A slightly different approach uses the relation between X_1 and X_2 (as is done for the integration of the rate equation),

$$X_{10} - X_1(t) = X_{20} - X_2(t) \qquad (16)$$

and allows to directly obtain the exact solution, Eq. (8). In fact, substitution of Eq. (16) into Eq. (11) gives

$$X_2(t) = X_{20} \exp\left[-k\left(X_{10} - X_{20}\right)t\right]\exp\left(-k\int_0^t X_2(u)\,du\right) = \frac{X_{20}}{X_{10}} \exp\left[-k\left(X_{10} - X_{20}\right)t\right]X_1(t) \quad (17)$$

and using again Eq. (16), Eq. (17) becomes

$$X_2(t) = \frac{X_{20}}{X_{10}} \exp\left[-k\left(X_{10} - X_{20}\right)t\right]\left[X_{10} - X_{20} + X_2(t)\right] \qquad (18)$$

which is an algebraic equation that yields Eq. (8). In this way the integral approach leads to the exact solution of a non-linear kinetic problem without solving any differential equation.

3.1.2 Consecutive reactions with one bimolecular step

Another example of application is the following scheme:

$$X_1 \xrightarrow{\ k_1\ } X_2$$
$$X_2 + X_3 \xrightarrow{\ k_2\ } X_4$$
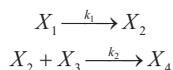
Scheme 2

where X_{10} and X_{30} are nonzero. This scheme has a known solution in terms of incomplete gamma functions [11]. Only when $X_{30} >> X_{10}$ (pseudo-unimolecular conditions) is the solution elementary. The system of differential equations is:

$$\frac{dX_1}{dt} = -k_1 X_1 \tag{19}$$

$$\frac{dX_2}{dt} = k_1 X_1 - k_2 X_2 X_3 \tag{20}$$

$$\frac{dX_3}{dt} = -k_2 X_2 X_3 \tag{21}$$

$$\frac{dX_4}{dt} = k_2 X_2 X_3 \tag{22}$$

The integral approach to Scheme 2 is now outlined.

If X_2 is generated at time t_0, then its survival probability reads

$$X_{2\delta}(t \mid t_0) = \exp\left(-\int_{t_0}^{t} k_2 X_3(u) \, du \right) \tag{23}$$

On the other hand, the production term for X_2 is

$$P_2(t) = k_1 X_1(t) \tag{24}$$

and therefore the time evolution of X_2 is

$$X_2(t) = \int_0^t P_2(u) X_{2\delta}(t \,|\, u) \, du = \int_0^t k_1 X_1(u) \exp\left(-\int_u^t k_2 X_3(v) \, dv\right) du \qquad (25)$$

As a check, it is confirmed that differentiation of this equation with respect to time yields the customary rate equation form,

$$\frac{dX_2}{dt} = k_1 X_1 - k_2 X_3 \int_0^t k_1 X_1 \exp\left(-\int_u^t k_2 X_3(v) \, dv\right) du = k_1 X_1 - k_2 X_2 X_3 \qquad (26)$$

as it should.

The formal solution for X_2, Eq. (25), is a function of the concentrations of X_1 and X_3, and further steps are needed to obtain the final result. In this case, the time evolution of X_1 is simply

$$X_1(t) = X_{10} \exp\left(-k_1 t\right) \qquad (27)$$

whereas the time evolution of X_3 obeys

$$X_3(t) = X_{30} \exp\left(-\int_0^t k_2 X_2(u) \, du\right) \qquad (28)$$

Insertion of Eqs. (27) and (28) in Eq. (25) yields

$$X_2(t) = k_1 X_{10} \int_0^t \exp\left[-k_1 u - k_2 X_{30} \int_u^t \exp\left(-k_2 \int_0^v X_2(w) \, dw\right) dv\right] du \qquad (29)$$

which is an integral equation for X_2 only. As in the previous example, this equation can be solved by the repeated insertion of the l.h.s. into the r.h.s., starting from the zero-order approximation which is $X_2^{(0)}(t) = X_{20} = 0$. The lower order approximations will be accurate only for short times. The first-order approximation is

$$X_2^{(1)}(t) = \frac{k_1 X_{10}}{k_2 X_{30} - k_1}\left(e^{-k_1 t} - e^{-k_2 X_{30} t}\right) \qquad (30)$$

which is the exact result when $X_{30} >> X_{10}$. The second-order approximation, which is better than $X_2^{(1)}(t)$, is obtained as

$$X_2^{(2)}(t) = k_1 X_{10} \int_0^t \exp\left(-k_1 u - k_2 X_{30} \int_u^t \exp\left[-\frac{k_1 k_2 X_{10}}{k_2 X_{30} - k_1}\left(\frac{1 - e^{-k_1 v}}{k_1} - \frac{1 - e^{-k_2 X_{30} v}}{k_2 X_{30}}\right)\right] dv\right) du \qquad (31)$$

A different approach is again to directly relate X_3 and X_2:

$$X_3(t) = X_{30} + X_2(t) + X_1(t) - X_{10} = X_{30} + X_2(t) - (1 - e^{-k_1 t}) X_{10} \qquad (32)$$

and then to insert this equation together with Eq. (27) into Eq. (25) to yield

$$X_2(t) = k_1 X_{10} \int_0^t \exp\left(-k_1 u - k_2 \left(X_{30} - X_{10}\right)(t - u) + \frac{k_2 X_{10}}{k_1}\left(e^{-k_1 t} - e^{-k_1 u}\right) - k_2 \int_u^t X_2(v) dv\right) du \quad (33)$$

which is equivalent but simpler than Eq. (29). The corresponding first-order approximation is in this case

$$X_2^{(1)}(t) = k_1 X_{10} \int_0^t \exp\left[-k_1 u - k_2 \left(X_{30} - X_{10}\right)(t - u) + \frac{k_2 X_{10}}{k_1}\left(e^{-k_1 t} - e^{-k_1 u}\right)\right] du \qquad (34)$$

With a software like *Mathematica* higher order approximations can be easily obtained numerically.

3.3 Reversible reactions

3.3.1 Elementary equilibrium with one bimolecular step

The simplest possible case is now the reaction

$$X_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X_2 + X_3$$
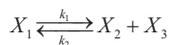
Scheme 3

with $X_{20} = X_{30} = 0$, whose solution, given in implicit form in [12], can be rewritten as

$$X_2(t) = \frac{X_{10}X_{2\infty}\left(1 - \exp\left[-\left(2\frac{X_{10}}{X_{2\infty}} - 1\right)k_1 t\right]\right)}{X_{10} + (X_{10} - X_{2\infty})\exp\left[-\left(2\frac{X_{10}}{X_{2\infty}} - 1\right)k_1 t\right]} \tag{35}$$

where $X_{2\infty}$ is the equilibrium concentration of X_2,

$$X_{2\infty} = \frac{2X_{10}}{1 + \sqrt{1 + \frac{4k_2 X_{10}}{k_1}}} \tag{36}$$

Application of the integral formalism gives

$$X_1(t) = \int_0^t [X_{10}\delta(u) + k_2 X_2(u)X_3(u)]\exp[-k_1(t - u)]\,du = \left(X_{10} + k_2\int_0^t X_2(u)X_3(u)e^{k_1 u}du\right)e^{-k_1 t} \tag{37}$$

$$X_2(t) = \int_0^t k_1 X_1(u)\exp\left(-\int_u^t k_2 X_3(v)\,dv\right)du \tag{38}$$

$$X_3(t) = \int_0^t k_1 X_1(u)\exp\left(-\int_u^t k_2 X_2(v)\,dv\right)du \tag{39}$$

Using $X_{10} - X_1(t) = X_2(t) = X_3(t)$, an integral equation for X_1 is obtained,

$$X_1(t) = \left(X_{10} + k_2\int_0^t [X_{10} - X_1(u)]^2 e^{k_1 u}du\right)e^{-k_1 t} \tag{40}$$

The first-order approximation is

$$X_1^{(1)}(t) = X_{10}e^{-k_1 t} \tag{41}$$

This is the irreversible reaction result. The second-order approximation is

$$X_1^{(2)}(t) = X_{10}\left[1 + k_2 X_{10}\int_0^t (1 - e^{-k_1 u})^2 e^{k_1 u}du\right]e^{-k_1 t} = X_{10}\left(1 + \frac{2k_2 X_{10}}{k_1}[\sinh(k_1 t) - k_1 t]\right)e^{-k_1 t} \tag{42}$$

and is valid for $k_2 X_{10} \ll k_1$. With a software like *Mathematica* calculation of analytical

higher order approximations using Eq. (40) is immediate. In Fig. 1 two approximations

for $X_1(t)$ are displayed along with the exact solution. The following results are observed: (i) The higher the approximation, the better the general agreement. (ii) For a given approximation, the agreement is good for short times, but deteriorates with time stabilizing at a constant difference for long times. (iii) The more important the reversibility, the worse the general agreement, and the earlier the time at which a given approximation starts to diverge significantly from the exact solution.

3.3.2 Enzyme kinetics

The simplest description of enzyme action is the so-called irreversible Henri-Michaelis-Menten (HMM) mechanism:

$$E + S \xrightleftharpoons[k_d]{k_a} ES \xrightarrow{k_{cat}} E + P$$
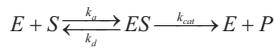
Scheme 4

where E is the enzyme, S the substrate, ES the enzyme-substrate complex, and P is the product. In this scheme, reversibility of the second step is neglected, and also the necessary existence of a second complex EP is not accounted for explicitly. No analytical solution is known for the HMM kinetics, that is, the system of kinetic differential equations has not been solved to yield expressions for the concentrations of all species in closed form, i.e., in terms of known functions. Interestingly, the more general reversible HMM scheme,

$$E + S \xrightleftharpoons[k_d]{k_a} ES \xrightleftharpoons[k_b]{k_{cat}} E + P$$
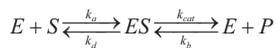
Scheme 5
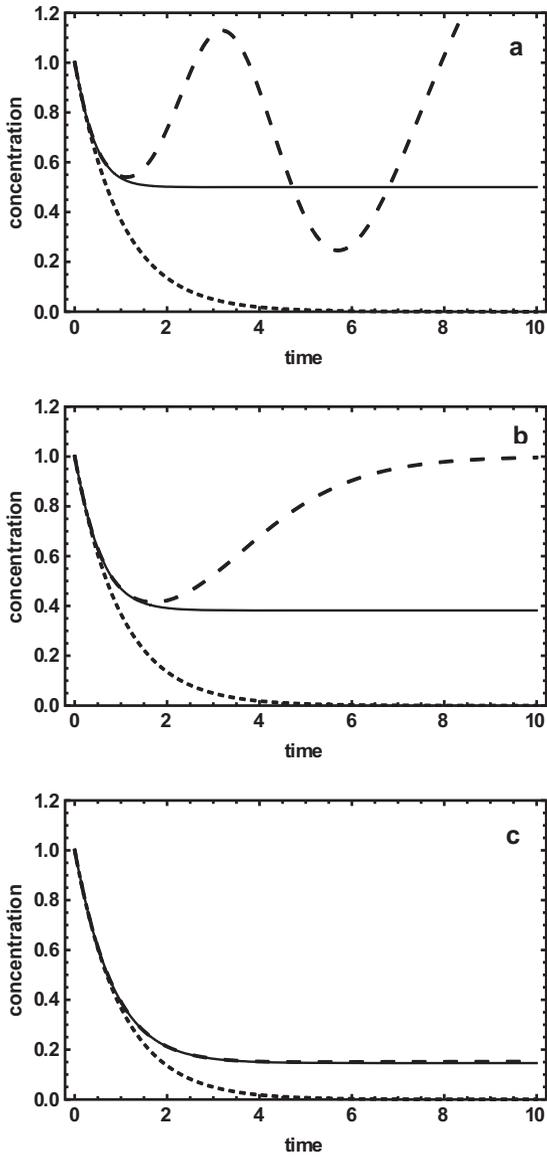
Fig. 1 First-order (---) and fourth-order (– –) approximations, along with the exact result (——), for $X_{10}=1$, $k_1=1$ and $k_2=2$ (a), $k_2=1$ (b), $k_2=0.2$ (c).

admits a simple analytical solution for the particular case $k_a = k_b$ [13].

Recently, an exact one-dimensional formulation of the irreversible HMM kinetics was developed [14], the whole problem being condensed in a second-order non-linear autonomous differential equation,

$$\Phi \frac{d^2\sigma}{d\tau^2} + \left(1 + e_0 + \sigma + \frac{d\sigma}{d\tau}\right)\frac{d\sigma}{d\tau} + e_0\sigma = 0 \tag{43}$$

where $e = [E]/K_m$, $s = [S]/K_m$, $c = [ES]/K_m$, $p = [P]/K_m$, $e_0 = [E]_0/K_m$, $s_0 = [S]_0/K_m$, and $\sigma = s + c = s_0 - p$ are dimensionless concentrations, $\tau = k_{cat}\, t$ is a dimensionless time, Φ is the enzyme efficiency, $\Phi = k_{cat}/(k_{cat} + k_d)$, and K_m is the Michaelis constant, $K_m = (k_d + k_{cat})/k_a$. The exact solution of HMM kinetics was then obtained as a set of Maclaurin series [14],

$$s(\tau) = s_0 - \frac{e_0 s_0}{\Phi}\tau + \frac{e_0 s_0}{\Phi^2}\left(1 - \Phi + e_0 + s_0\right)\frac{\tau^2}{2!} + \ldots, \tag{44}$$

$$c(\tau) = \frac{e_0 s_0}{\Phi}\tau - \frac{e_0 s_0}{\Phi^2}\left(1 + e_0 + s_0\right)\frac{\tau^2}{2!} + \ldots, \tag{45}$$

$$p(\tau) = \frac{e_0 s_0}{\Phi}\frac{\tau^2}{2} - \frac{e_0 s_0}{\Phi^2}\left(1 + e_0 + s_0\right)\frac{\tau^3}{3!} + \ldots. \tag{46}$$

From the same evolution equation, a number of approximate solutions, some known, some new, were derived in a systematic way. For instance, for $e_0 \ll 1 + s_0$ it was obtained that after the short transient phase, and up to near completion [14],

$$s(\tau) = W\left(s_0\, e^{s_0} e^{-e_0\tau}\right) - e_0 \frac{W\left(s_0\, e^{s_0} e^{-e_0\tau}\right)}{1 + W\left(s_0\, e^{s_0} e^{-e_0\tau}\right)}, \tag{47}$$

$$p(\tau) = s_0 - W\left(s_0\, e^{s_0} e^{-e_0\tau}\right) \tag{48}$$

where W is the Lambert function.

The evolution equation obtained is also well suited for the numerical computation of the concentrations of all species as a function of time for any given combination of parameters.

Application of the integral formalism to the irreversible HMM kinetics gives

$$E(t) = \int_0^t \left[E_0\delta(u) + (k_d + k_{cat})C(u) \right] \exp\left(-\int_u^t k_a S(v)dv \right) du \tag{49}$$

$$S(t) = \int_0^t \left[S_0\delta(u) + k_d C(u) \right] \exp\left(-\int_u^t k_a E(v)dv \right) du \tag{50}$$

Where E, S and C are the enzyme, substrate and enzyme-substrate complex concentrations, respectively. The following mass conservation relations are obeyed,

$$E_0 = E(t) + C(t) \tag{51}$$

$$S_0 = S(t) + C(t) + P(t) \tag{52}$$

Using Eq. (51), Eqs. (49) and (50) become, after simplification,

$$E(t) = E_0 \exp\left(-\int_0^t k_a S(v)dv \right) + (k_d + k_{cat}) \int_0^t [E_0 - E(u)] \exp\left(-\int_u^t k_a S(v)dv \right) du \tag{53}$$

$$S(t) = S_0 \exp\left(-\int_0^t k_a E(v)dv \right) + k_d \int_0^t [E_0 - E(u)] \exp\left(-\int_u^t k_a E(v)dv \right) du \tag{54}$$

Insertion of Eq. (54) in Eq. (53) gives an exact integral equation in E only,

$$E(t) = E_0 \exp(\Xi) + (k_d + k_{cat}) \times \int_0^t [E_0 - E(z)] \exp(\Xi) dz \tag{55}$$

where

$$\Xi = -\int_z^t k_a \left[S_0 \exp\left(-\int_0^u k_a E(w)dw \right) + k_d \int_0^u [E_0 - E(v)] \exp\left(-\int_v^u k_a E(w)dw \right) dv \right] du.$$

This equation is in a sense the integral counterpart of Eq. (43).

With the initial approximation $S^{(0)}(t) = S_0$ Eq. (53) reduces to

$$E(t) = E_0 \exp\left(-k_a S_0 t\right) + \left(k_d + k_{cat}\right) \int_0^t \left[E_0 - E(u)\right] \exp\left[-k_a S_0 \left(t - u\right)\right] du \qquad (56)$$

whose solution, obtained by the application of Laplace transforms, is the first-order approximation for $E(t)$,

$$E^{(1)}(t) = E_0 \frac{K_m + S_0 \exp\left[-k_a \left(K_m + S_0\right) t\right]}{K_m + S_0} \qquad (57)$$

This approximation is better than that obtained from Eq. (55) by setting $E = E_0$ in the r.h.s., and which is analogous to Eq. (14), as reversibility is neglected at this level,

$$E^{(1)}(t) = E_0 \exp\left[-\frac{S_0}{E_0}\left(1 - e^{-k_a E_0 t}\right)\right] \qquad (58)$$

Substitution of Eq. (57) into Eq. (54) gives a first-order approximation for $S(t)$ that is still valid only for short times. However, a different route for the calculation of an approximate $S(t)$ that yields a simpler and more accurate expression is to use Eqs. (51) and (57) to get

$$C^{(1)}(t) = E_0 S_0 \frac{1 - \exp\left[-k_a \left(K_m + S_0\right) t\right]}{K_m + S_0} \qquad (59)$$

and then to note that

$$P^{(1)}(t) = k_{cat} C^{(1)} \otimes 1 = \frac{k_{cat} E_0 S_0}{K_m + S_0}\left(t - \frac{1 - \exp\left[-k_a \left(K_m + S_0\right) t\right]}{k_a \left(K_m + S_0\right)}\right) \qquad (60)$$

Hence, using Eq. (52),

$$S^{(1)}(t) = S_0 - \frac{k_{cat} E_0 S_0}{K_m + S_0} t - \frac{E_0 S_0 \left(k_d / k_a + S_0\right)}{\left(K_m + S_0\right)^2}\left(1 - \exp\left[-k_a \left(K_m + S_0\right) t\right]\right) \qquad (61)$$

or, in reduced variables,

$$s^{(1)}(\tau) = s_0 - \frac{e_0 s_0}{1 + s_0}\tau - \frac{e_0 s_0 \left(1 - \Phi + s_0\right)}{\left(1 + s_0\right)^2}\left[1 - \exp\left(-\frac{1 + s_0}{\Phi}\tau\right)\right] \qquad (62)$$
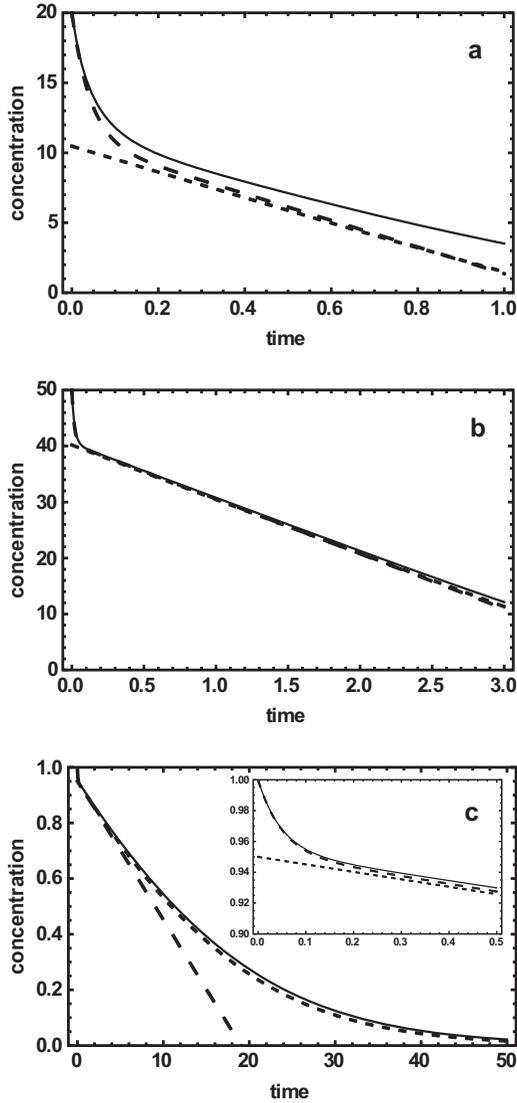
Fig. 2 Substrate reduced concentration in HMM kinetics: Approximate analytical solutions Eq. (47) (----), Eq. (62) (− −), and exact numerical integration (——) for $\Phi=0.9$, $e_0= 10$, and $s_0=20$ (a); $\Phi=0.9$, $e_0= 10$, and $s_0=50$ (b); and $\Phi=0.1$, $e_0= 0.1$, and $s_0=1$ (c). Note the early crossing of the two approximate solutions in case (c).

This is a simple formula that represents quite well the exact solution (obtained numerically) for short times, and also for intermediate times provided $S_0 > E_0$, see Fig. 2. It describes both the initial transient phase, with a duration of approximately $1/(k_a(K_m+S_0))$, and the subsequent steady-state with a linear time evolution.

For $S_0 >> K_m$ Eq. (61) further simplifies to

$$S^{(1)}(t) = S_0 - E_0 \left[k_{cat}t + 1 - \exp(-k_a S_0 t) \right]$$ (63)

Note that the differential equation formalism under the same approximation also allows obtaining Eq. (61), but only Eqs. (59) [15-17] and (60) [17] seem to have been previously derived. Indeed, Eq. (52) immediately yields *S(t)*, Eq. (61), if *E(t)* and *C(t)* are (approximately) known. This was nevertheless not done, probably as a result of the notion that making the initial assumption *S(t)=S₀* would render meaningless a subsequent bootstrap recalculation of *S(t)*. Indeed, the method as applied to the system of differential equations does not converge for higher approximations. On the other hand, convergence of the iterative solution of the system of Eqs. (53) and (54) is expected, but convergence is slow and does not lead in this case to simple analytical functions.

Higher order calculations of $E(t)$ can be made numerically using Eqs. (53) and (54), but are increasingly demanding in terms of computer time. In Fig. 3 are shown the first and second-order approximations, $E^{(1)}(t)$ and $E^{(2)}(t)$, and also an improved approximation $E_{imp}(t)$ obtained inserting Eq. (61) in Eq. (53). It is seen that this approximation remains valid for to longer times than the other two, as Eq. (61) is also closer to the exact solution for $S(t)$ and convergence is thus significantly improved.
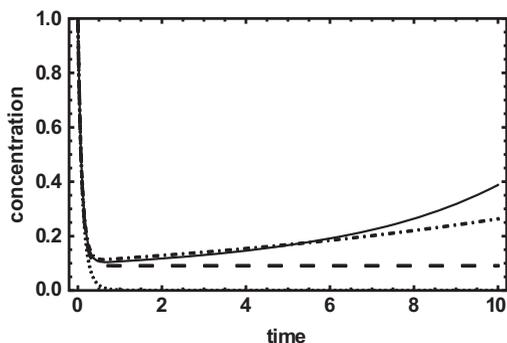
Fig. 3 Enzyme reduced concentration in HMM kinetics: First-order $e^{(1)}(\tau)$ ($\cdots$), second-order $e^{(2)}(\tau)$ ($--$), and improved $e_{\text{imp}}(\tau)$($-\cdot-$) approximations, along with the exact result $e(\tau)$ ($—$) for $\Phi = e_0 = s_0 = 1$.

4. CONCLUSIONS

The standard approach in formal kinetics is to proceed from the kinetic scheme to the corresponding system of first-order differential equations, and then to integrate this system in order to obtain the concentrations of selected species as a function of time. In the present work a completely general integral formulation of chemical and photochemical kinetics was developed. This approach does not require the writing and integration of the system of differential equations. The integral formulation, as shown with several examples, leads to complicated integral equations but for the simplest case. Nevertheless it allows obtaining approximate solutions to any desired degree of accuracy. Application of the integral approach to the Henri-Michaelis-Menten enzyme kinetics led to a general characteristic integral equation, along with a simple yet accurate particular analytical solution for the substrate concentration.

REFERENCES

[1] K. J. Laidler, *Chemical Kinetics*, Harper & Row, Cambridge, 1987.

[2] P. Érdi, J. Tóth, *Mathematical Models of Chemical Reactions*, Princeton University Press, Princeton, 1989.

[3] M. N. Berberan-Santos, The time dependence of rate coefficients and fluorescence anisotropy for non-delta production, *J. Lumin.* **50** (1991) 83-87.

[4] M. N. Berberan-Santos, J. M. G. Martinho, A linear response approach to kinetics with time-dependent rate coefficients, *Chem. Phys.* **164** (1992) 259-269.

[5] M. N. Berberan-Santos, L. Pogliani, J. M. G. Martinho, A convolution approach to the kinetics of chemical and photochemical reactions, *React. Kinet. Catal. Lett.* **54** (1995) 287-292.

[6] L. Pogliani, M. N. Berberan-Santos, J. M. G. Martinho, Matrix and convolution methods in chemical kinetics, *J. Math. Chem.* **20** (1996) 193-210.

[7] M. N. Berberan-Santos, J. M. G. Martinho, Kinetics of sequential energy-transfer processes, *J. Phys. Chem.* **94** (1990) 5847-5849.

[8] M. N. Berberan-Santos, J. M. G. Martinho, Diffusion-influenced excimer formation kinetics, *J. Chem. Phys.* **95** (1991) 1817-1824.

[9] M. N. Berberan-Santos, J. P. Farinha, J. M. G. Martinho, Linear and convolution methods for the analysis of ground and excited state kinetics. Application to the monomer-excimer scheme*, Chem. Phys.* **260** (2000) 401-414.

[10] C. Baleizão, M. N. Berberan-Santos, Thermally activated delayed fluorescence as a cycling process between excited singlet and triplet states. Application to the fullerenes, *J. Chem. Phys.* **126** (2007) 204510.

[11] J. Chien, Kinetic analysis of irreversible consecutive reactions, *J. Am. Chem. Soc.* **70** (1948) 2256-2261.

[12] Z. G. Szabó, Kinetic characterization of complex reaction systems, in: C. H. Bamford, C. F. H. Tipper (Eds.), *Comprehensive Chemical Kinetics*, Vol. 2, Elsevier, Amsterdam, 1969.

[13] W. G. Miller, R. A. Alberty, Kinetics of the reversible Michaelis-Menten mechanism and the applicability of the steady-state approximation, *J. Am. Chem. Soc.* **80** (1958) 5146-5151.

[14] M. N. Berberan-Santos, A general treatment of Henri-Michaelis-Menten enzyme kinetics: Exact series solution and approximate analytical solutions, *MATCH Commun. Math. Comput. Chem.* **63** (2010) 283-318.

[15] H. Gutfreund, Steps in the formation and decomposition of some enzyme-substrate complexes, *Disc. Faraday Soc.* **20** (1955) 167-173.

[16] A. R. Schultz, *Enzyme Kinetics*, Cambridge Univ. Press, Cambridge, 1994.

[17] K. J. Laidler, *The Chemical Kinetics of Enzyme Action*, Oxford Univ. Press, Oxford, 1958.