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A Windows program for the derivation of steady-state equations in enzyme systems

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Abstract

Despite the number of computer-assisted methods described for the derivation of steady-state equations of enzyme systems, most of them are focused on strict steady-state conditions or are not able to solve complex reaction mechanisms. Moreover, many of them are based on computer programs that are either not readily available or have limitations.

We present here a computer program called *WinStes*, which derives equations for both strict steady-state systems and those with the assumption of rapid equilibrium, for branched or unbranched mechanisms, containing both reversible and irreversible conversion steps. It solves reaction mechanisms involving up to 255 enzyme species, connected by up to 255 conversion steps. The program provides all the advantages of the Windows programs, such as a user-friendly graphical interface, and has a short computation time.

WinStes is available free of charge on request from the authors.

Keywords: Enzyme kinetics; Enzyme mechanisms; Rate equations; Kinetic equations; Rapid equilibrium; Steady state

1. Introduction

The foundation for steady–steady kinetics probably dates from 1930, with Haldane's enzyme book.

Today, 75 years later, the situation is quite different and very complete studies have been done on enzymes. The kinetic complexities of their properties and actions and also of their performance and regulatory mechanisms, when it is possible to discover them, are often based on extremely complicated calculations.

In spite of the increasing importance that analysis of the kinetic behavior of enzyme reactions in their transient phase has acquired in recent years, theoretical as well as experimental studies of the steady state of enzyme systems are still the fundamental instrument for their kinetic characterization and for the discrimination between possible reaction mechanisms.

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The strict steady-state equations of an enzyme system may be too complex to be of practical interest (see [1]). Thus, many enzyme reactions are simplified by assuming that one or more of the reversible steps in the mechanism are in rapid equilibrium. Cha (see [2]) proposed a simplifying modification of the King and Altman method (see [3]) for obtaining equations that describe these mechanisms. Computer implementations of Cha's method have been described by Cornish-Bowden (see [4]), Lam (see [1]) and Ishikawa (see [5]).

Besides Cha's method, the steady-state equations of a partial or total equilibrium mechanism can be obtained from the corresponding strict steady-state equations, deleting in the latter ones those terms that are relatively small because of the rapid equilibrium assumption. This elimination can be carried either manually (see [1]) or by adding suitable subroutines to the computer program that gives the strict steady-state equations. Such a computer program was developed by Kinderlerer and Ainsworth (see [6]). However, in spite of the indubitable merit of this program, it has some important limitations, e.g. it is restricted to mechanisms involving up to 10 enzyme intermediates, with up to six reactions between each enzyme state and with a maximum of eight reactants.

In 1995 Varón et al. (see [7]) developed a computer program written in the Visual BASIC programming language for MS DOS called Albass, which gives the strict steady-state equations as well as the corresponding ones if rapid equilibrium is assumed. Nevertheless, this program had some limitations arising from the programming language employed, e.g., the limited memory it was able to handle.

Two years later, Varón et al. (see [8]), developed a new program called Referass that overcame the limitations mentioned above. This program gave a straightforward printout of the results in an easily understood form. Though the program was written using C++ programming language, it ran in the MS DOS operating system.

More recently, Fromm and Fromm (see [9]) presented a two-step computer assisted procedure for deriving steady-state rate equations using the program *Mathematica*. This procedure does not require any software but *Mathematica*, which is an all-purpose software package. However, the method presents some limitations for kinetic analysis, such as

- only the strict steady-state equations are derived by this method.
- The rate of ligand species released in irreversible steps of the reaction mechanism are not directly obtained.
- In complex mechanism models, the calculation time is quite long.

The objective of the present contribution is to develop a computer program that allows the user to derive not only the strict steady-state equations but also those for rapid equilibrium conditions, via an user-friendly graphical interface. Running the program only requires choosing a symbolic notation for each of the enzyme species and for the rate constants (first or pseudo-first order) connecting each pair of enzyme species in the mechanism.

2. Materials and methods

The implementation of this program has been divided in two parts. The first of them is a dynamic link library (WinStes.dll), written in the C++ programming language and compiled using the Microsoft Visual C++ 6.0 compiler. It contains the functions needed for obtaining the kinetic equations as well as for handling the memory. The second part is an executable file (WinStes.exe), written in the C++ programming language using the Borland C++ Builder 5 compiler. It serves as interface for the user to input the data and output the results and from which the functions of the DLL are called when it is necessary.

In the implementation of the present version, we have used the algorithms developed by Varón et al. (see [10]). The example described in this article have been solved using a computer based on a Pentium IV 1.4 GHz with 256 Mbytes of RAM and a 40 Gbyte IDE hard disk.

3. Theory

3.1. The model

The general enzyme reaction model used in this contribution has been already described in other contexts (see [8,10,11]). It consists of n enzyme species denoted arbitrarily by X_i ($i = 1, 2, \ldots, n$) (where X_1 is the free

enzyme) and g ligand species (products, substrates, inhibitors and activators) denoted arbitrarily as Y_s ($s = 1, 2, \ldots, g$). Any single step in this model belongs to one of the following types (Scheme 1):

Either $k_{i,j}$ or $k_{j,i}$ may be zero in any of steps (a)–(c). Most enzyme reaction mechanisms can be described by a combination of reaction steps of type (a)–(f).

We assume that the only enzyme species present at the onset of the reaction is the free enzyme, which has an initial concentration of $[E]_0$, and that the concentration of any ligand species which reacts with an enzyme species remains constant during the entire course of the reaction. Under these conditions, any conversion step of the model is either of first or pseudo-first order.

By $K_{i,j}$ we denote either $k_{i,j}$ or $k_{i,j}[Y_s]_0$, depending on whether the conversion of X_i into X_j is a first or pseudo-first order reaction. In some mechanisms, two or more steps may exist between the same pair of enzyme species (parallel steps (see [4])). In these cases, the rate constants involved in each of the set of parallel steps is denoted by numbered symbols: $K_{i,j}(1)$, $K_{i,j}(2)$, etc. The sum of these constants $K_{i,j} = K_{i,j}(1) + K_{i,j}(2) + \cdots$ does not mean a first or pseudo-first rate constants.

In this paper, the term *rate constant* is used either for a first or a pseudo-first order rate constant. More details about the model are given by Varón et al. (see [8]).

3.2. The steady-state equations

3.2.1. The strict steady-state equations

A number of contributions giving the strict transient phase equations of a general enzyme system exist (see [8,10,12,13]). The following strict steady-state Eqs. (1)–(3) for the steady-state concentration of any of the enzyme species can be obtained easily from Eqs. (1)–(8) in Varón et al. (see [10]).

$$X_i \underset{k_{j,i}}{\overset{k_{i,j}}{\rightleftharpoons}} X_j \qquad (i \neq j) \qquad \text{(a)}$$

$$X_i + Y_s \underset{k_{j,i}}{\overset{k_{i,j}}{\rightleftharpoons}} X_j \qquad (i \neq j) \qquad \text{(b)}$$

$$X_i + Y_s \underset{k_{j,i}}{\overset{k_{i,j}}{\rightleftharpoons}} X_j + Y_w \qquad (i \neq j;\ s \neq w) \qquad \text{(c)}$$

$$X_i + Y_s \xrightarrow{k_{i,j}} X_j + \sum_{r \neq s} Y_r \qquad (i \neq j) \qquad \text{(d)}$$

$$X_j \xrightarrow{k_{i,j}} X_i + Y_s + \sum_{r \neq s} Y_r \qquad (i \neq j) \qquad \text{(e)}$$

$$X_j + Y_w \xrightarrow{k_{i,j}} X_i + Y_s + \sum_{r \neq s,w} Y_r \qquad (i \neq j) \qquad \text{(f)}$$

Scheme 1.

$$[X_i] = \frac{f_{i,u}[E]_0}{F_u} \quad (i = 1, 2, \ldots, n), \tag{1}$$

$$\alpha_{Ys} = \frac{N_{Ys,u}[E]_0}{F_u} \quad (s = 1, 2, \ldots, g), \tag{2}$$

$$N_{Ys,u} = \sum_{(i,j)} [K_{j,i} f_{j,u} - K_{i,j} f_{i,u}] \quad (s = 1, 2, \ldots, g), \tag{3}$$

where $[E]_0$ is the initial concentration of the free enzyme, X_1.

The meaning of the different symbols appears in Varón et al. (see [10]), but it is useful to summarize some of them here.

3.2.2. Meaning of u and F_u

Let $D(\lambda)$ be the secular determinant of the set of n differential linear equations with coefficients (the constants $K_{i,j}$ $(i,j = 1, 2, \ldots, n)$) describing the kinetics of the enzyme species in the reaction mechanism under study, i.e.:

$$D(\lambda) = \begin{vmatrix} K_{1,1} - \lambda & K_{2,1} & \ldots & K_{n,1} \\ K_{1,2} & K_{2,2} - \lambda & \ldots & K_{n,2} \\ \cdot & \cdot & \ldots & \cdot \\ \cdot & \cdot & \ldots & \cdot \\ \cdot & \cdot & \ldots & \cdot \\ K_{1,n} & K_{2,n} & \ldots & K_{n,n} - \lambda \end{vmatrix}. \tag{4}$$

The elements $K_{i,i}$ on the main diagonal are

$$K_{i,i} = -\sum_{\substack{r=1 \\ r \neq i}}^{n} K_{i,r} \quad (i = 1, 2, \ldots, n). \tag{5}$$

The expansion of this determinant yields (see [10,14]):

$$D(\lambda) = (-1)^n \lambda^c T(\lambda), \tag{6}$$

where

$$T(\lambda) = \sum_{q=0}^{u} F_q \lambda^{u-q} \tag{7}$$

c and u are the number of null and non-null roots of the polynomial $D(\lambda)$, respectively, and their values depend on the actual reaction mechanism. Hence, $n = c + u$.

The expressions for F_q $(q = 0, 1, 2, \ldots, u)$, and therefore for F_u, can be obtained by expanding the secular determinant $D(\lambda)$. However, they can be also obtained in an easy, systematic and recurrent way (see [10]). The coefficient F_0 is always unity, while the coefficient F_1 is the sum of all the rate constants $K_{i,j}$:

$$F_1 = \sum_{\substack{i,j=1 \\ i \neq j}}^{u} K_{i,j}. \tag{8}$$

The coefficient F_2 is obtained from F_1, the coefficient F_3 is obtained from $F_2, \ldots$, following the method described in Varón et al. (see [10]). The last coefficient obtained in this way is F_u, thus the u-value, the number of non-null roots of $D(\lambda)$, coincides with the number of $K_{i,j}$'s, in a term of F_u.

3.2.3. Meaning of the coefficient $f_{i,u}$

The coefficients $f_{1,u}$ and $f_{i,u}$ may be obtained easily from the coefficient F_u deleting in it the suitable terms as it is described in Varón et al. (see [10]).

As an illustration, we will use the Random Bi–Bi mechanism, followed by some dehydrogenases and kinases (see [15,16]) shown in Scheme 2.

The non-null constants $K_{i,j}$ $(i,j = 1,2,3,4,5,6,7;\ i \neq j)$ involved in Scheme 2 are

$$K_{1,2} = k_1[A],$$
$$K_{1,3} = k'_1[B],$$
$$K_{2,1} = k_{-1},$$
$$K_{2,4} = k_2[B],$$
$$K_{3,1} = k'_{-1},$$
$$K_{3,4} = k'_2[A],$$
$$K_{4,2} = k_{-2},$$
$$K_{4,3} = k'_{-2},$$
$$K_{4,5} = k_3,$$
$$K_{5,4} = k_{-3},$$
$$K_{5,6} = k_4,$$
$$K_{5,7} = k'_4,$$
$$K_{6,1} = k_5,$$
$$K_{7,1} = k'_5,$$

where $[A]$, and $[B]$ are the initial concentrations of the substrates A and B.

In this example, following the procedure described above, the last non-null coefficient F_u is F_6, from which f_{i6} $(i = 5,6,7)$ and $N_{Ys,u}$ can be easily derived.

3.2.4. The steady-state equations for the rapid equilibrium conditions

The rapid equilibrium assumption requires that all the rate constants of first and pseudo-first order involved in the reversible steps that are assumed to be in rapid equilibrium are much higher than all the other ones in the mechanism (see [2]) and mutually not very different. This last statement means that if a set of rate constants are mutually not very different, then the quotient of any pair of them neither goes to 0 nor to ∞. We say in this case that they are of the same infinite order (see [10]). The constants belonging to a set of rate constants much higher than the others and mutually not very different are called *high rate* constants (see [10]).

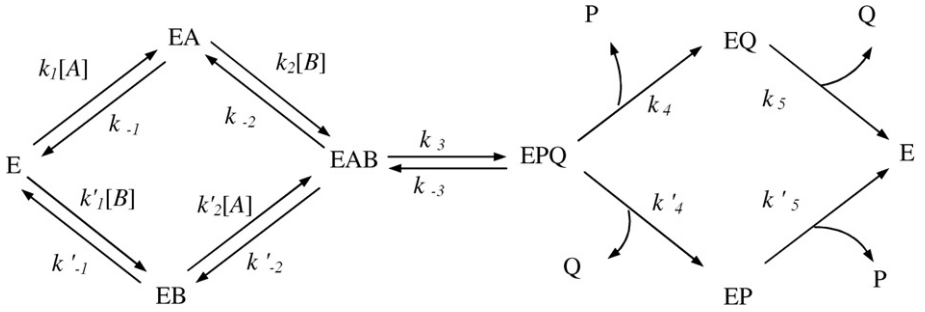
We can summarize the assumptions of rapid equilibrium as the fulfillment of the following conditions (see [10]):

$$\left.\begin{array}{l}\text{high rate constants} \to \infty,\\ \text{high rate constants are of the same infinite order.}\end{array}\right\} \tag{9}$$

We denote with m half of the number of *high rate* constants involved in a reaction mechanism.

3.2.5. Effect on the coefficient F_u

The insertion of conditions Eq. (9) in F_u will cause those terms of the coefficient containing fewer high rate constants than other terms to be neglected (see [10]). The coefficient F_u will have at least one term containing m *high rate* constants, but none with more than m of these constants (see [10]).



Scheme 2. Random Bi–Bi mechanism.

In our example, if we assume that the steps between E and EA, E and EB and between EAB and EPQ are in rapid equilibrium, the corresponding rate constants are *high rate constants*. Therefore, if condition Eq. (9) is applied, it is observed that

$$\left.\begin{array}{l} K_{12}, K_{21}, K_{31}, K_{13}, K_{45}, K_{54} \to \infty, \\ K_{12}, K_{21}, K_{31}, K_{13}, K_{45}, K_{54} \text{ are of the same infinite order.} \end{array}\right\} \tag{10}$$

To obtain the coefficient F_6 corresponding to the rapid equilibrium conditions, each one of the terms of coefficient F_6 mentioned above that contains fewer high rate constants than the others must be neglected, because it is of a smaller infinite order.

3.2.6. Effect on the coefficients $f_{i,u}$

In these coefficients, the terms containing fewer high rate constants than others of the same coefficient may be neglected because they are of a smaller infinite order.

3.2.7. Treatment of loops whose reaction steps are all reversible and in rapid equilibrium

For the purpose of this contribution we will name any loop with all its reaction steps in rapid equilibrium as an α-loop (see [7]). Our program checks the reaction scheme entered to find possible α-loops. Once an α-loop has been found, the program derives the corresponding relationship arising from the application of the mass-action law to each of the reversible reaction steps in the α-loop and which involves all the forward and reverse rate constants of these steps. Our computer program takes advantage of the fact that an α-loop is kinetically equivalent to the segment that results after removing any of its reversible steps (see [7]).

4. Implementation

The computer program has been written and compiled with C++ Borland Builder 5 under the name WinStes. It runs under the Windows operating system and thus WinStes represents a substantial improvement of our previous programs, one written using Microsoft Visual Basic for MS-DOS (see [7]) and the other written using C++, (see [8]) for MS-DOS.

The main characteristics of this program are

- the data input is very straightforward and intuitive, because of the graphical interface.
- The program automatically detects the irreversible steps, the reversible steps and the α-loops.
- Any notation for enzyme species and ligand species both is allowed.
- It can be applied to enzyme-catalyzed reactions with mechanisms containing up to 255 enzyme species, connected by up to 255 reactions.
- The results can be saved in a text file, which can be opened easily with most word processor programs.
- The data corresponding to the mechanism under study can be saved and loaded whenever the user wants.
- The computation time is very short.

In Table 1 is shown a comparison of the computation time of two of the programs mentioned in Section 1 and the program that is presented in this paper.

The computation time of WinStes is shorter than that of Albass and similar to the computation time of Referass; however, this last program has the serious disadvantage, among others mentioned previously, that it does not run under recent versions of the Windows operating system, such as Microsoft Windows XP.

4.1. Hardware requirements

The main requirement is for a 32-bit Windows operating system such as Windows 95/98, Millennium, 2000 or XP with enough free memory. In addition, for correct viewing of all the screens it is recommended that a graphical resolution of at least 800×600 pixels (with small fonts) displaying 256 colours or more be used (Fig. 1).

Table 1
Comparison of the elapsed computation time of three programs when they are applied to derive the equations for three different reaction enzyme mechanisms

Mechanism	Ref.	Albass	Referass	WinStes
General mechanism for two-substrate systems	[2]	35	2.91	2.95
		8	0.05	0.13
Reversible single substrate-single modifier mechanism	[2]	2	0.05	0.05
		<1	<0.01	<0.01
Mechanism with enzyme species not included in rapid equilibrium segment	[2]	4	0.05	0.09
		1	<0.01	0.02

The time is shown in columns 3, 4 and 5. For each program and each mechanism a pair of values is given. The top figure of each pair is the time elapsed in acquiring the strict equations and the other one is the time elapsed in acquiring the equations under full rapid equilibrium assumptions.

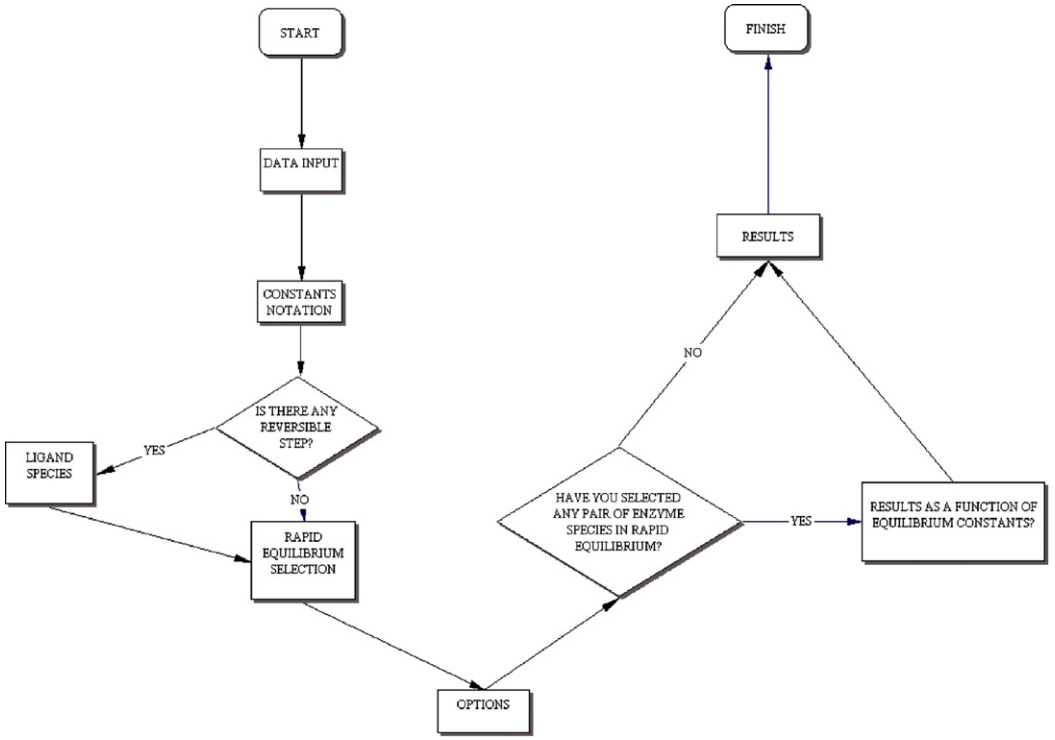


Fig. 1. Flow diagram of the computer program WinStes.

4.2. Data Input

The WinStes has been designed as a set of consecutive forms, in which the user must type using the notation that the program requires, or to select among several options.

4.2.1. Enzyme species notation

On executing the program, the first form that it is opened is the form Data Input.

The program allows any notation for enzyme species as characters e.g. E, ES, ESM, ..., characters followed by numbers X1, X2, ..., etc. The only restriction is that the free enzyme notation has to be typed in a different place from the remaining enzyme species.

To enter the free enzyme notation, type it in the box labeled ''Free enzyme notation'' and then click the button Add or press the Enter key. The rest of the enzyme species notation has to be typed in the box labeled

''Enzyme Species Notation''. The program also allows deleting any enzyme species from the list and replacing it. Once the whole notation has been typed, clicking the button Next will open the next form.

To use the data corresponding to a mechanism previously studied and saved, click the button Load. The program offers the list of the files containing these data, whose extension is ''*.INW''. On selecting one of these files, the program will fulfill the list of enzyme species and the rate constants corresponding to the mechanism.

4.2.2. Rate constants notation

The second form of the program, called Constants Notation, shows a grid in which are typed the rate constants between each pair of enzyme species of the mechanism. The user must type in each cell of the grid the symbolic first or pseudo-first order rate constants or constants notation of the step that links the enzyme species of the head of the column with the enzyme species of the head of the row. Only the non-null rate constants must be typed, if the program finds a blank cell, it automatically assigns a null rate constant to the pair of enzyme species. The expression for the rate constants of a reaction step consists of a lower-case k (or k') followed by a plus sign, which can be omitted, or a minus sign and a subindex, e.g.

$$k1, k-2, k4.$$

When using a lower case k followed by an apostrophe (k'), such as the mechanism corresponding to Scheme 2, place the apostrophe behind the subindex, e.g.:

$$k1', k-2' \quad \text{instead of } k'1 \quad \text{or} \quad k'-2.$$

If the rate constant is pseudo-first order, then the corresponding ligand species notation, written in square brackets, must be typed after the k.

$$k1[S], k-8[M].$$

For a reversible step between a pair of enzyme species, the rate constants of each reaction must be denoted using the same subindex, but one of them must be preceded by the minus sign. Each expression must be typed in different cells, with the head of the column of one cell coinciding with the head of the row of the other one, and vice versa.

In those cases in which there are two or more parallel steps between a pair of enzyme species, the user must type in the corresponding cell the rate constants notations of each step separated by a plus sign, e.g.:

$$k-2+k+3[S].$$

4.2.3. Ligand species notation

The program automatically saves the notation for the ligand species involved in pseudo-first order rate constants. But if there is any irreversible step in which a ligand species could be released, the program detects it and opens a form called Ligand Species. In this form, the irreversible steps are shown with a blank box to type the notation of the released ligand species. If no ligand species is released, the box is left empty.

4.2.4. Rapid equilibrium steps

If the program has detected any reversible step, it will open a form called Rapid Equilibrium Selection, in which any such steps are shown. If any of them is in rapid equilibrium, the user checks the small box on the left of the enzyme species notation.

4.2.5. Options

In the following two forms, the user can select which results are wanted and how they are wanted. The first form for options allows the user to select by checking the enzyme species whose steady-state concentrations are desired and/or the ligand species whose steady-state rates are wanted.

If there is any step in rapid equilibrium, the program asks if the user wants the results as a function of only the individual rate constants or including the corresponding equilibrium constants. By default, the results are given as a function of only the individual rate constants; clicking in the radio button beside the second question chooses this option.

Finally, the program sets the width of the output file to 80 columns. Other widths can be typed in, or selected from a list of values between 132 or 264. The minimum the program allows is 50 and it automatically changes to 50 any typed value below this limit.

Once all the options have been selected, pressing the Process button generates the results.

4.3. The results

The results of the calculations are shown in a form called WinStes. They include the expressions of the steady-state concentration of the enzyme species selected and/or the steady-state rate of the ligand species selected in the most simplified form.

In case one wishes to save the data of the current mechanism, this is achieved by clicking on the "Data" button, choosing the desired directory and typing a name for the file. The program will add the extension INW to the file. Saving the results follows a similar procedure, except that the program will add the extension LIS to the file.

4.3.1. The equations provided by the program

The general equations summarized above can be used for any enzyme reaction that fits the model that we have already described. However, when these equations are applied to a specific mechanism, it could happen that they may be simplified. When the different coefficients are expressed as a function of the rate constants, the resulting equations can be simplified if a common factor exists in each one of the terms of the coefficient F_u, $f_{i,u}$ and $N_{Ys,u}$ (see [10]).

In partial or total equilibrium mechanisms under rapid equilibrium conditions, the steady-state equations can also be given as a function of the equilibrium constants K_q's of the reversible reactions steps, which are assumed to be in rapid equilibrium. For this purpose, the coefficients F_u, $f_{i,u}$ and $N_{Ys,u}$ are divided by the product of the individual rate constants denoted with a positive subindex (see [10]).

The equations provided by the program include the simplifications described above and are printed as

$$[X_i] = \frac{N(X_i)[E]_0}{\text{Den}}, \tag{11}$$

$$V_{Ys} = \frac{M(Ys)[E]_0}{\text{Den}}. \tag{12}$$

Beside the equations, the expressions for $N(X_i)$, $M(Y_s)$ and Den are given.

The subroutine developed by us first searches for an α-loop in the proposed mechanism. If one is found, the subroutine derives and saves the relationship among the rate constants involved in the α-loop and deletes one of its reversible reaction steps. Next, the search for another α-loop begins in the resulting mechanism and the process is repeated until no more α-loops are found.

4.4. Example

We will derive the steady-state equations for our example, corresponding to the Random Bi–Bi mechanism, on the assumption that the reversible steps E ↔ EA, E ↔ EB and EAB ↔ EPQ are in rapid equilibrium.

4.4.1. Enzyme species notation

In this form, the user can give a name to the mechanism but it is not compulsory. Firstly the user must type the free enzyme notation, and then the notation of the rest of enzyme species using the notation of Scheme 2 as can be seen in Fig. 2.

4.4.2. Rate constants notation

Fig. 3 shows the rate constants notation corresponding to Scheme 2.

Once the all of rate constants have been typed, we click on Next, and the program automatically detects the reversible and irreversible steps and the next window is opened.

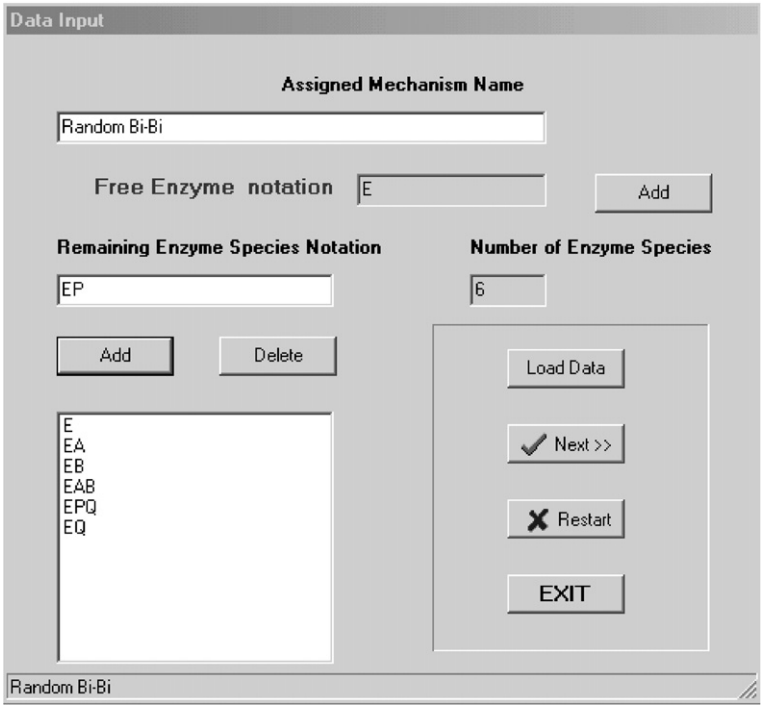
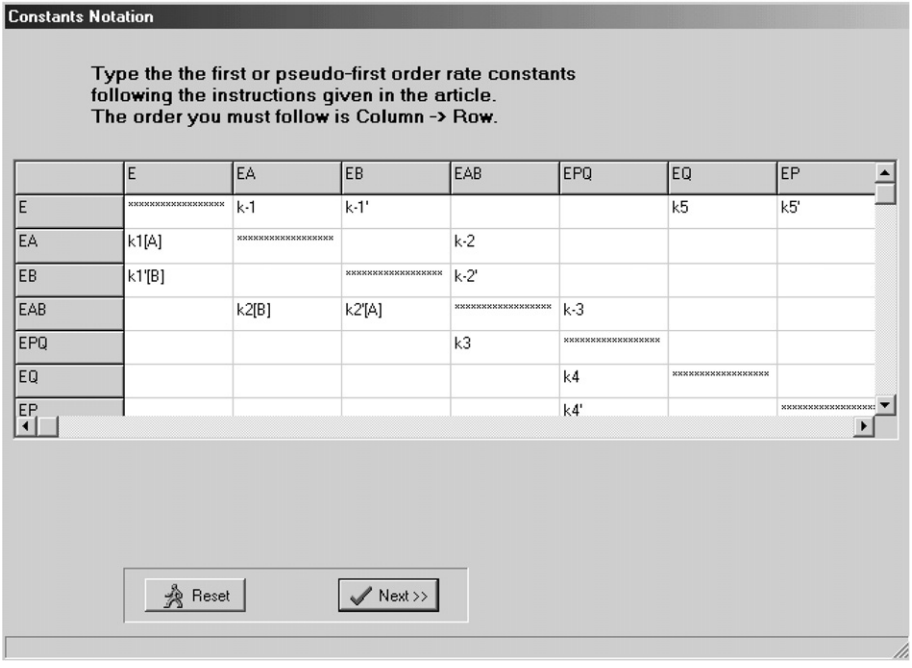


Fig. 2. Form Data Input. Enzyme species notation corresponding to Scheme 2.



	E	EA	EB	EAB	EPQ	EQ	EP
E	**********	k-1	k-1'			k5	k5'
EA	k1[A]	**********		k-2			
EB	k1'[B]		**********	k-2'			
EAB		k2[B]	k2'[A]	**********	k-3		
EPQ				k3	**********		
EQ					k4	**********	
EP					k4'		**********

Fig. 3. Form Constants Notation. Constants notation corresponding to Scheme 2.

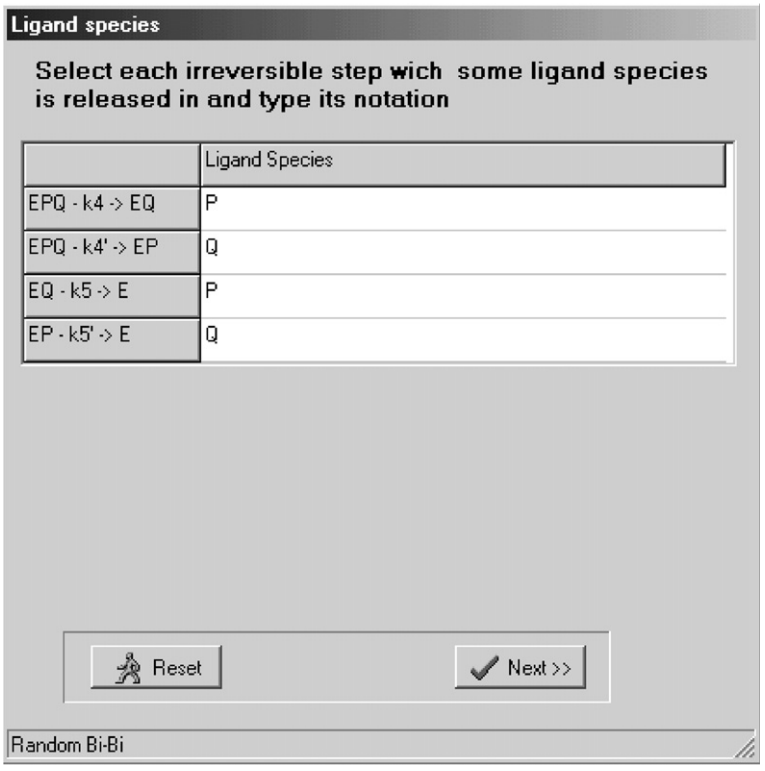


Fig. 4. Form Ligand Species. Ligand species notation corresponding to Scheme 2.

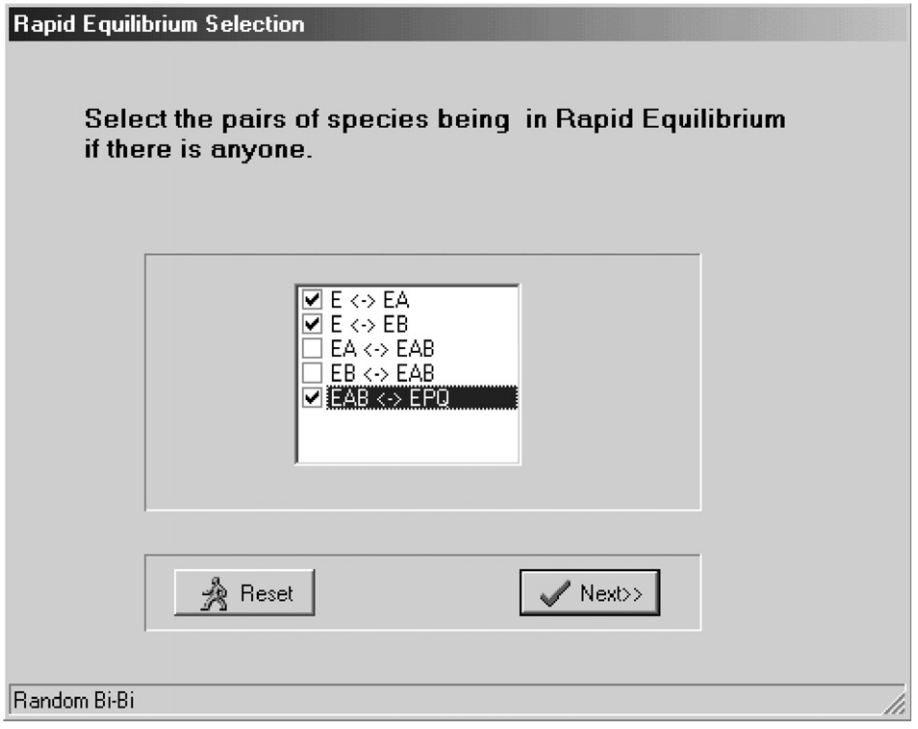


Fig. 5. Form Rapid Equilibrium Selection. Reversible steps corresponding to Scheme 2.

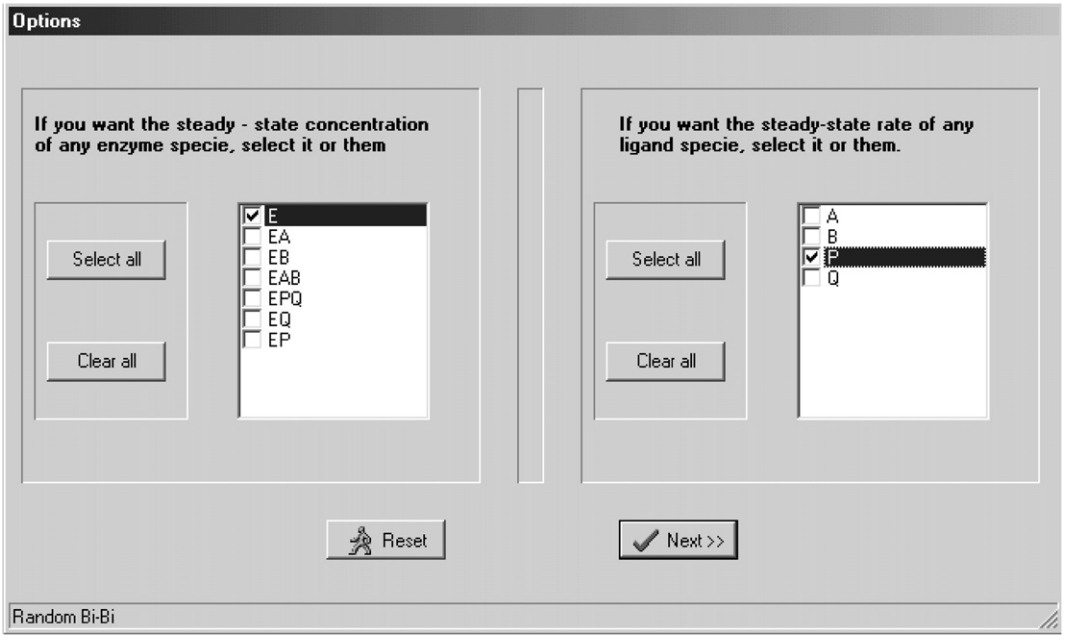


Fig. 6. Form Options. Enzyme species and ligand species notation corresponding to Scheme 2.

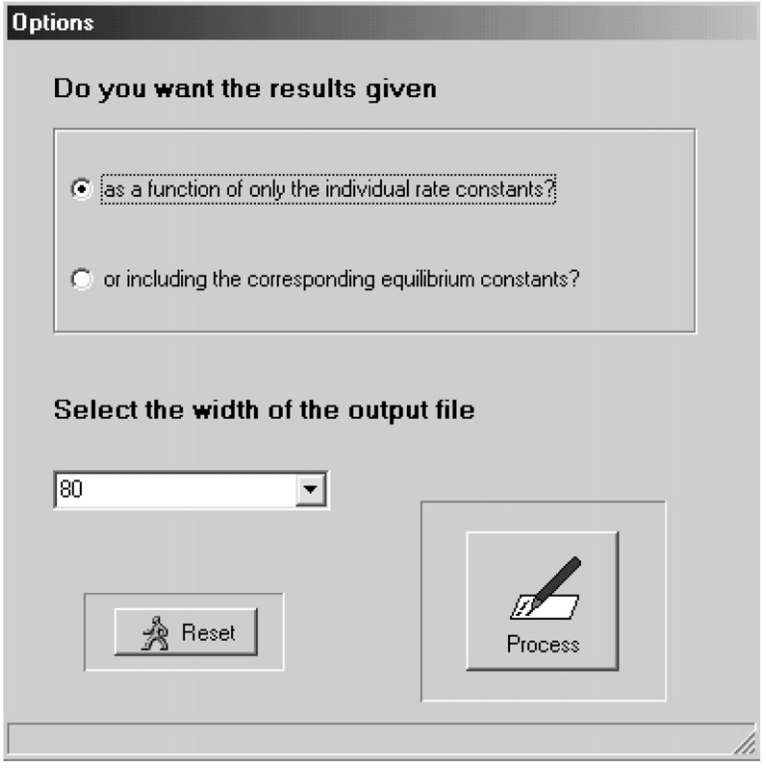


Fig. 7. Form Options.

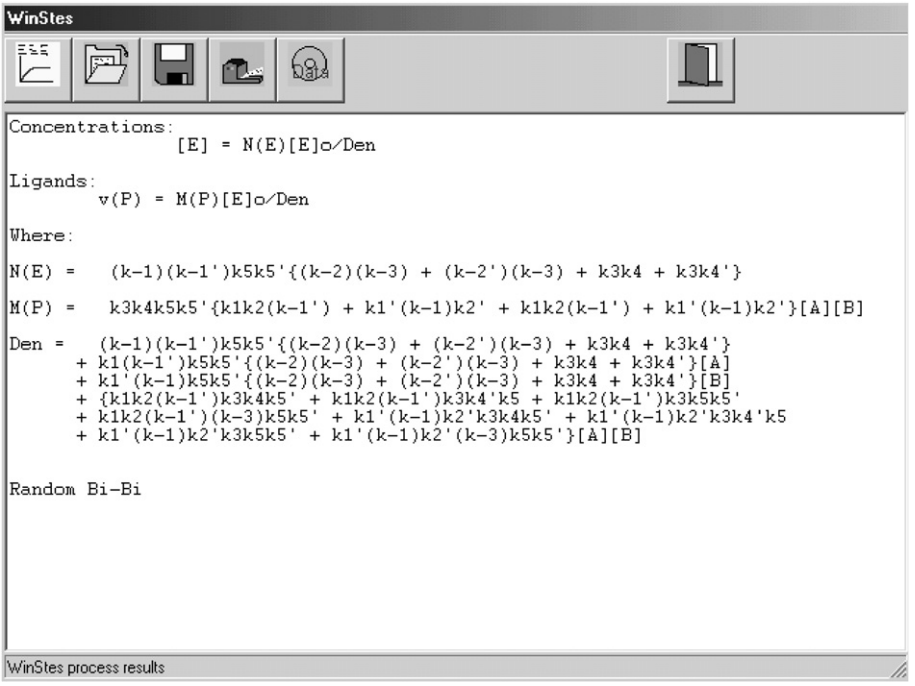


Fig. 8. WinStes. Equations corresponding to Scheme 2.

4.4.3. Ligand species notation

The program shows the four irreversible steps and asks the user for the ligand species released in them. In our example, two ligand species are released, each of them in two different steps. Thus, we have to type their notation in the corresponding cells, as is shown in Fig. 4.

4.4.4. Rapid equilibrium steps

The program has detected five reversible steps and asks the user to specify which of them are in rapid equilibrium. We can select one, two, ..., all or none of them. In this example, we select the steps between E and EA, E and EB and between EAB an EPQ are in rapid equilibrium (Fig. 5).

4.4.5. Options

In our example, we choose E and P (Fig. 6). Finally, we choose the option that shows the results as a function of only the individual rate constants and we click oPn the Process button (Fig. 7).

4.4.6. Results

The results are shown in Fig. 8. For further calculations, we recommend saving the data corresponding to this mechanism.

5. Results and discussion

Since Cha (see [2]) published his important contribution, a number of authors (see [1,4–6,9,11,17,18]) have also published methods that allows the computerized derivation of the strict steady-state solutions of reaction mechanisms, some of them rather complex (see [5,7–9,11]).

Usually, the kinetic study of the steady state with any conversion step in rapid equilibrium has been made with the Cha method.

An alternative procedure was implemented (see [7,8]). This involves first the computerized derivation of the strict steady-state solutions followed by the elimination of the terms that correspond to any or all the reaction

steps that are in rapid equilibrium. Those are, as far as we know, the only published methods that offer this possibility.

Our computer program allows the user to derive both the strict steady-state solutions as well as those with some steps at rapid equilibrium. The assumption of a rapid equilibrium mechanism usually results in much simpler equations compared to those corresponding to the strict steady-state conditions, particularly when the different assumptions are applied to systems composed of many reactions.

As an example, here we show the equations produced by our program for the rate of the ligand species P in the Random Bi–Bi mechanism (Scheme 2). Eq. (13) is the strict steady-state rate of P and Eq. (14) is the equation obtained when we make the rapid equilibrium assumption. The substantial simplification resulting from the latter assumption are easily seen.

$$V_P = \frac{a[A][B] + b[A]^2[B] + c[A][B]^2}{d + e[A] + f[B] + g[A][B] + h[A]^2 + i[B]^2 + j[A]^2[B] + k[A][B]^2}, \tag{13}$$

where

$$\begin{aligned}
a &= k3k4k5k5'\{k1k2(k-1') + k1'(k-1)k2' + k1k2(k-1') + k1'(k-1)k2'\},\\
b &= k1k2k2'k3k4k5k5'\{2\},\\
c &= k1'k2k2'k3k4k5k5'\{2\},\\
d &= (k-1)(k-1')k5k5'\{(k-2)(k-3) + (k-2)k4 + (k-2)k4' + (k-2')(k-3)\\
&\quad + (k-2')k4 + (k-2')k4' + k3k4 + k3k4'\},\\
e &= k5k5'\{k1(k-1')(k-2)(k-3) + k1(k-1')(k-2)k4 + k1(k-1')(k-2)k4'\\
&\quad + k1(k-1')(k-2')(k-3) + k1(k-1')(k-2')k4 + k1(k-1')(k-2')k4'\\
&\quad + k1(k-1')k3k4 + k1(k-1')k3k4' + (k-1)k2'(k-2)(k-3)\\
&\quad + (k-1)k2'(k-2)k4 + (k-1)k2'(k-2)k4' + (k-1)k2'k3k4 + (k-1)k2'k3k4'\},\\
f &= k5k5'\{k1'(k-1)(k-2)(k-3) + k1'(k-1)(k-2)k4 + k1'(k-1)(k-2)k4'\\
&\quad + k1'(k-1)(k-2')(k-3) + k1'(k-1)(k-2')k4 + k1'(k-1)(k-2')k4'\\
&\quad + k1'(k-1)k3k4 + k1'(k-1)k3k4' + k2(k-1')(k-2')(k-3) + k2(k-1')(k-2')k4\\
&\quad + k2(k-1')(k-2')k4' + k2(k-1')k3k4 + k2(k-1')k3k4'\},\\
g &= \{k1k2(k-1')k3k4k5' + k1k2(k-1')k3k4'k5 + k1k2(k-1')k3k5k5'\\
&\quad + k1k2(k-1')(k-3)k5k5' + k1k2(k-1')k4k5k5' + k1k2(k-1')k4'k5k5'\\
&\quad + k1k2(k-2')(k-3)k5k5' + k1k2(k-2')k4k5k5' + k1k2(k-2')k4'k5k5'\\
&\quad + k1'(k-1)k2'k3k4k5' + k1'(k-1)k2'k3k4'k5 + k1'(k-1)k2'k3k5k5'\\
&\quad + k1'(k-1)k2'(k-3)k5k5' + k1'(k-1)k2'k4k5k5' + k1'(k-1)k2'k4'k5k5'\\
&\quad + k1'k2'(k-2)(k-3)k5k5' + k1'k2'(k-2)k4k5k5' + k1'k2'(k-2)k4'k5k5'\\
&\quad + k2k2'k3k4k5k5' + k2k2'k3k4'k5k5'\},\\
h &= k1k2'k5k5'\{(k-2)(k-3) + (k-2)k4 + (k-2)k4' + k3k4 + k3k4'\},\\
i &= k1'k2k5k5'\{(k-2')(k-3) + (k-2')k4 + (k-2')k4' + k3k4 + k3k4'\},\\
j &= k1k2k2'\{k3k4k5' + k3k4'k5 + k3k5k5' + (k-3)k5k5' + k4k5k5' + k4'k5k5'\},\\
k &= k1'k2k2'\{k3k4k5' + k3k4'k5 + k3k5k5' + (k-3)k5k5' + k4k5k5' + k4'k5k5'\}.
\end{aligned}$$

$$V_P = \frac{m[A][B]}{n[A] + p[B] + q[A][B]}, \tag{14}$$

where

$$\begin{aligned}
m &= k3k4k5k5'\{k1k2(k-1') + k1'(k-1)k2' + k1k2(k-1') + k1'(k-1)k2'\},\\
n &= (k-1)(k-1')k5k5'\{(k-2)(k-3) + (k-2')(k-3) + k3k4 + k3k4'\}\\
&\quad + k1(k-1')k5k5'\{(k-2)(k-3) + (k-2')(k-3) + k3k4 + k3k4'\},\\
p &= k1'(k-1)k5k5'\{(k-2)(k-3) + (k-2')(k-3) + k3k4 + k3k4'\},\\
q &= \{k1k2(k-1')k3k4k5' + k1k2(k-1')k3k4'k5 + k1k2(k-1')k3k5k5' + k1k2(k-1')(k-3)k5k5'\\
&\quad + k1'(k-1)k2'k3k4k5' + k1'(k-1)k2'k3k4'k5 + k1'(k-1)k2'k3k5k5' + k1'(k-1)k2'(k-3)k5k5'\}.
\end{aligned}$$

The user-friendly graphical interface of our program requires the use of no special programming skills. The program handles reactions in which up to 255 enzyme species can be involved, with a limit of 255 non-null rate constants in the mechanism. It automatically detects the irreversible steps, the reversible steps and the α-loops. Any notation for both enzyme species and ligand species is allowed. The definition of the mechanism under study can be saved for further calculations and variations.

Our program is valid for those reaction mechanisms that fit the general model described in Section 3.1. Thus, it can be applied to almost any enzyme system, irrespective of whether it is a branched or unbranched mechanism, irrespective of whether there are parallel steps, irreversible steps, repeated rate constants, closed loops, etc. It is not applicable to mechanisms of reactions for zymogen activation or to mechanisms involving more than one enzyme acting simultaneously on one or more substrates.

The correct execution of the program has been checked using a number of different mechanisms; however, in order to make further improvements, we would appreciate any suggestions from readers.

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