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Use of a windows program for simulation of the progress curves of reactants and intermediates involved in enzyme-catalyzed reactions

Francisco García-Sevilla [a], Carmelo Garrido-del Solo [a], Ronald G. Duggleby [b], Francisco García-Cánovas [c], Ramón Peyró [d], Ramón Varón [a,*]

[a] *Departamento de Química-Física, Escuela Politécnica Superior, Universidad de Castilla-La Mancha, Campus Universitario, E-02071 Albacete, Spain*

[b] *Department of Biochemistry, University of Queensland, Brisbane, Qld 4072, Australia*

[c] *Departamento de Bioquímica y Biología Molecular A, Facultad de Biología, Universidad de Murcia, E-30071 Murcia, Spain*

[d] *Hospital General, Hermanos Falcó s/n, E-02006 Albacete, Spain*

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Abstract

A program that performs simulation of the kinetics of enzyme-catalyzed reactions with up to 32 species is described. The program is written in C++ for MS Windows 95/98/NT and uses a simple text file to define the kinetic model. The use of the program is illustrated with some examples. WES is available free of charge on request from the authors (e-mail: fgarcia@iele-ab.uclm.es).

Keywords: Enzyme kinetics; Computer program; Differential equations; Simulation; Progress curves

1. Introduction

The kinetics of enzyme-catalyzed reactions depends upon an underlying system of differential equations. When this system is linear, then it can be solved using procedures described in the mathematical literature (Spiegel, 1981; Gerald and Wheatley, 1989). Generally, this system of equations is not linear and, therefore, it is possible neither to solve it nor to find analytical solutions. In this case one must resort to one of the following two procedures: linearize the system or solve it by using numerical calculus.

The linearization process assumes certain conditions (concerning the initial concentrations of some of the species involved or particular relationships between the rate constants) so that a set of differential equations is obtained that is approximately linear. The analytical solutions thus derived are, therefore, only applicable to a real system under these restrictive conditions. Recently computer programs have been published (Varón

* Corresponding author. Tel.: +34-967599200; fax: +34-967599224.

E-mail address: rvaron@pol-ab.uclm.es (R. Varón)

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et al., 1990, 1991, 1999) that rely on this linearization approach for the acquisition of the algebraic kinetic equations for the time course of enzyme reactions as a function of the rate constants and initial concentrations. Although these programs and that to be presented here by us can be complementary, they are totally independent.

It is always possible to solve, by using procedures of numerical calculus (Carnahan et al. 1969; Press et al., 1992), systems of differential equations that have no analytical solution. The solution in these cases is given as a table of time versus concentration and/or time versus rate and provides a simulation of the kinetics of the reaction.

The most useful application of the simulation is for testing whether the kinetic equations are realistic by comparing a plot of experimental data with the simulated curves.

A key step in this process is obtaining the simulated curves and this step is limited by the availability of efficient computer programs. Irrespective of whether the enzyme system is linear or not, such programs must take into account the specific characteristic of these systems and provide a convenient interface for input of the data and presentation of the results for the user. Several programs have been described that can be used for simulation and/or fitting of the progress curves of enzyme-catalyzed reactions and related systems (Chandler et al., 1972; Duggleby and Morrison, 1977; Canela and Franco, 1986; Franco et al., 1986; Cox and Boeker, 1987; Hensley et al., 1992; Royer and Beechem, 1992; Duggleby, 1994; Frieden, 1994; Ehlde and Zacchi, 1995; Mendes and Kell, 1998). Despite the fact that many of these programs have proved useful in specific contexts, none has a user interface (input, output or both) that is particularly simple and in some cases these programs require a great deal of experience to use effectively.

In addition to the programs mentioned above, there are general mathematical software packages that will provide numerical solutions of sets of differential equations. However, their great versatility is difficult to exploit because the use of these programs is usually very complicated. Moreover, most of these programs do not furnish all of the information in which an enzymologist is interested. Some of these requirements of workers in enzyme kinetics are:

1. the progress curves of the concentrations and/or the rates of various individual reactant and enzyme species;
2. progress curves corresponding to any combination of concentrations (e.g. sums, quotients, etc);
3. the equations of the straight lines tangent to the concentration progress curves at both the initial and final integration times;
4. the singular points of each of the progress curves of the concentrations;
5. the facility to rescale the curves; the facility to zoom and move through the curves; and
6. the facility to save graphic images of the curves generated and/or a text file report of the tangent straight lines mentioned above, their intercept, tables of values of the concentrations and/or rates at various times, etc.

A few years ago, Garrido del Solo et al. (Garrido-del Solo et al., 1992) published a computer program for MS DOS written in TURBO PASCAL for simulating any enzyme reaction with up to 20 species. Nevertheless, the program runs slowly due to the algorithm used while the input of the equations and the output of the results is not particularly easy for the user.

The above limitations for enzymological studies using existing programs encouraged us to implement a specific computer program for MS Windows 95/98/NT that covers almost all needs for the simulation of the kinetics of enzyme systems.

The main characteristics of this program are:

1. It allows reaction mechanisms containing up to 32 species. This number could be easily increased if necessary, the memory requirements being the limiting factor.
2. The computation time is very short, even for a complex mechanism. Obviously, this time depends on the computer used. Thus, when a 133 MHz Pentium was used, the computer time was less than 60 s for all examples, whereas using a 400 MHz Pentium II machine the computation times were less than 10 s.
3. The input of the data is easy and versatile.

4. It provides the results as graphic images of progress curves and as ASCII tables.

2. Systems and methods

The implementation of the program has been divided in two major blocks. The first block is a dynamic link library (wes.dll), written in ANSI C language and compiled using the Visual C++ 6.0 compiler, in which the functions needed for integrating the differential equations, handling of memory and files, and calculus of the x and y coordinates for each of the required plots have been included. The second block corresponds to an executable file (wes.exe), written using the Borland Delphi Desktop 4.0 compiler and which serves as the input/output interface for the user and from which the functions of the DLL are called when it is necessary. In the implementation of the present version, the Runge–Kutta–Fehlberg integration algorithm has been used. The examples described in this article have been solved using a computer based on a Pentium 133 MHz with 32 Mbytes of RAM and a 2 Gbytes IDE hard disk.

3. Algorithm

Integration algorithms that use a fixed step size are easy to program, but they are not appropriate for enzyme systems in which the most rapid changes in the concentrations and rates usually occur early in the reaction. The selection of such a method would require the use of a step size (h) that is sufficiently small so that it can cope with the transition phase and, consequently, the processing time would be excessively high (inversely proportional to h).

Thus we preferred a variable step size method to carry out the numerical integration. Choosing the particular method involved several tests on enzyme systems of varying complexity, using different values for the tolerance, comparing the Adams–Bashforth–Moulton (ABM) and Runge–Kutta–Fehlberg (RKF) algorithms (Fehlberg, 1970; Burden and Faires, 1985). The latter was found to be more efficient for the integration, even for tolerance values of the order of 10^{-21} times the concentration unit used in the integration. This observation, together with the easier programming and the possibility to vary h in the last iteration (in order to coincide with the time period established by the user) inclined us to choose the RKF algorithm.

This method evaluates the concentrations of all of the species at time $t+h$ from their values at time t. Therefore, this method requires knowledge of the concentrations of all of the species at time t_0, the initial time of the simulated curves (which may or may not coincide with the onset of the reaction, i.e. $t_0 \neq 0$). This t_0-value must be entered by the user as part of the input of data (see below). The algorithm requires a value for the maximum error allowed (ε) in any iteration as well as a value for the integration step size (h) for the first iteration. After various trials it was decided to use for ε the value 2.22×10^{-16} (which is the smallest non-zero value for which $1+\varepsilon > 1$ in the IEEE-754 format for 64 binary digits) and to set the initial h-value as $\varepsilon^{1/4} = 1.22 \times 10^{-4}$. Then, the concentrations at t_0+h are evaluated and the integration error quantified. If this error is higher than ε the iteration is repeated using a lower h-value. On the other hand, if the error is small enough the integration step is accepted, a new h-value is determined and the evaluation of the next set of concentrations is initiated. In those cases in which the h-value falls below 10^{-31} or in which accuracy is lost in the sum (i.e. if it happens that $t = t+h$ for $h > 0$), then the integration process stops and a warning is issued to the user.

Several existing programs for simulations oblige the user to compile and link the differential equations with the executable program. The objective of this is to reduce calculation time but it can be tedious for the user. In the program we have designed an analyser for expressions. The analyser interprets the equations and transforms them into an intermediate code which is calculated almost as quickly as the compiled expression. The penalty for this is that the differential equations must be written using a defined syntax, the format of which is indicated in the section input of the data below.

4. Implementation

The user interface has been designed so that it is relatively simple and does not require the user to search through a large number of menu options and suboptions to do something so simple as to print or to expand the graph that is on the screen. The program presents a screen with four tabs, one for the input of the data, a second for viewing a graphic representation of the results of the simulation, a third for a tabular representation of the major features of the results and a fourth for predicting the instantaneous concentrations and/or rates of the species at different chosen times.

4.1. Hardware requirements

The principal requirement is for a 32-bit Windows operating system such as Windows 95/98 or Windows NT with enough free memory to store the values corresponding to each of the differential equations involved in the system under study. In addition, for correct viewing of all of the screens it is recommended that a graphical resolution of at least 800 × 600 pixels (with small fonts) displaying 256 colours or more is used.

4.2. Input of the data

The tab 'Input', which is selected automatically when the program is started (Fig. 1), presents at the top three small buttons that, respectively, define a new system to be simulated, open a previous case already solved and saved, or save the system that is currently defined. This screen contains four windows (Fig. 1) in which text can be entered. Editing uses the same keystroke combinations as a simple text editor such as, for example, 'Notepad' for Windows in which one can copy, cut and paste text with Ctrl + C, Ctrl + X and Ctrl + V, respectively.

In the window labelled Description the user may write any arbitrary text to describe the reaction mechanism under study; this text can occupy any number of lines, although only three of them will be visible at any time.

In the window labelled Rate constants must be written the values chosen for the rate constants involved in the reaction mechanism under study,

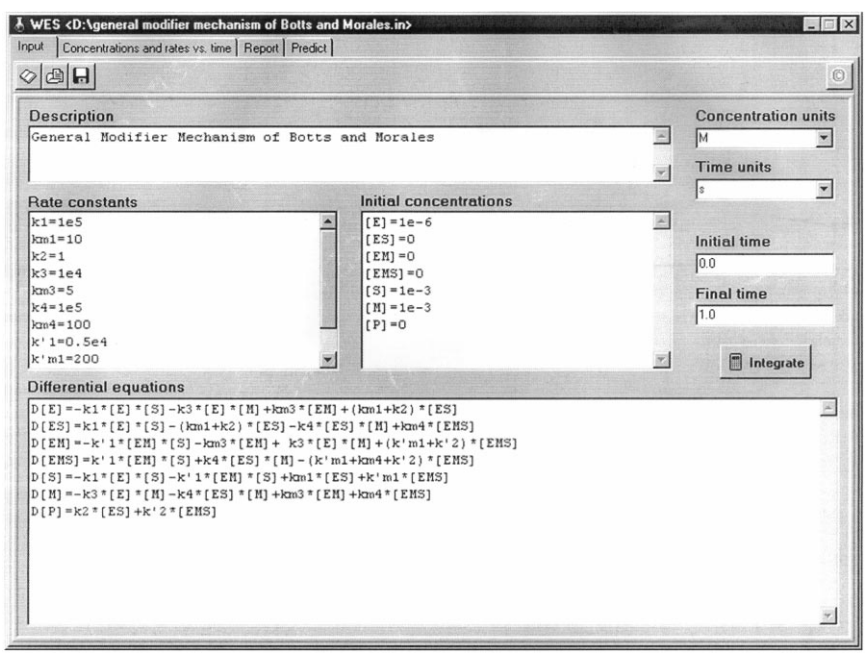


Fig. 1. Data input screen corresponding to Scheme 1 below.

one value per line. Each line must begin with an obligatory *k* (upper- or lower-case) followed by any combination of letters and/or digits, the symbol equal (=) and the numeric value assigned to the rate constant. For example:

k1 = 0.03

kon = 1e − 6

K4 = 10

These definitions imply that the units to be used for concentrations and for time have been chosen. The program uses M (molar) and s (seconds), respectively, as the defaults but these units can be changed. Most commonly this will be done by clicking on the drop arrow corresponding to the 'Concentration units' or 'Time units' boxes at the right of the screen and selecting the desired unit from among those offered in the list (M, mM, μM and nM for concentration and s, min, h, d, ms, μs and ns for time). Alternatively, any other unit of interest can be typed in directly.

In the bottom window labelled Differential equations the differential equations that describe the kinetics of the enzyme system under study are written. Each equation is written on a separate line with the left-hand side (i.e. the time derivative of the species concentration) written as a capital D followed by the symbol used for the species, between square brackets. The right-hand side will be one term or a sum of two or more terms. Each term must be a product of powers of concentrations, multiplied by a coefficient. The coefficient may be any expression containing fixed numerical values and/or rate constants. Thus, for example, the following differential equations:

$$\left.\begin{aligned}
&\frac{d[X_1]}{dt} = 0\\
&\frac{d[X_1]}{dt} = k_1[X_1][X_2]\\
&\frac{d[X_1]}{dt} = (k_1 + k_2)[X_1] - (k_3 + k_4)[X_2][X_3]\\
&\frac{d[X_1]}{dt} = -\left(\frac{k_1}{k_2} + k_3\right)k_4[X_2]^2\\
&\frac{d[X_1]}{dt} = k_1\frac{[X_2][X_3]^2}{X_4} + k_2[X_5]^2[X_6] + k_3[X_7]^3
\end{aligned}\right\} \quad (1)$$

must be written as:

D[X1] = 0 (i.e. [*X*1] *is constant*)

D[X1] = k1*[X1]*[X2]

D[X1] = (k1 + k2)*[X1] − (k3 + k4)*[X2]*[X3]

D[X1] = −(k1/k2 + k3)*k4*[X2]^2

D[X1] = k1*[X2]*[X3]^2*[X4]^ − 1
+ k2*[X5]^2*[X6] + k3*[X7]^3

Special attention must be paid to the name given to the species, because the program distinguishes between upper- and lower-case letters, so that [X1] and [x1] will be treated as different quantities. In each term on the right-hand side, the concentrations can appear in any order and a concentration can appear more than once but the coefficient that multiplies each term must be written first.

After introduction of the equations, the time interval for calculation of the simulated curves must be specified, i.e. the initial (0 as default) and final (10 units of time as default) time values must be provided.

Finally, in the window labelled Initial concentrations, the values of the concentrations at the initial time chosen for the simulation must be written, using one line for each concentration value. For example:

[E] = 0.001

[A] = 10

The initial concentrations values for any species not appearing in the list will be interpreted as zero by the program, so it is sufficient to write only those species that have non-zero initial concentrations.

4.3. Starting the integration of the differential equations

Once all the inputs have been completed, clicking on the Integrate button will start the integration process. A dialog box will appear that indicates the estimated processing time, the time elapsed from the beginning of the process and a progress bar showing an estimate of the current

percentage of the integration process. The user can, at any time, click the Cancel button to stop the process.

The integration process comprises two parts. The first consists of an initialisation in which the program determines the number of points that are necessary for the correct plot of the curves as well as the memory necessary for their storage. The second part carries out the numerical integration of the system and saves in memory the values of times, instantaneous concentrations and instantaneous rates of all of the species involved in the reaction mechanism under study. If the process is cancelled during the integration the values calculated until that time are obtained; of course, if the process is stopped during the initialisation, then no values are obtained because they have not yet been calculated.

If the system entered cannot be integrated, i.e. an excessively small step size (h) is needed, then the process stops and an indicative message is displayed. In this case the integration can be tried again reducing the final time of integration.

4.4. Graphical output of the results

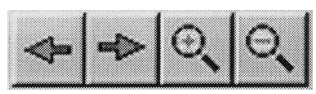
Once the integration is completed the progress dialog box disappears and the second tab Concentrations and rates versus time is automatically selected (Fig. 2). The progress curves of the concentrations of all of the species is displayed using ordinate and abscissa scales that allow all the points to be plotted within the area of the screen.

At the bottom of the screen appears a button for each of the species involved in the reaction mechanism labelled with the name used for the corresponding species, colour-coded to match with the lines on the graph. The colours used are: red, blue, green, yellow, aqua, fuchsia, lime or navy solid lines for the first eight species and the same series of colours but as dotted lines for the next eight species. When there are more than 16 species, the colours and type of lines are repeated so that species 1 and 17 will be the same. One can choose which species are displayed and suppressed by clicking on the corresponding button. When a species is not selected, then the label on the corresponding button will appear white.

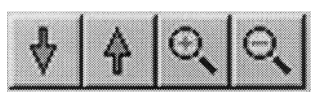
At the top of the screen are four groups of buttons that allow the user to control the range of times and concentrations or rates to be plotted.

The first button from the left is Autoscale and will calculate both the upper and limits (of concentrations or rates) of the species currently selected and will set the limits for the time axis as the initial and final integration times chosen previously.

The second button is Axes limits and brings up a dialog box in which the user can enter any numerical values for the limits of the abscissa and ordinate axes to facilitate viewing of any region of the simulation.



The next group of four buttons, which act only on the time axis, allow the user to move left or right and to expand or reduce the scale used.



The third group of four buttons carry out the same function as the previous group, except that they act on the ordinate axis.

The buttons in the last group have the following functions:

The Combination of concentrations button allows mathematical combinations of the concentrations to be calculated. Clicking on this button invokes a dialog box in which can be written equations corresponding to the desired combina-

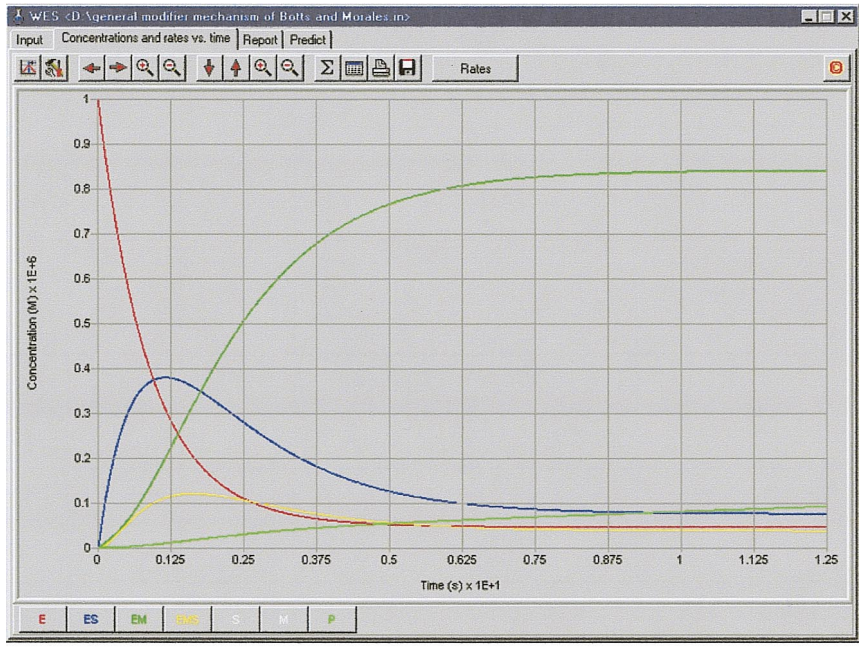


Fig. 2

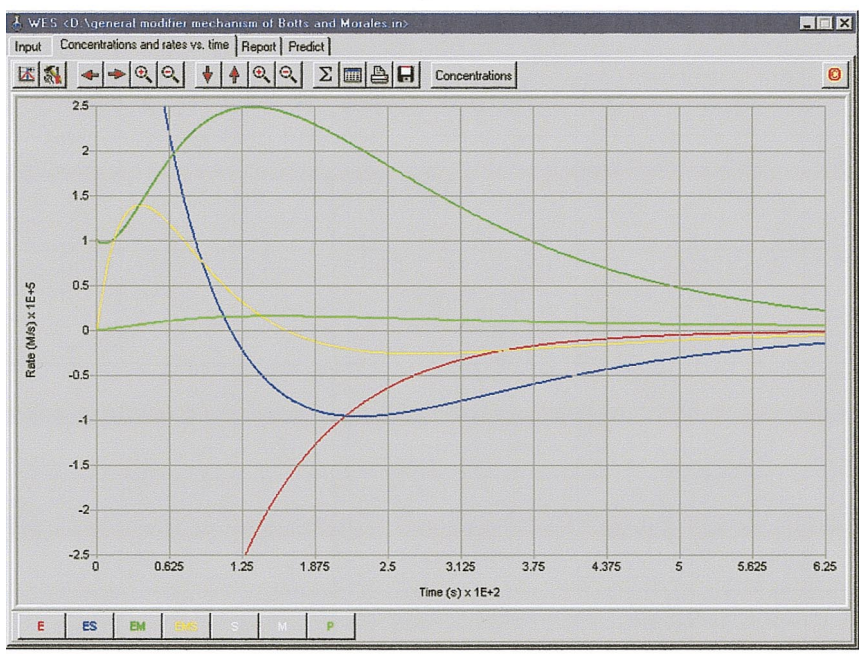


Fig. 3

Fig. 2. Simulated progress curves of the concentrations furnished by the program using the model defined in Fig. 1.
Fig. 3. Simulated progress curves of the rates furnished by the program using the model defined in Fig. 1.

tions. On the left hand side of each equation a name is assigned while on the right hand side is written the desired combination of concentrations following the same rules which were given to enter the right hand side of the differential equations. Thus, for example:

$$sum = [X1] + [X2] + 2*[X3]$$

creates a new quantity named sum that will be shown on the screen and can be manipulated as explained above for the other species.

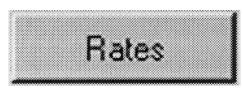
The Import button allows superimposition on the plot of an ASCII file containing experimental data or data resulting from another simulation. The file must consist of a minimum of two columns of which the first must be labelled as Time and must contain two or more time values. The remaining columns contain the corresponding concentrations and/or rates. Any columns containing concentrations must be labelled with the name given to the species written in square brackets, e.g. [X1], [X2], etc., while columns with labels that do not begin with the '[' character will be considered as columns of rates. An example of a valid data file to be imported is:

Time	[X1]	[X2]	RX1
0	0	1	1
1	1	0.9	2
2	2	0.8	2.3

Once the file has been imported, buttons corresponding to the columns of concentrations and/or rates are created automatically with the appropriate labels and plots of the data are added to the screen. For the buttons corresponding to information derived from the imported file there are four possible modes for representing the data: lines (solid or dotted), points (a circle around the datum), lines and points and, finally, 'no display'. When a species is not selected, then the label on the corresponding button will appear white, in the others modes a small graphic on the left side of the button indicates which display mode is used. Note that points, instead of lines, are used as the default option for the drawing of the importing data, although the user can change it by clicking on the button.

The Print button sends to the printer whatever is currently shown on the screen using the printer chosen as the default for Windows.

The Copy button creates a bitmap file, with a resolution 800×600 pixels and 256 colours, of whatever is currently shown on the screen.



Finally, clicking on the Swap button switches between plots of the concentrations and of rates for the selected species (Fig. 3).

4.5. Tabular output of the results

The third tab labelled Report provides some characteristics of the progress curves which can be of additional utility in kinetic data analysis and experimental design. In addition, the points defining each of the progress curves can be viewed in a table or be saved (Fig. 4).

- Tangent straight lines contains the values of the slope and ordinate intercepts of the straight lines tangent to each of the progress curves of the concentrations, at the initial and final integration times. In addition, the coordinates of the intersection between these two straight lines are given.
- Singular Points reports all maxima, minima and inflection points, within the simulation interval, of each of the progress curves of the concentrations ordered by species and time.
- Save/View allows the above data as well as the instantaneous concentrations and rates of all of

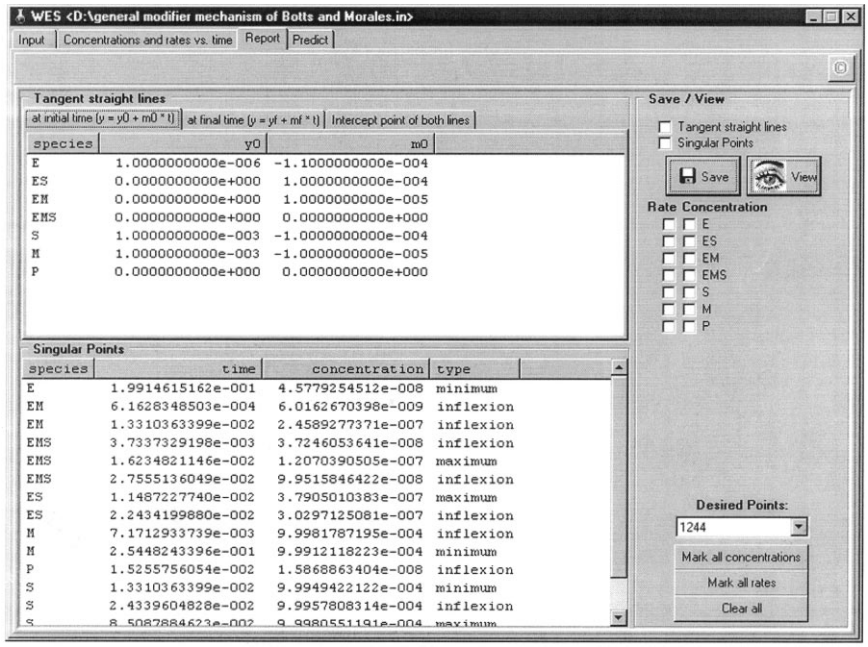


Fig. 4. Report screen corresponding to Fig. 1.

the species to be saved in an ASCII file for subsequent printing or analysis with other programs (spreadsheet, database, word processor, etc.). The user selects check boxes corresponding to the data to be included in the file, as well as the number of points that are to appear in the table. The number of points can range from all of the points calculated down to every tenth point. After the data desired have been selected, the user then clicks on the button Save.

The View button operates in a similar manner. After selecting the required concentrations and/or the rates using the check boxes, clicking on this button generates a table in which the number of points in each column will be reduced, if necessary, so that its size does not exceed 64 Kbytes, which is the usual limit for ASCII text editors.

4.6. Concentrations and rates at specific chosen times

The fourth tab labelled Predict has been implemented in order to obtain the value of concentrations and/or rates of any species at specific arbitrarily chosen times (Fig. 5).

First of all the user must enter in the window labelled as Desired times, the chosen time values (one per line) at which the concentrations and/or the rates of one or more of the species involved in the enzyme reaction are to be calculated. The chosen time values must be greater than or equal to t_0, i.e. the initial integration time. To reduce the CPU usage in the integration process for each desired time t_i, if $t_0 < t_i \leqslant t_f$ then the RKF algorithm is started with the concentrations already calculated for the greatest time $t_j < t_i$. On the other hand, if $t_i > t_f$ then the values for the greatest time calculated t_f are used. Once the times have been entered and the species have been selected, calculation is initiated by the Predict button. The Processing dialog box will be displayed as described in Starting the integration of the differential equations section above and the user may press the Cancel button before the integration is completed. When all points have been calculated the dialog box is hidden and the results are shown in the window labelled Concentrations and/or rates. The user can then save the contents of this window as an ASCII file by clicking in the Save button.

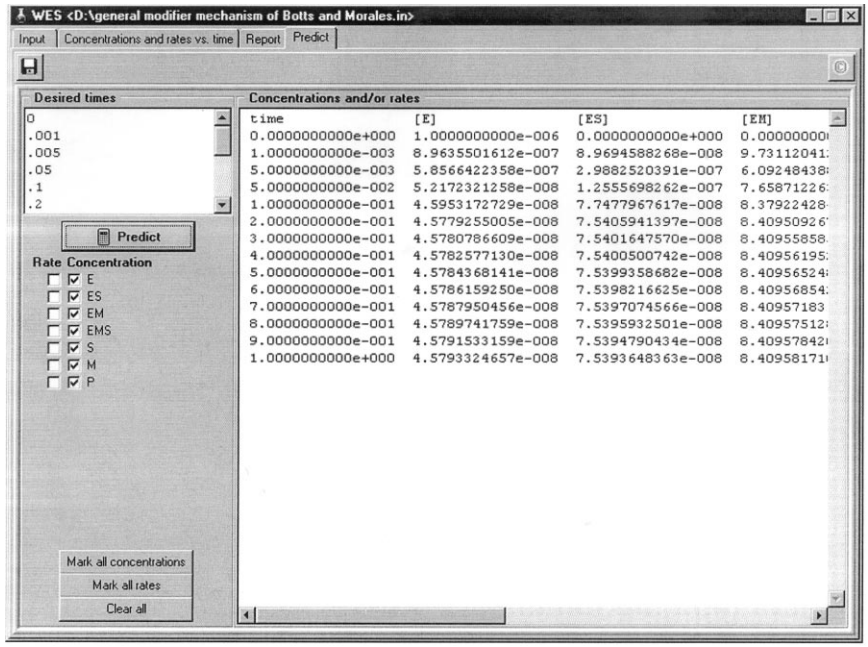


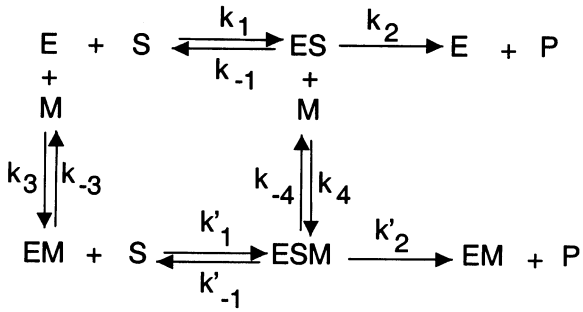
Fig. 5. A predict screen corresponding to Fig. 1.

5. Example

As an example, we will show how to simulate the enzyme reaction which evolves according to the well known general modifier mechanism of Botts and Morales (1953). This is given in Scheme 1, where S, M and P are the substrate, the modifier and the product, respectively, E is the free enzyme and ES, EM and ESM are the intermediate enzyme forms involved.

The system of differential equations describing the kinetics of this reaction is:

$$\left.\begin{aligned}
\frac{d[E]}{dt} &= -k_1[E][S] - k_3[E][M] + k_{-3}[EM] + (k_{-1} + k_2)[ES] \\
\frac{d[ES]}{dt} &= k_1[E][S] - (k_{-1} + k_2)[ES] - k_4[ES][M] + k_{-4}[EMS] \\
\frac{d[EM]}{dt} &= -k'_1[EM][S] - k_{-3}[EM] + k_3[E][M] + (k'_{-1} + k'_2)[EMS] \\
\frac{d[EMS]}{dt} &= k'_1[EM][S] + k_4[ES][M] - (k'_{-1} + k_{-4} + k'_2)[EMS] \\
\frac{d[S]}{dt} &= -k_1[E][S] - k'_1[EM][S] + k_{-1}[ES] + k'_{-1}[EMS] \\
\frac{d[M]}{dt} &= -k_3[E][M] - k_4[ES][M] + k_{-3}[EM] + k_{-4}[EMS] \\
\frac{d[P]}{dt} &= k_2[ES] + k'_2[EMS]
\end{aligned}\right\} \quad (2)$$



Scheme 1. General modifier mechanism of Botts and Morales.

5.1. Input of data:

The data input necessary to use the program here presented for simulation of this reaction will now be described.

In the window Description we may use any text but for the purposes of this illustration we will write:

General modifier mechanism of Botts and Morales

The differential equations must be written as:

$$D[E] = -k1*[E]*[S] - k3*[E]*[M] + km3*[EM] + (km1 + k2)*[ES]$$

$$D[ES] = k1*[E]*[S] - (km1 + k2)*[ES] - k4*[ES]*[M] + km4*[EMS]$$

$$D[EM] = -k'1*[EM]*[S] - km3*[EM] + k3*[E]*[M] + (k'm1 + k'2)*[EMS]$$

$$D[EMS] = k'1*[EM]*[S] + k4*[ES]*[M] - (k'm1 + km4 + k'2)*[EMS]$$

$$D[S] = -k1*[E]*[S] - k'1*[EM]*[S] + km1*[ES] + k'm1*[EMS]$$

$$D[M] = -k3*[E]*[M] - k4*[ES]*[M] + km3*[EM] + km4*[EMS]$$

$$D[P] = k2*[ES] + k'2*[EMS]$$

where the subindex m is used instead of the sign '-'.

The rate constants have been assigned the arbitrary values:

k1 = 1e5

km1 = 10

k2 = 1

k3 = 1e4

km3 = 5

k4 = 1e5

km4 = 100

k'1 = 0.5e4

k'm1 = 200

k'2 = 10

which are entered in the window Rate constants. Finally, in the Initial concentrations window we write the following arbitrary values for the initial concentrations:

[E] = 1e − 6

[ES] = 0

[EM] = 0

[EMS] = 0

[S] = 1e − 3

[M] = 1e − 3

[P] = 0

although the lines corresponding to ES, EM, EMS and P are not strictly necessary because the program would assume values of zero had they not been specified.

As units for the concentration and time we use the default values given by the program, i.e. M (molar) for the concentrations and s (seconds) for the time.

The integration period has been set from $t = 0$ to $t = 1$ second.

5.2. Graphical and tabular output:

Once the system has been integrated, the program produces the results that were shown in Figs. 2–5.

6. Results and discussion

In this contribution, we present a program that addresses the most important and frequent simu-

involving up to 32 species. Nevertheless, it could be modified easily for simulations involving a higher number of species; the limit of 32 species is a compromise between the RAM necessary for the storage of the curves and the number of species involved. It should be noted that 32 is the maximal number of curves that the program can simultaneously handle, i.e. if a reaction scheme consists of N species ($N \leqslant 32$) then the maximal number of curves arising from combinations and/or imported data can not exceed of $32 - N$.

The program provides information on the concentrations and rates of change for all reactants and enzyme intermediates, which can then be compared to experimental observations. In addition, the tangent straight lines and the singular points furnished in the Report tab can be used to formulate experimental designs and data analyses. Thus, for some enzyme systems, it is of interest to know the rate for one or more of the species involved at $t = 0$ (Espín and Tudela, 1993; Sculley et al., 1996; Wang et al., 1996). Also the tangent straight lines at the final integration time is of interest because it can furnish the steady-state rate and/or the induction period and, in some reactions involving inactivations or irreversible inhibitions, the final concentration of a product (Wang, 1990; Wang and Tsou, 1990). Likewise, the intersection point of the tangent straight lines at $t = 0$ and at the final reaction time may be also used in kinetic analyses (Zhao and Tsou, 1992; Zhao and Wang, 1996). More recently the singular points have been also used in enzyme kinetic analyses (Garrido-del Solo et al., 1994, 1996). An accurate knowledge of these straight lines and singular points allows a better comparison between a model of enzyme reaction and the possible derived analytical solutions. In any case, these reports given by the program are additional information that completes the quantitative simulation of the progress curves.

The program solves numerically the set of differential equations that describe the enzyme system from the individual rate constants and initial concentrations. Although it is designed to solve the set of differential equations that can be directly written from the reaction scheme, it can also be adapted to systems in which one or more of the reversible steps are at steady-state or are in rapid equilibrium. An example is the unstable alkaline phosphatase model described previously (Pike and Duggleby, 1987).

The underlying differential equations for this system are:

$$\left.\begin{aligned} \frac{\mathrm{d}[E']_t}{\mathrm{d}t} &= \frac{-k_1[E']}{1 + \dfrac{[A]_t}{K_a} + \dfrac{[P]_t}{K_p}} \\ \frac{\mathrm{d}[P]_t}{\mathrm{d}t} &= \frac{\dfrac{k_3[E']_t[A]_t}{K_a}}{1 + \dfrac{[\mathrm{A}]_t}{K_a} + \dfrac{[P]_t}{K_p}} \end{aligned}\right\} \quad (3)$$

and it should be recalled that the equation analyser requires that the differential equations are written in the general form:

D[Xi] = coef*[Xj]^Nj*[Xk]^Nk*... *[Xm]^Nm + ...

+ other possible terms like this one

where coef is an expression composed of numerical values and rate constants (ki or Ki), [Xi] is the instantaneous concentration of the species Xi and the exponents Nj, Nk,... can be any real number. It is therefore necessary to define a fictitious auxiliary species in order to write the differential equations in the required form:

D[E'] = −k1*[E']*[aux]^−1

D[P] = (k3/Ka)*[E']*[A]*[aux]^−1

D[A] = −(k3/Ka)*[E']*[A]*[aux]^−1

D[aux]

= −(1/Ka − 1/Kp)*(k3/Ka)*[E']*[A]*[aux]^−1

where

[aux] = 1 + [A]/Ka + [P]/Kp

In the section Algorithm we considered the limitations of the integration method used and saw that the integration process could need a very high CPU time or even the integration might fail what depends on the enzyme system under study, the final time for calculation of the simulated curves and the values assigned to the initial concentrations and the rate constants. Thus, if in the example corresponding to Scheme 1 the initial values of

[E], [S] and [M] and the k1-value are changed to 10^{-5}, 0.1, 10^{-6} and 10^{9}, respectively, then the program cannot carry out the integration.

It should be pointed out that this program is intended primarily to simulate enzyme reactions but it can equally well be used to simulate any other type of chemical reaction whose kinetics can be described by a set of differential equations that can be written in the syntax that is recognised by the program. Moreover, given that the program can handle up to 32 species, it can be used to simulate two or more independent systems simultaneously, or a single system for different values of the initial concentrations and/or rate constants. The versatility of the program is limited only by the needs and the imagination of the user.

Finally, the program has been tested and shown to be effective using many different systems other than the examples given here. Nevertheless, in order to improve future versions, we would appreciate any suggestion or comments from readers.

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