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ORIGINAL PAPER

Linear compartmental systems. III. Application to enzymatic reactions

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Abstract In this paper we extend to enzyme systems the results previously obtained in paper I of this series for linear compartmental systems. We obtain the time course equations for both the enzyme and ligand species involved in the reaction mechanisms,

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which fit a general enzyme system model when the connections between the different enzyme species are of first or pseudofirst order. The kinetic equations obtained here for a given species, enzyme or ligand have the advantage over all previous equations described in the literature, in that they are in the most simplified form possible, since they only contain the kinetic parameters and initial concentrations of the enzymatic reaction which really have some influence on the time progress curves of the species under study. These kinetic equations are denominated optimized equation to distinguish them from the others, which shall call non-optimized equations. We discuss those cases when both types of equation coincide and we show how, when they do not coincide, the non-optimized equations can be simplified to the optimized ones. Therefore, we show that the optimized equations could be used in all cases to avoid the need of subsequent simplifications to eliminate the parameters that play no role in the corresponding time equations. To illustrate the use of this procedure we will apply it to two simple examples of enzymatic reactions.

Keywords Compartmental system · Enzyme system · Kinetic equation · Enzyme species · Ligand species

1 Introduction

Compartmental systems are important for describing many aspects of biotechnology and many other biological sciences; for example pharmacokinetic processes, namely the absorption, distribution and elimination of drugs, metabolite kinetics, residence times [1,2], enzyme kinetics [3,4], nuclear medicine [5], the study of basic nutritional processes, e.g. digestion, nutrient uptake and metabolism [6], toxickinetics [7], various aspects of cell growth [8] and of tumor cell growth [9].

Recently, Garcia-Sevilla et al. published two contributions [10,11] on obtaining symbolic optimized kinetic equations, i.e. in the simplest possible form, valid in any linear compartmental system, open or closed, with zero inputs. The kinetic equations were established in [10], and a software was implemented in [11] to facilitate the process of obtaining these equations, circumventing the slow and laborious manual work and hence the possibility of human errors. The results obtained in these contributions can be applied directly to any linear compartmental system, thus providing a powerful and interesting tool.

To illustrate the potential of these results we propose applying them to the kinetic analysis of enzyme systems which, in the most usual experimental conditions, can be modeled as a linear compartmental system, whose compartments are the different enzyme forms involved in the reaction mechanism.

Most enzymatic reactions in the usual limiting enzyme experimental conditions can be considered special cases of linear compartmental systems. In this contribution we establish a general model of enzymatic reaction that fits a linear system of compartments so that it can be applied to the results given in [10]. This is the starting point of the present contribution. However, as already mentioned, this requires further analysis, which is carried out here, to obtain the time course equations of the ligand species (substrate, product, activators and inhibitors) involved in the enzymatic reaction.

This analysis could be done directly without considering the enzyme system as a compartmental system. However, this would be unnecessarily difficult, tedious and slow and would limit the possibilities and the mathematical elegance that emanates when one particularizes from a general model.

The aim of this analysis is to obtain analytical solutions, more general and optimized than those currently available [12–22]. We provide the kinetic equations corresponding to any enzymatic reaction that fits a general model corresponding to first or pseudofirst order interconversions, reversible or irreversible, between the enzyme forms. Most enzymatic reactions fit this model under certain initial conditions previously proposed by our group [15–20,22].

In paper IV of this series we will implement specific software that allows these optimized equations to be obtained easily and with a short computational time, thus avoiding human errors.

2 Enzyme systems as compartmental systems

For enzymatic reactions, there are three experimental ways to achieve first or pseudofirst order interconversions between enzyme forms: (1) Setting initial ligand species concentration in excess with respect to the enzyme species to which the former binds [13,14]. (2) Setting the initial free enzyme concentration in excess of the ligand species with which the free enzyme combines [23–26] and (3) Assuming that the concentration of one or more enzyme or ligand species remains constant throughout the course of the reaction, regardless of whether or not it is in excess with respect to other species [26–38]. Options 1) and 2) correspond to the conditions of limiting enzyme or limiting ligand, respectively.

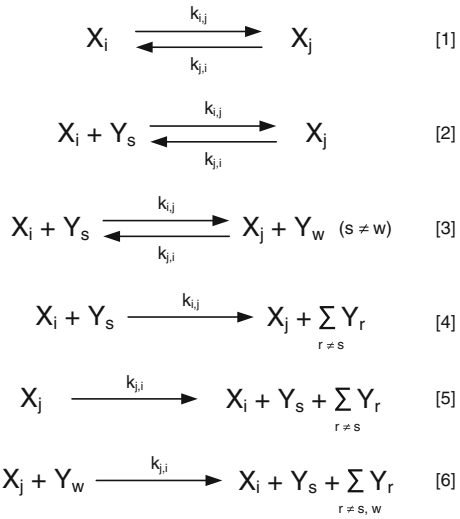
Varon [39], Galvez and Varon [4] and Galvez et al. [40–42] analyzed the transition phase of enzymatic reactions as a special case of closed linear compartmental systems. This analogy regarding the kinetic behavior of enzymatic reactions as compartmental systems has also been treated by Garcia-Meseguer [43] and Garcia-Meseguer et al. [20]. But all the equations used by these authors to analyze the enzymatic reactions as compartmental systems suffer from the same limitations: the equations are not in the most simplified form because they contain kinetic parameters and initial concentrations that do not really have any influence on the instantaneous concentrations of interest. In this contribution, we adapt optimized kinetic equations for compartmental systems, Eqs. (44)–(46) in [10], to the enzymatic systems, to obtain the optimized kinetic equations for the enzyme species involved in the reaction mechanism and, from these equations, we obtain the optimized time course equations for the ligand species.

2.1 Enzymatic reactions as compartmental systems

Since this contribution is an extension of [10], the notation used here is the same as used there. Any additional notation will be introduced during the course of this work, setting the following adaptations, when necessary:

1. The number of compartments, n, is used as the number of enzyme species.
2. Compartment X_i is used as the enzyme species X_i $(i = 1, 2, \ldots, n)$.

Scheme 1 Possible elementary reactions [1]–[6] of the general model of enzymatic reaction of a linear compartmental system



3. The fractional transfer coefficient $K_{i,j}$ $(i, j = 1, 2, \ldots, n)$ between two compartments X_i and X_j $(i, j = 1, 2, \ldots, n; i \neq j)$ is used as a rate constant of first or pseudofirst order if, in the conversion of the enzyme species X_i into X_j, there are no parallel steps. If there are parallel steps, then $K_{i,j}$ is replaced by a sum of rate constants of first or pseudofirst order that are represented by $K_{i,j}(1)$, $K_{i,j}(2)$, ... Obviously, in the case of parallel steps, $K_{i,j} = K_{i,j}(1) + K_{i,j}(2) + \ldots$
4. The initial amount of matter in the compartment X_i, x_i^o, is used as the initial concentration of the enzyme species X_i, $[X_i]_0$
5. The instantaneous amount of matter in the compartment X_i, x_i, is used as the instantaneous concentration of enzyme species X_i, $[X_i]$.

The general model of enzymatic reaction, which can be treated as a closed linear compartmental system with zero input, is shown in Scheme 1 [4,39,44]. This general enzymatic reaction model consists of n enzyme species, denoted by X_i $(i = 1, 2, \ldots, n)$, and g ligand species, denoted by Y_s $(s = 1, 2, \ldots, g)$. The reaction steps of the model may be of types [1]–[6] indicated in Scheme 1. Rate constants, $k_{i,j}$, can be zero in any of the steps [1]–[3]. In steps [4]–[6] the subscript r normally takes one or two values, except in step [4], where it takes at least two values, otherwise this step would coincide with step [3].

2.1.1 Initial conditions and rate constants

It is assumed that at the onset of the reaction more than one enzyme species can be present, which in the notation of [10] belong to set Ω. It is also assumed that if a ligand species, Y_s, binds to an enzyme species, then the concentration of this ligand species remains approximately constant throughout the reaction, which can be reached in limiting enzyme conditions or as a result of a metabolic regulation of the concentration.

In these conditions, at any reaction time, the instantaneous concentration of Y_s, $[Y_s]$, is approximately equal or equal to its initial concentration, $[Y_s]_0$.

The direct connection between the different enzymatic forms is expressed through rate constants, $K_{j,i}(i, j = 1, 2, \ldots, n; i \neq j)$. If the enzyme species X_j reacts with the ligand species Y_s to form the enzyme species X_i, then $K_{j,i} = k_{j,i}[Y_s]_0$. If X_j becomes X_i in a unimolecular step, then $K_{j,i} = k_{j,i}$. If one enzyme species, X_j, is not converted into another, X_i, then $K_{j,i} = 0$. If there are parallel steps between a pair of enzyme species, X_j and X_i, that connect them directly, then rate constants need to be distinguished in some way, for example, by giving a number to each one, e.g. $[K_{j,i}(1), K_{i,j}(1)]$, $[K_{j,i}(2), K_{i,j}(2)]$, ... for parallel steps 1, 2,...In these cases:

$$K_{j,i} = K_{j,i}(1) + K_{j,i}(2) + \cdots \tag{1}$$

$$K_{i,j} = K_{i,j}(1) + K_{i,j}(2) + \cdots \tag{2}$$

3 Concentration-time equations for enzyme species

If the notations of Eqs. (44)–(46) in [10] are adapted for enzymatic reactions, as described above, the following equations are obtained for the instantaneous concentration of the enzyme species:

$$[X_i] = A_{i,0} + \sum_{h \in z(i)} A_{i,h} e^{\lambda_h t} \qquad (i = 1, 2, \ldots, n) \tag{3}$$

$$A_{i,0} = \frac{\sum_{k \in \omega(i)} (f_{k,i})_{u(i)}(E_i)\,[X_k]_0}{F_{u(i)}(E_i)} \qquad (i = 1, 2, \ldots, n) \tag{4}$$

$$A_{i,h} = \frac{(-1)^{u(i)-1} \sum_{k \in \omega(i)} [X_k]_0 \left\{ \sum_{q=0}^{u(i)} (f_{k,i})_q (E_i) \lambda_h^{u(i)-q} \right\}}{\lambda_h \prod\limits_{\substack{p \in z(i) \\ p \neq h}} (\lambda_p - \lambda_h)}$$

$$[i = 1, 2, \ldots, n;\ h \in z(i)] \tag{5}$$

where the meaning of $u(i)$, $\lambda_h[h = 1, 2, \ldots, u(i)]$, $\omega(i)$, $(f_{k,i})_q[q = 0, 1, \ldots, u(i)]$ and $z(i)$ are those described in [10].

Equations (3)–(5) are the optimized general kinetic equations for the enzyme species, with the possibility that more than one enzyme species could be present at the onset of the reaction.

4 Concentration-time equations for ligand species

The differential equations giving the variation of the concentration of any ligand species, Y_s, have the form:

$$\frac{d[Y_s]}{dt} = \sum_{(i,j)} \left(K_{j,i}[X_j] - K_{i,j}[X_i] \right) \qquad (s = 1, 2, \ldots, g) \tag{6}$$

where (i, j) means that the sum is extended to all pairs of values (i, j) where i and j are the subscripts that appear in reaction steps [2]–[6] described in Scheme 1. If in the reaction scheme there are parallel steps between two enzyme species X_j and X_i, and Y_s is involved in any of these steps, then the pair (i, j) for each of the steps and the $K_{j,i}$ and $K_{i,j,}$ values in Eqs. (8)–(11), which appear below, should be replaced by the corresponding numbered symbol. For example, in a parallel phase in which $K_{j,i}(2)$ and $K_{i,j}(2)$ symbols are to be used, the distinction $(i, j)(2)$ must be included.

If Eqs. (3)–(5) are taken into account in Eq. (6) and both sides are integrated, we obtain, after some rearrangement and using the previous notation:

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t + f_s(t) \tag{7}$$

where:

$$\alpha_s = \sum_{(i,j)} \left\{ K_{j,i} \frac{\sum_{k\in\omega(j)} (f_{k,j})_{u(j)} (E_j)[X_k]_0}{F_{u(j)}(E_j)} - K_{i,j} \frac{\sum_{k\in\omega(i)} (f_{k,i})_{u(i)} (E_i)[X_k]_0}{F_{u(i)}(E_i)} \right\} \tag{8}$$

and $f_s(t)$ is the following function of time that depends on the ligand Y_s, that is, on s:

$$f_s(t) = \sum_{(i,j)} \left\{ K_{j,i} \sum_{h\in z(j)} \frac{(-1)^{u(j)-1} \sum_{k\in\omega(j)} [X_k]_0 \sum_{q=0}^{u(j)} (f_{k,j})_q (E_j) \lambda_h^{u(j)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(j) \\ p\neq h}} (\lambda_p - \lambda_h)} e^{\lambda_h t} \right.$$

$$\left. - K_{i,j} \sum_{h\in z(i)} \frac{(-1)^{u(i)-1} \sum_{k\in\omega(i)} [X_k]_0 \sum_{q=0}^{u(i)} (f_{k,i})_q (E_i) \lambda_h^{u(i)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(i) \\ p\neq h}} (\lambda_p - \lambda_h)} e^{\lambda_h t} \right\} \tag{9}$$

If in Eq. (7) we make $t = 0$, then:

$$\beta_s = -\sum_{(i,j)} \left\{ K_{j,i} \sum_{h\in z(j)} \frac{(-1)^{u(j)-1} \sum_{k\in\omega(j)} [X_k]_0 \sum_{q=0}^{u(j)} (f_{k,j})_q (E_j) \lambda_h^{u(j)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(j) \\ p\neq h}} (\lambda_p - \lambda_h)} \right.$$

$$\left. - K_{i,j} \sum_{h\in z(i)} \frac{(-1)^{u(i)-1} \sum_{k\in\omega(i)} [X_k]_0 \sum_{q=0}^{u(i)} (f_{k,i})_q (E_i) \lambda_h^{u(i)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(i) \\ p\neq h}} (\lambda_p - \lambda_h)} \right\} \tag{10}$$

In "Appendix 1" we demonstrate that Eq. (10) can be written as:

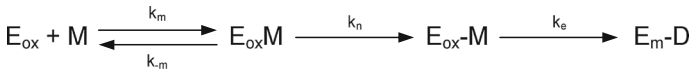
$$\beta_s = \sum_{(i,j)} \left[K_{j,i} \sum_{k\in\omega(j)} [X_k]_0 \left\{ \frac{(f_{k,j})_{u(j)-1}(E_j)}{F_{u(j)}(E_j)} - \frac{(f_{k,j})_{u(j)}(E_j) F_{u(j)-1}(E_j)}{F^2_{u(j)}(E_j)} \right\} - \right.$$
$$\left. - K_{i,j} \sum_{k\in\omega(i)} [X_k]_0 \left\{ \frac{(f_{k,i})_{u(i)-1}(E_i)}{F_{u(i)}(E_i)} - \frac{(f_{k,i})_{u(i)}(E_i) F_{u(i)-1}(E_i)}{F^2_{u(i)}(E_i)} \right\} \right] \quad (11)$$

5 Examples

To illustrate the use of the obtained equations we will apply them to two simple examples of enzymatic reaction schemes. The chosen examples, despite their simplicity, are suitable for observing the advantages of the equations proposed in this contribution.

5.1 Example 1

In this first example we will obtain the instantaneous concentration of the enzymatic form E_{ox} of Scheme 2, which corresponds to one segment of the mechanism of *oxi*tyrosinase acting on monophenols, as proposed by Fujieda et al. [45]. The corresponding graph is shown in Fig. 1.



Scheme 2 Enzymatic reaction model proposed by Fujieda et al. [45], where E_{ox} is the *oxi* form of tyrosinase, M is a monophenol and E_m-D complex type *met* of tyrosinase-diphenol

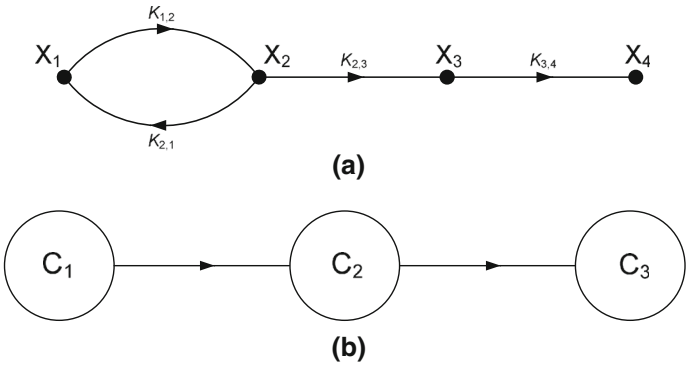


Fig. 1 **a** Directed graph related to the enzymatic reaction scheme shown in Scheme 2. X_1, X_2, X_3 and X_4 denote the compartments that correspond to the enzyme species E_{ox}, $E_{ox}M$, E_{ox}-M and E_m-D, respectively. $K_{1,2}$, $K_{2,1}$, $K_{2,3}$ and $K_{3,4}$ are the fractional transfer coefficients. **b** Condensation diagram corresponding to directed graph of (**a**). Classes are $C_1 = \{X_1, X_2\}$, $C_2 = \{X_3\}$ and $C_3 = \{X_4\}$. The initial class is C_1, C_2 is the transit and C_3 is the final one

Initial conditions We assume that the only enzyme species present at t=0 is the free enzyme E_{ox}, which corresponds to the compartment X_1, whose initial and instantaneous concentrations are denoted by $[E_{ox}]_0$ and $[E_{ox}]$, respectively. We denote by $[M]_0$ and $[M]$ the initial and instantaneous concentrations, respectively, of M. We also assume that $[M]$ remains practically constant during the course of the reaction and, therefore, approximately equal to $[M]_0$ (which can be achieved if $[E_{ox}]_0 << [M]_0$). Thus, any reaction step of this mechanism is of first or pseudofirst order, so that it fits our general model.

Fractional transfer coefficient notation The nonzero fractional transfer coefficients of Fig. 1 are related with the velocity constants of Scheme 2 as follows:

$$\left.\begin{array}{l} K_{1,2} = k_m[\mathrm{M}]_0 \\ K_{2,1} = k_{-m} \\ K_{2,3} = k_n \\ K_{3,4} = k_e \end{array}\right\} \tag{12}$$

Instantaneous concentration of E_{ox} The equations for the time evolution of the enzyme species can be manually obtained by applying Eqs. (3)–(5) and proceeding as explained in [10]. The parameters and coefficients involved in these equations are:

$$i = 1, u(i) = 2, z(i) = \{1, 2\}, \omega(i) = \{1\}, \Omega = \{\mathrm{X}_1\}, \mathrm{E}(\Omega, \mathrm{X}_1) = \mathrm{E}_1 = \{\mathrm{C}_1\} \tag{13}$$

Taking into account these values, Eq. (3) is:

$$[X_1] = A_{1,0} + A_{1,1}e^{\lambda_1 t} + A_{1,2}e^{\lambda_2 t} \tag{14}$$

where λ_1 and λ_2 are the roots of the polynomial $T_1(\lambda) = \lambda^2 + F_1(1)\lambda + F_2(1)$ with:

$$F_1(1) = K_{1,2} + K_{2,1} + K_{2,3} \tag{15}$$

$$F_2(1) = K_{1,2}K_{2,3} \tag{16}$$

Since $E_1 = \{C_1\}$, then

$$F_1(E_1) = F_1(1) \tag{17}$$

$$F_2(E_1) = F_2(1) \tag{18}$$

In Eq. (14) expressions of $A_{1,0}$, $A_{1,1}$ and $A_{1,2}$ are:

$$A_{1,0} = -\frac{[X_1]_0 (f_{1,1})_2(E_1)}{F_2(E_1)} \tag{19}$$

$$A_{1,1} = -\frac{[X_1]_0 \sum_{q=0}^{2} (f_{1,1})_q(E_1)\lambda_1^{2-q}}{\lambda_1(\lambda_2 - \lambda_1)} \tag{20}$$

$$A_{1,2} = -\frac{[X_1]_0 \sum_{q=0}^{2} (f_{1,1})_q (E_1) \lambda_2^{2-q}}{\lambda_2 (\lambda_1 - \lambda_2)} \tag{21}$$

Coefficients $(f_{1,1})_q(E_1)$, which are obtained from coefficients $F_q(E_1)$, as described by Garcia-Meseguer [20], are:

$$\left.\begin{array}{l} (f_{1,1})_0 = 1 \\ (f_{1,1})_1 = K_{2,1} + K_{2,3} \\ (f_{1,1})_2 = 0 \end{array}\right\} \tag{22}$$

Taking into account Eqs. (15)–(16), the relationship between the fractional transfer coefficients and rate constants, λ_1 and λ_2 are the roots of the equation:

$$\lambda^2 + (k_m[\mathrm{M}]_0 + k_{-m} + k_n)\,\lambda + k_m k_n [M]_0 = 0 \tag{23}$$

and moreover:

$$A_{1,0} = 0 \tag{24}$$

$$A_{1,1} = -\frac{[E_{ox}]_0 \left(\lambda_1^+ k_{-m} + k_n\right)}{\lambda_2 - \lambda_1} \tag{25}$$

$$A_{1,2} = -\frac{[E_{ox}]_0 (\lambda_2 + k_{-m} + k_n)}{\lambda_1 - \lambda_2} \tag{26}$$

so that Eq. (14) reduces to:

$$[E_{ox}] = A_{1,1} e^{\lambda_1 t} + A_{1,2} e^{\lambda_2 t} \tag{27}$$

5.2 Example 2

Now let us obtain the instantaneous concentration of the product, P, released in an enzymatic reaction whose mechanism is shown in Scheme 3 [46–48]. For ligands S, M and P, we will use the arbitrary notation Y_1, Y_2 and Y_3, respectively. The corresponding directed graph and condensation diagram are shown in Fig. 2.

Initial conditions. We assume that, at t = 0, the only enzyme species present is the free enzyme E, which corresponds to the compartment X_1, whose initial and instantaneous concentrations are denoted by $[E]_0$ and [E], respectively. We also assume that, at t=0, there is no product, i.e., $[P]_0 = 0$. The initial concentrations of S and M are denoted by $[S]_0$ and $[M]_0$, respectively, which we will consider much greater than $[E]_0$ so that instantaneous concentrations of S, [S], as well as of M, [M], remain practically constant during the reaction course and, therefore, approximately equal to $[S]_0$ and $[M]_0$. Thus, under these conditions, any reaction step of the mechanism described is of first or pseudofirst order and therefore corresponds to our model.

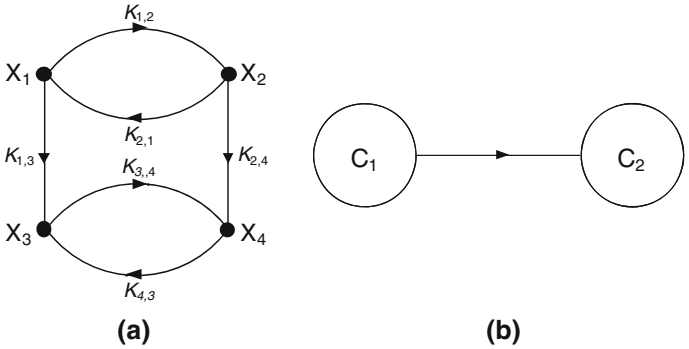
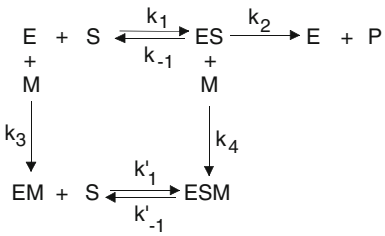


Fig. 2 **a** Directed graph of Scheme 3. X_1, X_2, X_3 and X_4 denote the compartments that correspond to the enzyme species E, ES, EM and ESM, respectively. $K_{1,2}$, $K_{2,1}$, $K_{3,4}$ and $K_{4,3}$ are the fractional transfer coefficients. **b** Condensation diagram corresponding to directed graph (**a**). The classes are: $C_1 = \{X_1, X_2\}$ and $C_2 = \{X_3, X_4\}$. The initial class is C_1 and C_2 is the final one

Scheme 3 Enzymatic reaction model consisting of a general mechanism of Botts and Morales [47] where the modifier is irreversible and the product, P, is released only from ES



Fractional transfer coefficient notation. The nonzero fractional transfer coefficients shown in Fig. 2 are related to the rate constants in Scheme 3 as follows:

$$\left.\begin{array}{l} K_{1,2} = k_1[S]_0 \\ K_{2,1} = K_{2,1}(1) + K_{2,2}(2) \\ K_{2,1}(1) = k_{-1} \\ K_{2,1}(2) = k_2 \\ K_{1,3} = k_3[M]_0 \\ K_{2,4} = k_4[M]_0 \\ K_{3,4} = k'_1[S]_0 \\ K_{4,3} = k'_{-1} \end{array}\right\} \tag{28}$$

Instantaneous concentration of P. In this example $Y_3 = P$ and so $[Y_3] = [P]$ and also $[Y_3]_0 = 0$, so that Eq. (7) can now be written as:

$$[P] = \beta_3 + \alpha_3 t + f_3(t) \tag{29}$$

Product P is obtained in only one step, which is irreversible. This step is one of the two parallel steps (2,1) in which the enzyme species X_2 ($j = 2$) becomes X_1($i = 1$), characterized by $K_{2,1}(2) = k_2$. Since this step is irreversible, $K_{1,2}(2) = 0$. The above considerations allow us rewrite Eqs. (8), (9) and (11) to give β_3, α_3 and f_3 involved in Eq. (7) as follows:

$$\beta_3 = K_{2,1}(2) \sum_{k\in\omega(2)} [X_k]_0 \left\{ \frac{(f_{k,2})_{u(2)-1}(E_2)}{F_{u(2)}(E_2)} - \frac{(f_{k,2})_{u(2)}(E_2) F_{u(2)-1}(E_2)}{F^2_{u(2)}(E_2)} \right\} \tag{30}$$

$$\alpha_3 = K_{2,1}(2) \frac{\sum_{k\in\omega(2)} (f_{k,2})_{u(2)}(E_2)[X_k]_0}{F_{u(2)}(E_2)} \tag{31}$$

$$f_3(t) = K_{2,1}(2) \sum_{h\in z(2)} \frac{(-1)^{u(2)-1} \sum_{k\in\omega(2)} [X_k]_0 \sum_{q=0}^{u(2)} (f_{k,2})_q(E_2) \lambda_h^{u(2)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(2)\\ p\neq h}} (\lambda_p - \lambda_h)} e^{\lambda_h t} \tag{32}$$

Proceeding as explained in [10] gives:

$$\Omega = \{X_1\} \tag{33}$$
$$\omega(2) = \{1\} \tag{34}$$
$$E\,(\Omega, X_2) = E_2 = \{C_1\} \tag{35}$$
$$T_1(\lambda) = \lambda^2 + F_1(1)\lambda + F_2(1) \tag{36}$$
$$F_1(1) = K_{1,2} + K_{1,3} + K_{2,1} + K_{2,4} \tag{37}$$
$$F_2(1) = K_{1,2}K_{2,4} + K_{1,3}K_{2,1} + K_{1,3}K_{2,4} \tag{38}$$
$$u(2) = 2 \tag{39}$$
$$T_{E_2}(\lambda) = \lambda^2 + F_1(E_2)\lambda + F_2(E_2) \tag{40}$$
$$F_1(E_2) = F_1\,(1) \tag{41}$$
$$F_2(E_2) = F_2\,(1) \tag{42}$$
$$z(2) = \{1, 2\} \tag{43}$$
$$(f_{1,2})_0 = (f_{1,2})_2 = 0 \tag{44}$$
$$(f_{1,2})_1 = K_{1,2} \tag{45}$$

Taking into account that $[\mathrm{X_1}]_0$ is equal to $[\mathrm{E}]_0$, then $\alpha_3 = 0$ and

$$\beta_3 = \frac{k_1 k_2 [S]_0 [E]_0}{k_1 k_4 [S]_0 [M]_0 + k_3\,(k_{-1} + k)\,[M]_0 + k_3 k_4 [M]_0^2} \tag{46}$$

$$f_3(t) = \frac{k_1 k_2 [S]_0 [E]_0}{\lambda_1 - \lambda_2} \left(\frac{e^{\lambda_1 t}}{\lambda_1} - \frac{e^{\lambda_2 t}}{\lambda_2} \right) \tag{47}$$

$$[P] = \beta_3 + f_3(t) \tag{48}$$

where λ_1 and λ_2 in Eq. (47) are the roots of the next polynomial $T_1(\lambda)$:

$$T_1(\lambda) = \lambda^2 + \{k_1[\mathrm{S}]_0 + k_{-1} + k_2 + (k_3 + k_4)\,[M]_0\}\,\lambda + k_1 k_4 [S]_0 [M]_0 + k_3\,(k_{-1} + k)\,[M]_0 + k_3 k_4 [M]_0^2 = 0 \tag{49}$$

6 Results and discussion

In this work we have adapted the kinetic equations corresponding to zero input closed linear compartmental system, obtained in [10], for use in the general enzymatic reaction model shown in Scheme 1, to acquire, by the direct application of the results there obtained, the time course equations of the different enzyme species involved, Eqs. (29)–(32). Hence, from the above equations, the time course equations for the ligand species involved have been derived: Eqs. (7)–(9) and (11).

6.1 Optimized kinetic equations

Equations (3)–(5), as well as (7)–(9) and (11), are in the simplest possible form in a double sense: (1) only the kinetic parameters and initial concentrations that have any influence on the instantaneous concentration of the species under study are involved in these equations and (2) according to the species under study, the arguments involved, i.e., the roots λ_h [$h \in z(i)$ in Eqs. (3)–(5) and $h \in z(j)$ and $h \in z(i)$ for different pairs (j, i) involved in Eq. (7)], are given as the roots of the irreducible polynomials $T_r(\lambda)$ $(r = 1, 2, \ldots, \delta)$ associated with the corresponding classes C_r whose enzymatic forms influence the wanted concentration. For this reason we shall call these equations optimized equations. To include more particular cases, in deriving these equations we have assumed that, in the general model, more than one enzyme species may be present at the onset of the reaction, as frequently occurs in enzymatic reactions [14,49–53].

6.2 Additional notation

For convenience, hereafter we will use the following additional notation:

IM (initial mechanism): initial mechanism proposed for a particular enzymatic reaction.
G: A generic species, enzymatic or ligand, involved in IM, whose instantaneous concentration, [G], is to be obtained. For example, G in Scheme 2 may be any of the species E_{ox}, M, $E_{ox}M$, E_{ox}-M or E_m-D and in Scheme 3 any of the species E, ES, EM, ESM, S, M or P.
oE(G): Optimized equations used to obtain [G]. For example, the optimized equations to obtain [E_{ox}] in Scheme 2, i.e., oE(E_{ox}), are Eqs. (14), (19)–(21) and the optimized equations to obtain [P] in Scheme 3, i.e., oE(P), are Eqs. (29)–(32).
oR(G): Optimized results obtained when using oE(G). These results are in the most simplified form. For example, oR(E_{ox}), corresponding to Example 1, are given by Eqs. (27), (25) and (26), and oR(P), corresponding to Example 2, are given by Eqs. (46)–(48). Figure 3 shows schematically how the oR(G) are obtained for any IM from the corresponding oE(G).
non-oE(G): Non optimized kinetic equations used to obtain [G].
non-oR(G): Results obtained when non-oE(G) are applied in mechanism IM to obtain [G].

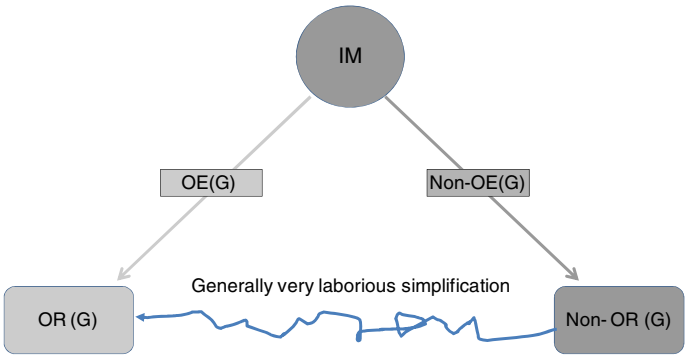


Fig. 3 Schematic illustration of the two possible ways for deriving the time course equation of a species, G, involved in IM. On the left, the oE(G) are used and the oR(G) are obtained. On the right, the non-oE(G) are used and the non-oR(G) are obtained. When the oR(G) and non-oR(G) do not match, as occurs in Examples 1–3, a process of simplification leading from the non-oR(G) to the oR(G), generally laborious and prone to errors, is necessary as is performed in the main text

6.3 Non optimized kinetic equations

There are several contributions that provide the time course equations, i.e. the kinetic equations, of both the enzyme and ligand species for different general models of enzymatic reactions in which all interconversions between the enzyme forms are of first or pseudofirst order [13–16,19]. Thus, kinetic equations have been derived for a general model in which all the reaction steps are reversible and only the free enzyme [13] or more than one enzyme species [14] is present at the onset of the reaction. There are also different contributions in the literature [16,19,39] that allow the instantaneous concentration of the ligand and enzyme species involved in the general model shown in Scheme 1 (in which irreversible reaction steps may exist) to be obtained for the case that one [15] or more [16] enzyme species are present at the onset of the reaction. Varon et al. published an algorithm that allows the symbolic expressions of coefficients involved in these equations to be obtained without expansion of any determinant, and they implemented a software that provides these coefficients [15,20]. In "Appendix 2", the same equations obtained by Varon et al. [16] are shown for the first time but they are improved because the coefficients used in them are always non-negative.

Several contributions present kinetic analyses of enzymatic reaction models which can be considered as particular cases of the general model analyzed in this work. In Fig. 4 we have indicated, in chronological order, only some of these contributions stating the most important characteristic of each.

Both optimized and non-optimized equations are applicable to enzymatic systems whose reaction mechanisms fit the general mechanism indicated in Scheme 1. In many enzyme systems, but not in all, the results obtained with the optimized and non-optimized equations coincide, i.e., oR(G) = non-oR(G), for any [G]. The only possible condensation diagrams of enzyme systems in which this occurs are indicated in Fig. 5, i.e., this coincidence is given only in those cases, where the compartmental system corresponding to the reaction mechanism consists of one only class or one initial class, together with one or more final class, each containing one only compartment

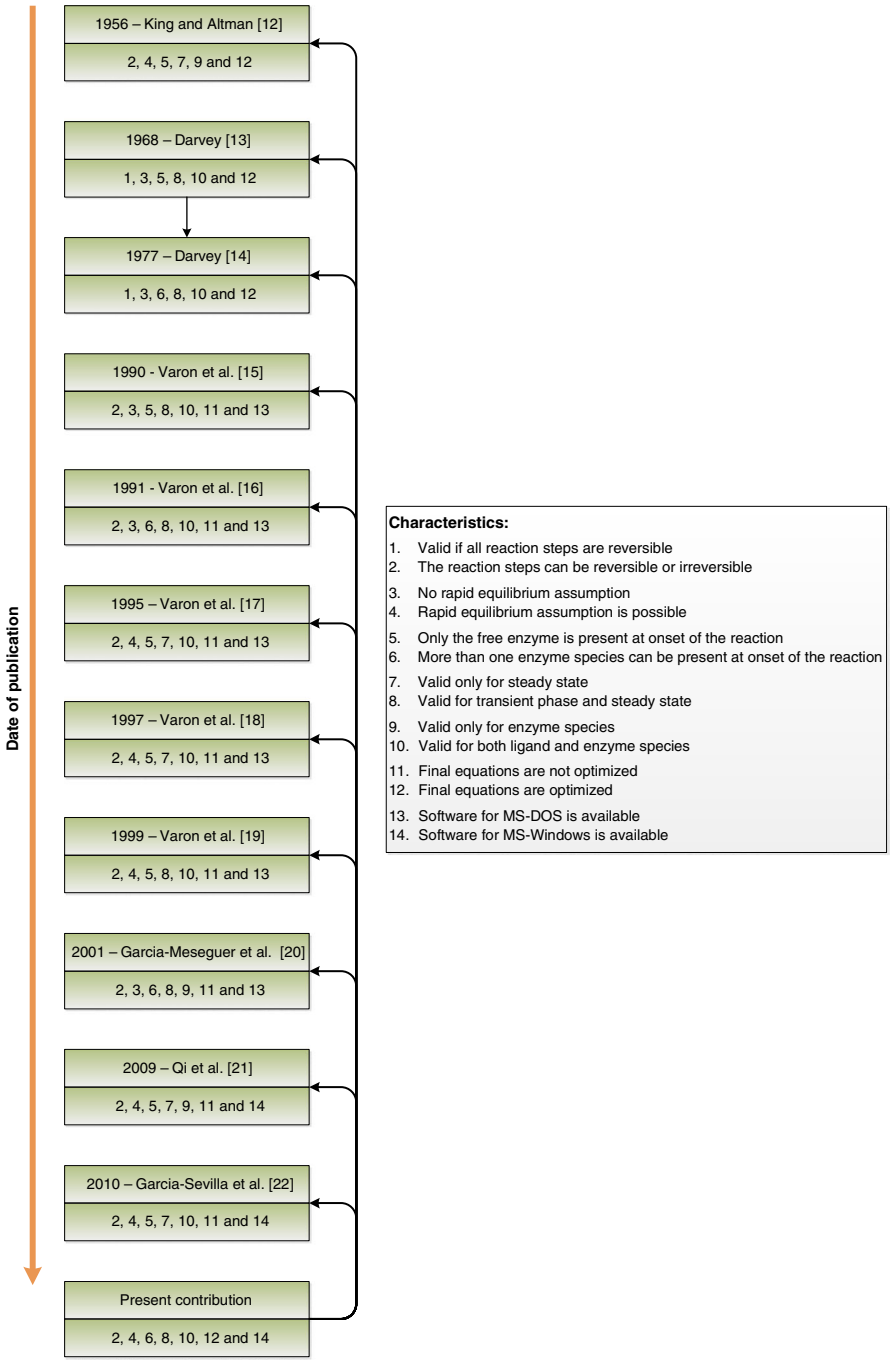


Fig. 4 Schematic chronological representation for some enzymatic models presented in previous contributions with their characteristics and relationship with the general model studied here

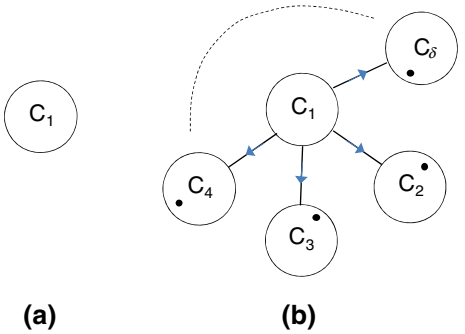
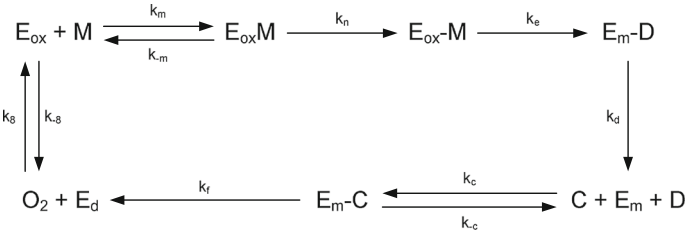
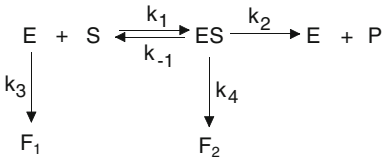


Fig. 5 Condensation diagrams corresponding to those enzyme systems in which expressions of the concentration of any of species involved, G, using both optimized and non optimized equations, coincide. **a** A single final class C_1, where all enzyme species are directly or indirectly connected with all others. **b** An initial class C_1, to which one or more enzyme species may belong. This class is connected to one or more final classes, C_2, $C_3, \ldots, C_\delta$, each of them consisting of only one enzyme species (indicated by a *black dot*)



Scheme 4 Simplified mechanism for kinetic evaluation of the activity of tyrosinase proposed by Yamazaki and Itoh [55]

Scheme 5 Michaelis-Menten mechanism with inactivation of free enzyme and enzyme-substrate complex [56]



(enzyme species). In Schemes 4 and 5 and the corresponding Figs. 6 and 7, examples of enzymatic reaction mechanisms where this happens are shown.

However, there are many other mechanisms where it does not happen that oR(G) = non-oR(G) for any [G]. These enzyme systems are those whose condensation diagram does not correspond to any of those shown in Fig. 5. The three examples studied above are of this type. A further example, among the many possible, is outlined in Scheme 6 and corresponding Fig. 8.

Obviously, both optimized and non optimized equations can be applied to any IM fitting general Scheme 1 to obtain [G]. But if the non-oE(G) is used for any IM whose condensation diagram is not one of those included in Fig. 5, then the results obtained must be simplified either by eliminating those kinetic parameters and/or

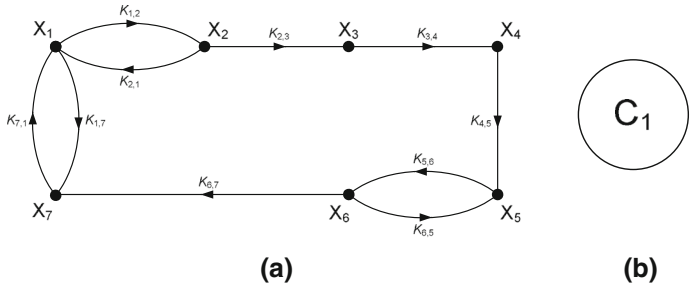


Fig. 6 **a** Directed graph concerning enzymatic reaction scheme depicted in Scheme 4. X_1, X_2, X_3, X_4, X_5, X_6 and X_7 denote compartments corresponding to enzyme species E_{ox}, $E_{ox}M$, $E_{ox} - M$, $E_m - D$, E_m, $E_m - C$ and E_d, respectively. $K_{1,2}$, $K_{2,1}$, $K_{2,3}$ $K_{3,4}$, $K_{4,5}$, $K_{5,6}$ $K_{6,5}$, $K_{6,7}$, $K_{7,1}$ and $K_{1,7}$ are fractional transfer coefficients. **b** Condensation diagram corresponding to directed graph (**a**). All compartments belong to a single class C_1, which is, therefore, a final class

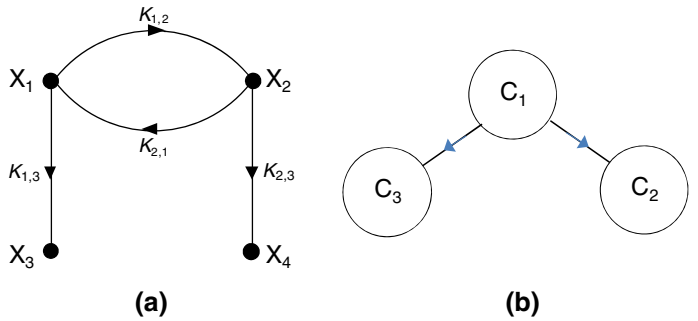
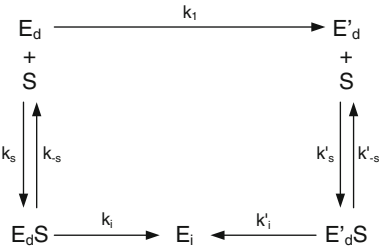


Fig. 7 **a** Directed graph concerning enzymatic reaction scheme depicted in Scheme 5. X_1, X_2, X_3 and X_4 denote the compartments that correspond to the enzyme species E, ES, F_1 and F_2, respectively. $K_{1,2}$, $K_{2,1}$, $K_{1,3}$ and $K_{2,4}$ are fractional transfer coefficients. **b** Diagram of condensation corresponding to directed graph (**a**). Class C_1 is initial and includes enzyme species X_1 and X_2, i.e., $C_1 = \{X_1, X_2\}$. Classes C_2 and C_3 are final and they consist of a single enzyme species each one: $C_2 = \{X_3\}$ and $C_3 = \{X_4\}$

Scheme 6 Tyrosinase inactivation enzymatic reaction model proposed by Muñoz-Muñoz et al. [57]



initial concentrations with no influence on [G] or expressing the arguments as roots of more simple polynomials.

In this contribution we provide a tool to derive kinetic equations of any enzymatic reaction fitting Scheme 1, with the security that they will not require subsequent simplification in any case, irrespective of the reaction mechanism involved.

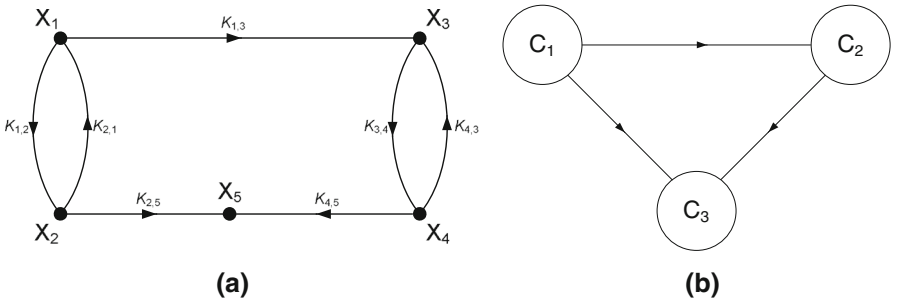


Fig. 8 **a** Directed graph concerning enzymatic reaction scheme depicted in Scheme 6. X_1, X_2, X_3, X_4 and X_5 denote the compartments that correspond with enzyme species E_d, E_dS, E'_d, E'_dS and E_i, respectively. $K_{1,2}$, $K_{2,1}$, $K_{1,3}$ $K_{3,4}$, $K_{4,3}$, $K_{2,5}$ and $K_{4,5}$ are fractional transfer coefficients. **b** Diagram of classes that correspond to the connectivity diagram shown (**a**) consisting of three classes: $C_1 = \{X_1, X_2\}$, $C_2 = \{X_3, X_4\}$ and $C_3 = \{X_5\}$. C_1 is an initial class, C_2 is a transit class and C_3 is a final class

6.4 Examples of simplification of non-oR(G) when oR(G) and the non-oR(G) differ

To demonstrate how non-oR(G) can be simplified, we will use the non-oR(G) results for the above Examples 1 and 2.

6.4.1 Example 1

If we apply the general non-optimized equations (89)–(91), given in "Appendix 2", for enzyme species, to IM in Scheme 2 we obtain:

$$[E_{ox}] = A_{1,1}e^{\lambda_1 t} + A_{1,2}e^{\lambda_2 t} + A_{1,3}e^{\lambda_3 t} \tag{50}$$

where λ_1, λ_2 and λ_3 are the roots of the equation:

$$\lambda^3 + F_1\lambda^2 + F_2\lambda + F_3 = 0 \tag{51}$$

where:

$$F_1 = k_m[M]_0 + k_{-m} + k_n + k_e \tag{52}$$

$$F_2 = k_m\,(k_n + k_e)\,[\mathrm{M}]_0 + k_e\,(k_{-m} + k_n) \tag{53}$$

$$F_3 = k_m k_n k_e[\mathrm{M}]_0 \tag{54}$$

$$A_{1,1} = \frac{[E_{ox}]_0\left\{\lambda_1^2 + (k_{-m} + k_n + k_e)\lambda_1 + k_e(k_{-m} + k_n)\right\}}{(\lambda_2 - \lambda_1)\,(\lambda_3 - \lambda_1)} \tag{55}$$

$$A_{1,2} = \frac{[E_{ox}]_0\left\{\lambda_2^2 + (k_{-m} + k_n + k_e)\lambda_2 + k_e(k_{-m} + k_n)\right\}}{(\lambda_1 - \lambda_2)\,(\lambda_3 - \lambda_2)} \tag{56}$$

$$A_{1,3} = \frac{[E_{ox}]_0\left\{\lambda_3^2 + (k_{-m} + k_n + k_e)\lambda_3 + k_e(k_{-m} + k_n)\right\}}{(\lambda_1 - \lambda_3)\,(\lambda_2 - \lambda_3)} \tag{57}$$

The non-oR(E_{ox}) given by Eqs. (50), (55)–(57) should coincide with the oR(E_{ox}) given by Eqs. (27), (25), (26) because they arise from the same enzyme system.

The simplifications carried out in Sect. 6.4, to go from the non-oR(G) to the oR(G) in examples 1 and 2 are not very difficult, but, for other systems, this process could become very laborious and prone to human errors, and also unnecessary if the oE(G) is directly applied to the IM.

Hence we will show that Eqs. (50), (55)–(57) can be simplified to Eqs. (27), (25), (26). Indeed, by handling Eqs. (51)–(54), the first one can also be written as:

$$\left\{\lambda^2 + (k_m[\mathrm{M}]_0 + k_{-m} + k_n)\lambda + k_m k_n[\mathrm{M}]_0\right\}(\lambda + k_e) = 0 \tag{58}$$

so that two of the three roots λ_1, λ_2 and λ_3, e.g. λ_1 and λ_2, are the roots of the first polynomial factor in Eq. (58), and λ_3 is:

$$\lambda_3 = -k_e \tag{59}$$

If Eq. (59) is taken into account in Eq. (57), the numerator on the right side becomes null and therefore:

$$A_{1,3} = 0 \tag{60}$$

Note that according to Eq. (59):

$$\lambda_1^2 + (k_{-m} + k_n + k_e)\lambda_1 + k_e(k_{-m} + k_n) = -(\lambda_1 + k_{-m} + k_n)(\lambda_3 - \lambda_1) \tag{61}$$
$$\lambda_2^2 + (k_{-m} + k_n + k_e)\lambda_2 + k_e(k_{-m} + k_n) = -(\lambda_2 + k_{-m} + k_n)(\lambda_3 - \lambda_2) \tag{62}$$

Inserting Eqs. (61) and (62) in Eqs. (55) and (56), respectively, and simplifying, we obtain the optimized Eqs. (25) and (26).

6.4.2 Example 2

If we apply the general non-optimized equations (92), (98)–(100), in "Appendix 2", for ligand species, to IM in Scheme 3 we obtain:

$$[\mathrm{P}] = \beta_3 + \sum_{h=1}^{3} \gamma_{3,h} e^{\lambda_h t} \tag{63}$$

where λ_1, λ_2 and λ_3 are the roots of the equation:

$$\lambda^3 + G_1\lambda^2 + G_2\lambda + G_3 = 0 \tag{64}$$

with

$$G_1 = \left(k_1 + k_1'\right)[S]_0 + (k_3 + k_4)[M]_0 + k_{-1} + k_2 + k_{-1}' \tag{65}$$
$$G_2 = \left(k_1 k_4 + k_3 k_1' + k_4 k_1'\right)[S]_0[M]_0 + k_1 k_1'[S]_0^2 + k_3 k_4[M]_0^2$$

$$+\left\{k_1k'_{-1}+k'_1\left(k_{-1}+k_2\right)\right\}[S]_0$$
$$+\left\{k_3\left(k_{-1}+k_2+k'_{-1}\right)+k_4k'_{-1}\right\}[M]_0+\left(k_{-1}+k_2\right)k'_{-1} \quad (66)$$

$$G_3=k_1k_4k'_1[S]_0^2[M]_0+k_3k_4k'_1[S]_0[M]_0^2+k_3k_4k'_{-1}[M]_0^2$$
$$+\left\{k_1k_4k'_{-1}+k_3k'_1\left(k_{-1}+k_2\right)\right\}[S]_0[M]_0+k_3\left(k_{-1}+k_2\right)k'_{-1}[M]_0 \quad (67)$$

and

$$\beta_3=\frac{k_1k_2(k'_1[S]_0+k'_{-1})[S]_0[E]_0}{G_3} \quad (68)$$

$$\gamma_h=k_1k_2\frac{\lambda_h+k'_1[S]_0+k'_{-1}}{\lambda_h\prod_{\substack{p=1\\p\neq h}}^{3}\left(\lambda_p-\lambda_h\right)}[S]_0[E]_0 \quad (h=1,2,3) \quad (69)$$

Note that the kinetic parameters k'_1 and k'_{-1} involved in Eqs. (65)–(67) have no influence on [P], according to Scheme 3, and therefore, we must simplify the time course equation of P, so that these two parameters do not appear. By comparing Eq. (63) with Eq. (29), we observe that in the first one there is an extra exponential term that can be removed by simplifying Eq. (63). This simplification of Eqs. (63)–(69) to obtain the optimized equivalents will be carried out below, and is indicated by a tortuous line in Fig. 3. However, this can be avoided by using our optimized equations, as will be seen below.

Equations (65)–(67) allow us to write Eq. (64) as:

$$\left\{\lambda^2+F_1(E_2)\lambda+F_2(E_2)\right\}\left(\lambda+k'_1[S]_0+k'_{-1}\right)=0 \quad (70)$$

where the expressions for $F_1(E_2)$ and $F_2(E_2)$ are given by Eqs. (41) and (42). From Eq. (70) we see that two of the three roots λ_1, λ_2 and λ_3, e.g. λ_1 and λ_2, are the roots of the first polynomial factor in Eq. (70), and λ_3 is:

$$\lambda_3=-\left(k'_1[S]_0+k'_{-1}\right) \quad (71)$$

and so Eq. (69) can be written as:

$$\gamma_h=k_1k_2\frac{\lambda_h-\lambda_3}{\lambda_h\prod_{\substack{p=1\\p\neq h}}^{3}\left(\lambda_p-\lambda_h\right)}[S]_0[E]_0 \quad (h=1,2,3) \quad (72)$$

which implies that:

$$\gamma_1=\frac{k_1k_2}{\lambda_1\left(\lambda_1-\lambda_2\right)}[S]_0[E]_0 \quad (73)$$

$$\gamma_2 = \frac{k_1 k_2}{\lambda_2 (\lambda_2 - \lambda_1)} [S]_0 [E]_0 \tag{74}$$

$$\gamma_3 = 0 \tag{75}$$

Moreover, careful observation of the expression of G_3 allows us to write:

$$G_3 = F_2(E_2)(k'_1[S]_0 + k'_{-1}) \tag{76}$$

and therefore Eq. (68) becomes:

$$\beta_3 = \frac{k_1 k_2 [S]_0 [E]_0}{F_2(E_2)} \tag{77}$$

Briefly, the non-oR(P) obtained using the non-oE(P) is simplified to the oR(P) obtained by applying the oE(P) through the simplification process. Note: (1) In the oR(P) only those kinetic parameters and initial concentrations that influence [P] are involved and (2) To obtain λ_1, λ_2 and λ_3 it is not necessary to analytically solve a cubic equation, which is a very complicated process, but they can be obtained as the roots of a quadratic equation and of a first order equation.

6.5 Applicability of the oE(G)

The optimized equations, describing the behavior of the enzyme and ligand species, are applicable to the transition phase and to the steady state of enzymatic reactions whose enzymatic reaction scheme conforms to the model described in this paper. This model includes most enzyme systems, whether or not the mechanism has branches and whether or not there are parallel steps, the velocity constants are repeated, there are inactivation steps, irreversible inhibition steps, or one or more species are present at the onset of the enzymatic reaction, etc. However no such equations can be applied to mechanisms of enzymatic reactions involving zymogen activation or cyclic reversible enzyme cascades.

At the steady state, the exponential terms of Eqs. (3) and (7) [see Eq. (9)] are negligible, so that these two equations can be reduced to:

$$[X_i] = A_{i,0} \qquad (i = 1, 2, \ldots, n) \qquad \text{(Steady State)} \tag{78}$$

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t \qquad (s = 1, 2, \ldots, g) \qquad \text{(Steady State)} \tag{79}$$

where expression of $A_{i,0}$, β_s and α_s are given by Eqs. (4), (11) and (8).

6.6 Concluding remarks

In this contribution we have obtained, for the first time and based on [10], time concentration equations for both enzyme and ligand species involved in any specific mechanism of an enzymatic reaction, IM, that fits the general model shown in Scheme 1, regardless of whether one or more enzyme species are present at the onset of the

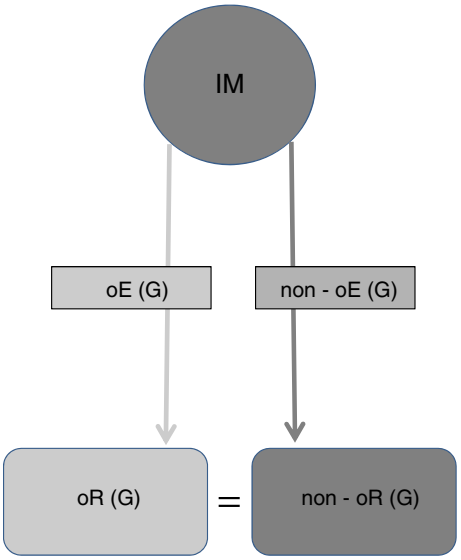


Fig. 9 The oR(G) and the non-oR(G) only coincide, for any G, when the condensation diagram is one of those indicated in Figs. 5a and 6b, i.e., when the condensation diagram consists of a single class

reaction. These equations, which are given in the most simplified form possible, are denominated optimized kinetic equations, oE(G), to distinguish them from those existing in the literature for the same purpose, which we have called non-optimized kinetic equations, non-oE(G). The oE(G) for a specific instantaneous concentration, [G] have two important characteristics: (1) the symbolic expression of [G] involves only those kinetic parameters and initial concentrations that have some influence on [G], (2) the arguments involved in the exponential terms are obtained by solving the polynomials which are factors and, therefore, of lower degree than the characteristic polynomial corresponding to the enzyme system.

It is true that in many enzymatic reaction schemes, especially in those whose condensation diagram is that indicated in Fig. 5, when determining the instantaneous concentration of any involved species, G—both oE(G) and non-oE(G)—lead to the same result, as is schematically indicated in Fig. 9. However, since this does not happen for all enzymatic reactions, it is advantageous to always use the optimized equations proposed here.

Besides advantages (1) and (2) mentioned above, the optimized equations for the instantaneous concentration of any ligand species, Y_s, involved in the reaction mechanisms are given in a form that clearly distinguish the separate contribution to $[Y_s]$ of any of the enzyme species from which Y_s is released or with which Y_s combines. This form of the optimized equations for Y_s is very useful for fully understanding the partial contribution to $[Y_s]$ of each of the enzyme species involved. Nevertheless, for other purposes, e.g. for a computerized treatment, it may be more convenient to express them in a more compact and suitable form. This, together with the implementation of a suitable software, will be attempted in paper IV of this series.

7 Appendix 1: Derivation of Eq. (11) from Eq. (10)

The first summatory

$$\sum_{h\in z(j)} \frac{(-1)^{u(j)-1}\sum_{k\in\omega(j)}[X_k]_0\sum_{q=0}^{u(j)}(f_{k,j})_q(E_j)\lambda_h^{u(j)-q}}{\lambda_h^2\prod\limits_{\substack{p\in z(j)\\ p\neq h}}\left(\lambda_p-\lambda_h\right)} \tag{80}$$

of Eq. (10) can be expressed as:

$$(-1)^{u(j)-1}\sum_{k\in\omega(j)}[X_k]_0\sum_{q=0}^{u(j)}\left\{(f_{k,j})_q(E_j)\sum_{h\in z(j)}\frac{1}{\lambda_h^{2-u(j)+q}\prod\limits_{\substack{p\in z(j)\\ p\neq h}}\left(\lambda_p-\lambda_h\right)}\right\} \tag{81}$$

Using the algorithm presented in "Appendix A" of Arribas et al. [54] we know that:

$$\sum_{h\in z(j)}\frac{1}{\lambda_h^{2-u(j)+q}\prod\limits_{\substack{p\in z(j)\\ p\neq h}}\left(\lambda_p-\lambda_h\right)}=\begin{cases}0 & \text{if } q<u(j)-1\\ \frac{1}{P_{u(j)}} & \text{if } q=u(j)-1\\ \frac{P_{u(j)-1}}{P_{u(j)}^2} & \text{if } q=u(j)\end{cases} \tag{82}$$

The meaning of $P_{u(j)}$ is the product of all non null roots, $u(j)$, of the polynomial $T_{E_j}(\lambda)$, i.e., those non null roots whose subscripts belong to the set $z(j)$. $P_{u(j)-1}$ is the sum of all different products of order $[u(j)-1]$ that can be formed with these roots.

The polynomial $T_{E_j}(\lambda)$ is given by:

$$T_{E_j}(\lambda)=F_0(E_j)\lambda^{u(j)}+F_1(E_j)\lambda^{u(j)-1}+\ldots+F_{u(j)-1}(E_j)\lambda+F_{u(j)}(E_j) \tag{83}$$

and is fulfilled that:

$$P_{u(j)-1}=(-1)^{u(j)-1}F_{u(j)-1}(E_j) \tag{84}$$

$$P_{u(j)}=(-1)^{u(j)}F_{u(j)}(E_j) \tag{85}$$

Using Eqs. (84) and (85) in Eq. (82), we obtain:

$$\sum_{h\in z(j)} \frac{1}{\lambda_h^{2-u(j)+q} \prod\limits_{\substack{p\in z(j)\\ p\neq h}} (\lambda_p - \lambda_h)} = \begin{cases} 0 & \text{if } q < u(j)-1 \\ (-1)^{u(j)} \frac{1}{F_{u(j)}(E_j)} & \text{if } q = u(j)-1 \\ (-1)^{u(j)-1} \frac{F_{u(j)-1}(E_j)}{F^2_{u(j)}(E_j)} & \text{if } q = u(j) \end{cases} \tag{86}$$

Taking into account these results in Eq. (80) one obtains:

$$\sum_{h\in z(j)} \frac{(-1)^{u(j)-1} \sum\limits_{k\in\omega(j)} [X_k]_0 \sum\limits_{q=0}^{u(j)} (f_{k,j})_q(E_j) \lambda_h^{u(j)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(j)\\ p\neq h}} (\lambda_p - \lambda_h)}$$
$$= \sum_{k\in\omega(j)} [X_k]_0 \left\{ -\frac{(f_{k,j})_{u(j)-1}(E_j)}{F_{u(j)}(E_j)} + \frac{(f_{k,j})_{u(j)}(E_j) F_{u(j)-1}(E_j)}{F^2_{u(j)}(E_j)} \right\} \tag{87}$$

Similarly, for the second summatory of Eq. (10) we have:

$$\sum_{h\in z(i)} \frac{(-1)^{u(i)-1} \sum\limits_{k\in\omega(i)} [X_k]_0 \sum\limits_{q=0}^{u(i)} (f_{k,i})_q(E_i) \lambda_h^{u(i)-q}}{\lambda_h^2 \prod\limits_{\substack{p\in z(i)\\ p\neq h}} (\lambda_p - \lambda_h)}$$
$$= \sum_{k\in\omega(i)} [X_k]_0 \left\{ -\frac{(f_{k,i})_{u(i)-1}(E_i)}{F_{u(i)}(E_i)} + \frac{(f_{k,i})_{u(i)}(E_i) F_{u(i)-1}(E_i)}{F^2_{u(i)}(E_i)} \right\} \tag{88}$$

If Eqs. (87) and (88) are inserted into Eq. (10) we obtain the expression given by Eq. (11) for β_s.

8 Appendix 2: Non-optimized general kinetic equations

8.1 About the enzyme species

Non-optimized progress equations of an enzyme species belonging to a reaction scheme that fits the above general model are given by Eqs. (3)–(5), which are reproduced here:

$$[X_i] = A_{i,0} + \sum_{h\in z(i)} A_{i,h} e^{\lambda_h t} \qquad (i = 1, 2, \ldots, n) \tag{89}$$

$$A_{i,0} = \frac{\sum_{k\in\omega} (f_{k,i})_u [X_k]_0}{F_u} \qquad (i = 1, 2, \ldots, n) \tag{90}$$

$$A_{i,h} = \frac{(-1)^{u-1} \sum_{k\in\omega} [X_k]_0 \left\{ \sum_{q=0}^{u} (f_{k,i})_q \lambda_h^{u-q} \right\}}{\lambda_h \prod\limits_{\substack{p=1 \\ p\neq h}}^{u} (\lambda_p - \lambda_h)}$$

$$(i = 1, 2, \ldots, n; h = 1, 2, \ldots, u) \tag{91}$$

where u, λ_h, ω, $(f_{k,i})_q$ are as described in [10]. $[X_i]$ is the instantaneous concentration of X_i and $[X_k]_0$ is the initial concentration of X_k.

Equations (89)–(91) are the general non-optimized kinetic equations for the enzyme species, with the possibility that there may be more than one enzyme species present at the onset of the reaction.

8.2 About the ligand species

Varon [39] and Galvez and Varon [4] established the following equations for a ligand species Y_s $(s = 1, 2, \ldots, g)$:

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t + \sum_{h=1}^{u} \gamma_{s,h} e^{\lambda_h t} \ (s = 1, 2, \ldots, g) \tag{92}$$

$$\alpha_s = \frac{(-1)^{n+1} \sum_{(i,j)} \left\{ K_{j,i} \sum_{k\in\omega} (-1)^{j+k} (a_{k,j})_u [X_k]_0 - K_{i,j} \sum_{k\in\omega} (-1)^{i+k} (a_{k,i})_u [X_k]_0 \right\}}{F_u} \tag{93}$$

$$\beta_s = \frac{(-1)^{n+1} \sum_{(i,j)} \left\{ K_{j,i} \sum_{k\in\omega} (-1)^{j+k} (a_{k,j})_{u-1} [X_k]_0 - K_{i,j} \sum_{k\in\omega} (-1)^{i+k} (a_{k,i})_{u-1} [X_k]_0 \right\}}{F_u} - \frac{F_{u-1}}{F_u} \alpha_s \tag{94}$$

$$\gamma_{s,h} = \frac{(-1)^c \sum_{(i,j)} \left\{ K_{j,i} \sum_{k\in\omega} (-1)^{j+k} [X_k]_0 \sum_{q=0}^{u} (a_{k,j})_q \lambda_h^{u-q} - K_{i,j} \sum_{k\in\omega} (-1)^{i+k} [X_k]_0 \sum_{q=0}^{u} (a_{k,i})_q \lambda_h^{u-q} \right\}}{\lambda_h^2 \prod\limits_{\substack{p=1 \\ p\neq h}}^{u} (\lambda_p - \lambda_h)}$$

$$(h = 1, 2, \ldots, u) \tag{95}$$

where n is the number of enzyme species involved in the reaction mechanism, c is the number of final classes in the condensation diagram and the meanings of u, λ_h, ω and $[X_k]_0$ are the same as in Eqs. (89)–(91). If $u = 1$, then the denominator in Eq. (95) is λ_1^2, i.e. F_1^2. The coefficients $(a_{k,i})_q$ $(k \in \omega; i = 1, 2, \ldots, n; q = 0, 1, \ldots, u)$ in Eqs. (93)–(95) are those involved in the expansion of determinant $D_{k,i}(\lambda)$ defined in [10], as follows:

$$D_{k,i}(\lambda) = \lambda^{c-1} \sum_{q=0}^{u} (a_{k,i})_q \lambda^{u-q} \qquad (k \in \omega; i = 1, 2, \ldots, n) \tag{96}$$

Hence, coefficients $(a_{k,i})_q$ $(k \in \omega; i = 1, 2, \ldots, n; q = 0, 1, \ldots, u)$ are related with coefficients $(f_{k,i})_q$ $(k \in \omega; i = 1, 2, \ldots, n; q = 0, 1, \ldots, u)$, defined in [10] and already used in this contribution for the relationships [20,39,44]:

$$(a_{k,i})_q = (-1)^{n+i+k-1} (f_{k,i})_q \qquad (k \in \omega; i = 1, 2, \ldots, n; q = 0, 1, \ldots, u) \tag{97}$$

If in the reaction scheme there are parallel steps between two enzyme species, X_j and X_i, and Y_s is involved in any of these steps, then the pair (i, j) for each of the steps and the values of $K_{j,i}$ and $K_{i,j}$ in Eqs. (93)–(95) must be replaced by the corresponding numbered symbol, as already indicated in the main text.

If, in Eqs. (93)–(95), Eq. (6) of [10] and Eq. (97) are borne in mind, then the first ones can be written in a more simplified form as:

$$\alpha_s = \frac{\sum_{(i,j)} \left\{ K_{j,i} \sum_{k\in\omega} (f_{k,j})_u [X_k]_0 - K_{i,j} \sum_{k\in\omega} (f_{k,i})_u [X_k]_0 \right\}}{F_u} \tag{98}$$

$$\beta_s = \frac{\sum_{(i,j)} \left\{ K_{j,i} \sum_{k\in\omega} (f_{k,j})_{u-1} [X_k]_0 - K_{i,j} \sum_{k\in\omega} (f_{k,i})_{u-1} [X_k]_0 \right\}}{F_u} - \frac{F_{u-1}}{F_u} \alpha_s \tag{99}$$

$$\gamma_{s,h} = \frac{(-1)^{u-1} \sum\limits_{(i,j)} \left\{ K_{j,i} \sum\limits_{k\in\omega} [X_k]_0 \sum\limits_{q=0}^{u} (f_{k,j})_q \lambda_h^{u-q} - K_{i,j} \sum\limits_{k\in\omega} [X_k]_0 \sum\limits_{q=0}^{u} (f_{k,i})_q \lambda_h^{u-q} \right\}}{\lambda_h^2 \prod\limits_{\substack{p=1 \\ p\neq h}}^{u} (\lambda_p - \lambda_h)} \tag{100}$$

Equations (92) and (98)–(100) are general non-optimized kinetic equations for ligand species, presented here for the first time, taking into account the possibility that more than one enzyme species may be present at the onset of the reaction.

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