

A Computer Application to Simulate the Kinetics of Any Enzyme System

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Abstract. Almost all intra- or extra-cellular biochemical reactions are catalyzed by enzymes, hence the importance of studying the structural, functional kinetics of enzymes. This paper contributes to the use of numerical integration to study the kinetic behavior of enzyme reactions, and describes the implementation and management of a new computing environment called PM-w2011 to simulate the kinetics of any enzyme system. This program emphasizes the graphical presentation of progress curves and features numerous display options and different formats for generating graphics files. From the differential equations describing the enzyme system under study, this computer application performs their integration by numerical methods, covers most of the requirements needed to facilitate the tasks of those who investigate the kinetics of enzymes, and even allows simultaneous studies based on the variation of any system parameter. PM-w2011 has been developed using Microsoft's .NET (C# and C++) technology and has been designed for any current MS-Windows operating system version: XP, Vista or 7. It can be downloaded free from the following address: <http://oretano.iele-ab.uclm.es/~fgarcia/PM-w2011/>

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1. Introduction

The kinetics of an enzyme reaction is determined by a system of differential equations describing it. Generally, this differential equations system is not linear, therefore to obtain analytical solutions approximately, it should be linear and use reasonable assumptions as that are easy to obtain experimentally. Having been linearized, the system can be solved analytically by various mathematical methods, e.g., Laplace transform.

Regardless of whether the system is linear or not, it can always be solved through numerical integration. In this case however, the solutions obtained are not symbolic, but come in the form of tables and/or graphics. Using numerical procedures [1, 2], it is always possible to solve any differential equations system regardless of the solution being analytical or not. The solution is obtained through a set of concentration-time and/or rate-time value pairs that determine the kinetics of an enzyme system.

This contribution intends to describe not only the characteristics of the PM-w2011 computer application implemented for the numerical resolution of any linear or non linear differential equations system, but also the behavior kinetics of any enzymatic reaction.

The most important numerical integration application is to obtain the kinetics behavior of any enzyme reaction under any boundary conditions to provide simulated progress curves which, in certain cases, can act as experimental time progress curves. Moreover, other important numerical integration applications offer the possibility of: 1) checking the degree of validity of the approximate analytical equations by comparing the simulated progress curves with the curves obtained for an approximate analytical equations graph; 2) verifying the goodness of any experimental design and analyzing analytical equations-based kinetic data; 3) ascertaining the behavior of the reaction for any set of initial concentrations from the kinetic parameters values in an enzyme reaction.

Therefore obtaining simulated progress curves is a crucial point, and a fact that is subject to the availability of appropriate, efficient software. Regardless of whether the enzyme system to be solved is linear or not, for it to be useful to researchers in this field, any program should take into account enzyme systems' specific characteristics and provide convenient data input and result outputs. Several programs that can be used for the simulation or fitting of progress curves of

enzyme systems, or other related programs have been described [3-18]. Although these programs have proved most effective in the specific context for which they were designed, in our opinion none is straightforward for data entry and/or presenting results and, in general, they all require ample experience to be properly employed.

There are also mathematical software packages, such as Mathematica or Matlab which, in addition to many other features, allow differential equations systems to be solved numerically. However, their versatility makes them very complex and difficult to handle by users without advanced knowledge. In addition, they do not permit data input and output in a way to which a researcher tends to be accustomed to work in; furthermore, most require previous programming.

2. System and methods

Some of the most important features for the researchers who work in enzymology require this type of computer applications, such as:

- Concentration vs. time and rate vs. time curves of all the enzyme species involved in the reaction mechanism.
- Concentration and velocity curves of all the species involved in the reaction mechanism represented vs. the concentration of any other species, or even itself.
- Achievement curves of any combination of concentrations (such as sum, quotient, etc.).
- The ease to add or remove curves to/from the graph and its automatic scaling.
- The chance to save the curves requested in a graphic form and/or in the form of tables of values: concentration-time or rate-time.
- The possibility of varying one of the system parameters (initial concentration, rate constants, etc.) for a list of possible values by graphically comparing how this variation affects the concentration-time and velocity-time curves.

3. Algorithm

Several years ago, Garcia-Sevilla et al. [16] implemented a software called WES for MS-Windows 32-bit operating systems written in C and Delphi, which simulates reactions with up to 32 enzyme species and the variable step algorithm of Runge-Kutta-Fehlberg [19, 20]. Although in this application, which was designed for Windows, data input and the presentation of results were straightforward, it did not implement all the characteristics that would be desirable for a program of this type, some of which are listed above.

The overall objective of this paper is to present a computer application which, from the differential equations system that describes an enzyme system, performs their integration by numerical methods and also circumvents the problems presented by previous programs by covering most of the requirements needed to facilitate the tasks of those people who investigate enzyme kinetics. This general objective will be achieved through the following specific objectives:

- (1) Implement a different version of the Runge-Kutta algorithm with a variable stepsize in the C++ programming language [21]; then compare the computation time employed in the resolution and the results obtained when applied to an enzyme system with a low level of complexity.
- (2) Improve calculations accuracy by using the functions implemented in the GMP, MPFR and MPFRCPP libraries (<http://gmplib.org/>; <http://www.mpfr.org/>; <http://zedgraph.org/>).
- (3) Repeat the previous tests for more complex mechanisms, and select the algorithm and precision to obtain an admissible error in an acceptable execution time when simulating most enzyme systems of an average or high level of complexity.
- (4) Implement an intuitive graphical environment in C# that allows users to write the differential equations and values of the constant and initial concentrations in a text or copy from any other application using the same nomenclature normally used in the bibliography.
- (5) Expand the graphical environment to represent the progress curves calculated for both the concentrations and rates of all the species involved in the reaction mechanism.

- (6) Provide a graphical environment with more interactive benefits, such as automatic axis scaling, viewing area selection or exact value determination (x, y) which the user is concerned about.
- (7) Represent the concentrations and rates calculated not only vs. the time, but also vs. the concentration of any kind of simulated enzyme system.
- (8) Allow the results to be exported to different formats: graphics files, tables with plain text and/or printers.
- (9) Create the necessary routines to simultaneously simulate with different initial concentrations and/or the values of the system's rate constants, and to represent them all simultaneously, thus providing an easier interpretation of the simulation results.

4. Implementation

This computer application has been designed for the MS-Windows operating system in any of its current versions: XP, Vista or 7. The development environment, Visual Studio 2008 Professional, provided by Microsoft, has been employed, and the C++ language has been utilized to implement a dynamic link library (DLL) into which all the calculation routines have been introduced. It makes good use of modern technology (i.e., programming Microsoft .NET in C#) to create a graphical environment that interacts with the user and also uses the DLL library to perform the required calculations.

C++ is a programming language that allows objects management. Currently there is a standard, ISO C++, to which the majority of more modern compilers manufacturers have joined, which is the case of Microsoft Visual C++, that has been used in this work. One peculiar aspect of C++ is the possibility of redefining operators (operators overload) and the ability to create new types of data that perform as basic types. Calculation routines have been packaged in a DLL library, which may be used from any executable program regardless of the language used.

C# is an object-oriented programming language that has been developed and standardized by Microsoft as part of its .NET framework, which has been adopted as a standard by ECMA (the

European Computer Manufacturers Association) and ISO. Its basic syntax derives from C/C++ and uses the object model of the .NET framework, which is similar to Java but includes improvements from other languages (including Delphi). The most recent version, 4.0, which is that employed in the final compilation of the application herein described, appeared on April 12, 2010. Although C# is part of the .NET framework, it is an Application of Programming Interface (API), while C# is an independent programming language that has been designed to generate programs in this framework.

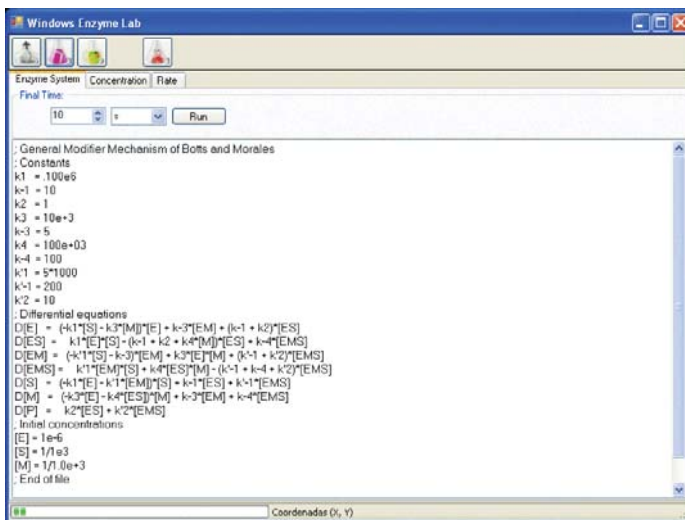


FIG. 1. Screenshot corresponding to the main window of the graphical environment implemented: tab for data input with a description of the mechanism of Scheme 1.

The graphical environment of the application, which is accessed by an executable file (EXE) that presents the main screen to the user, has been designed simply and intuitively. This main screen presents three tabs: one for data input (Figure 1) and the other two to graphically represent progress curves as concentrations or rates, respectively.

To help the user find the option concerned, there is a rapid access button bar located at the top of the window which allows access to all the program functions and shows only the buttons that are useful for each time. In addition, there is a status bar at the bottom of the window showing the status of the window.

4.1. Installation

The application does not need to be installed; the user simply copies the executable program (using the .exe extension) and the library file (using the .dll extension) in the folder required. Bear in mind that it is recommended to enable the MS-Windows operating system upgrades. If the system uses does not use the updated .NET library provided by Microsoft, the program may not carry out graphical representations correctly. In this case, it is necessary to download any updates.

4.2. Data Input

There are three kinds of data required to describe the kinetics of an enzyme system: rate constants, differential equations and initial concentrations; these are entered from the keyboard into the same main application window by selecting the “*Enzyme System*” tab (Figure 1) which appears by default to start the implementation. It is also possible to use not only the MS Windows operating system’s copy and paste options, but the text written beforehand with other applications, such as notepad or any other usual text editor. The syntax to be used for correct data input interpretation is that described below.

Rate constants

The definition of a (rate or not) constant should match the following syntax:

```
identifier = value | expression
```

where the identifier is a string of characters and/or digits that must begin with a letter, followed by the = symbol, and then by the numerical value assigned, or an algebraic expression of another constant defined in advance. For example, constant k_1 with a value of 10^5 could be defined by any of the following expressions:

```
k1 = 100000
```

```
k1 = 1e5  
k1 = .1e6
```

or we can use scale factors and also write them as follows:

```
kilo = 1000  
k1 = 100*kilo  
or, if preferred:  
kilo = 1e3  
mega = kilo*kilo  
k1 = mega/10
```

For the specific case of the rate constants (only those whose notation begins with k are considered as such), the characters prime ('), second (''), more (+) and less (-) may form part of the constant's name. In this way, the same notation used in the literature can be employed when reading and writing equations. Some examples of rate constants definitions in which these symbols are used are:

```
k'1 = 1e5  
k+3 = 10e+3  
k'-2 = 1e3/5  
k''5 = 2*(k'-2)/10
```

If the notation of any of the rate constants of the mechanism that simulates fails to conform to the syntax described above, it should be modified so that the expressions introduced into the program are interpreted correctly by the application. It should be noted that the concentration unit in the numerical values of the second-order constants must be molar, M.

Differential equations

To represent the concentration of a species, X, which is customary in the literature, the characters “[” and “]” are used. Therefore, the X concentration is written as [X]. Likewise for the time derivative of a concentration, we use the letter D (uppercase); in this way, D[X] means the time derivative of the concentration of species X. By bearing this in mind, all the system's differential equations were introduced into a different line with the syntax:

```
D[species] = differential equation
```


where the letter D must be written at the beginning of the line, while the name assigned to the species can contain letters and numbers, but must begin with a letter. Note that the implementation distinguishes between uppercase and lowercase; therefore, [X] and [x] will be taken into account as concentrations of different species: X and x. In the algebraic expression of the differential equation, the rate constants defined in advance may appear, and the concentrations of the species involved next to the operators add (+), subtract (-), multiply (*), quotient (/) and power (^) will be evaluated in the normal order of priority, in addition to the bracket needed to change priority. By way of example, the expression of three differential equations appears below: bear in mind that constants k1, k-1, k2, k3, k-3, k4 and k-4 must be defined in advance. The first of the three differential equations means that we wish the concentration of species X1 to remain constant during the simulation time.

```
D[X1]=0
D[X2]=(-k1*[X2]-k3*[X3])*[X1]+k-3*[X4]^2
D[X3]=(k-1+k2+k4*[X2])/[X4]+k-4*[X5]^-1
```

The text string used to describe the species can only contain letters (upper or lower case), digits and the underscore character “_”. If the notation of any of the species involved in the scheme of the enzyme reaction to be simulated does not conform to this syntax, it will be necessary to modify this notation, as indicated for the rate constants.

Initial concentrations

To establish the boundary conditions, the concentration values of all species that take t=0 which participate in the reaction mechanism should be written, even though it is not necessary to indicate those species whose initial concentration is zero. The syntax used is the same as that explained above for the rate constants except, in this case, for the initial concentration of species X that must be represented by [X] following the nomenclature used for instantaneous concentrations:

```
[species] = value | expression
```

Below several examples have been written which illustrate different ways of introducing the initial concentrations values:

```
kilo = 1000
```

```
[E] = 1e-6  
[S] = 1/kilo
```

It should be noted that although both notations match, when [X] appears to the left of the equal sign (=), it refers to the initial concentration of species X, yet when [X] appears to the right of the equal sign in the differential equation expression, it denotes the species instantaneous concentration. The units of all the numerical initial concentrations values must always be molar, M.

Comments

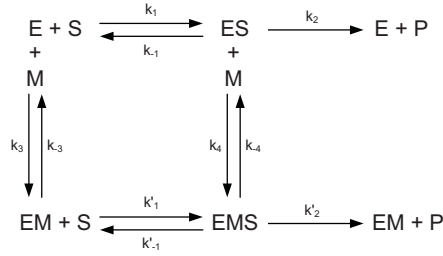
To perform any annotation of interest within the window upon defining the enzyme system to be simulated, a semicolon (;) should be used at the beginning of the line; in this way, the analyzer will ignore it completely. It is also possible to leave one or several blank lines to help read input data, and the use of several spaces or consecutive tabs to separate the fields of each data type is even possible as these characters are ignored by the application before the lexical analysis is carried out.

Final integration time selection

Finally, after performing the data input needed to describe the differential equations system, the user merely has to select the final simulation time and the units in which it is expressed in the top panel located below the button bar. The default value offered by the program is 10. It is imperative that the time unit selected in this paragraph is the same as that used when introducing the rate constants values. Otherwise although the integration process is implemented, the results will not be correct.

Complete example

The mechanism of Botts and Morales [22] explains how the PM-w2011 program is managed. The reaction scheme of this mechanism is represented in Scheme 1, while the corresponding differential equations system is described in Eqs. (1)-(7).



SCHEME 1. *The general modifier mechanism of Botts and Morales*

$$\frac{d[E]}{dt} = -k_1[E][S] - k_3[E][M] + k_{-3}[EM] + (k_{-1} + k_2)[ES] \quad (1)$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES] - k_4[ES][M] + k_{-4}[EMS] \quad (2)$$

$$\frac{d[EM]}{dt} = -k'_1[EM][S] - k_{-3}[EM] + k_3[E][M] + (k'_{-1} + k'_2)[EMS] \quad (3)$$

$$\frac{d[EMS]}{dt} = k'_1[EM][S] + k_4[ES][M] - (k'_{-1} + k_{-4} + k'_2)[EMS] \quad (4)$$

$$\frac{d[S]}{dt} = -k_1[E][S] - k'_1[EM][S] + k_{-1}[ES] + k'_{-1}[EMS] \quad (5)$$

$$\frac{d[M]}{dt} = -k_3[E][M] - k_4[ES][M] + k_{-3}[EM] + k_{-4}[EMS] \quad (6)$$

$$\frac{d[P]}{dt} = k_2[ES] - k'_2[EMS] \quad (7)$$

; General Modifier Mechanism of Botts and Morales

;

; Constants

k1=1e5

k-1=10

k2=1

```
k3=1e4
k-3=5
k4=1e5
k-4=100
k' 1=5e3
k' -1=200
k' 2=10
;
; Differential equations
D[E] = (-k1*[S]-k3*[M]) * [E] + k-3*[EM] + (k-1+k2) * [ES]
D[ES] = k1*[E] * [S] - (k-1+k2+k4*[M]) * [ES] + k-4*[EMS]
D[EM] = (-k' 1*[S]-k-3) * [EM] + k3*[E] * [M] + (k' -1+k' 2) * [EMS]
D[EMS] = k' 1*[EM] * [S] + k4*[ES] * [M] - (k' -1+k-4+k' 2) * [EMS]
D[S] = (-k1*[E]-k' 1*[EM]) * [S] + k-1*[ES] + k' -1*[EMS]
D[M] = (-k3*[E]-k4*[ES]) * [M] + k-3*[EM] + k4*[EMS]
D[P] = k2*[ES] + k' 2*[EMS]
;
; Initial concentrations
[E]=1e-6
[S]=1e-3
[M]=1e-3
; End
```

4.3. Beginning the integration process

After completing the necessary data input, pressing the button labeled “Run” starts the differential equations integration process. The computing time depends on the power of the CPU used, the end time and the complexity of the system under study. During this process, a progress bar is displayed at the bottom of the window showing the percentage of integration performed. The calculation can be stopped at any time by pressing the upper right button labeled “Stop”. The calculated values will not be lost and the results generated until that time will be displayed. Once the integration has reached the end time chosen by the user (or when the “Stop” button is

pressed), the application automatically switches to the “Concentration” tab to show the concentration progress curves for all the species involved in the reaction mechanism.

4.4. Graphic representation of the results

To display the graph with the rates of the species involved (Figure 2), the user must change the active tab by pressing on the “Rate” tab. The user may choose (in the left-hand side panel of the screen) the individual curves (of concentration and/or rate) to be included in or removed from the graphic panel. There is also a button labeled “Mark All/None” which selects all the curves, or none, instead of having to mark or unmark each one individually.

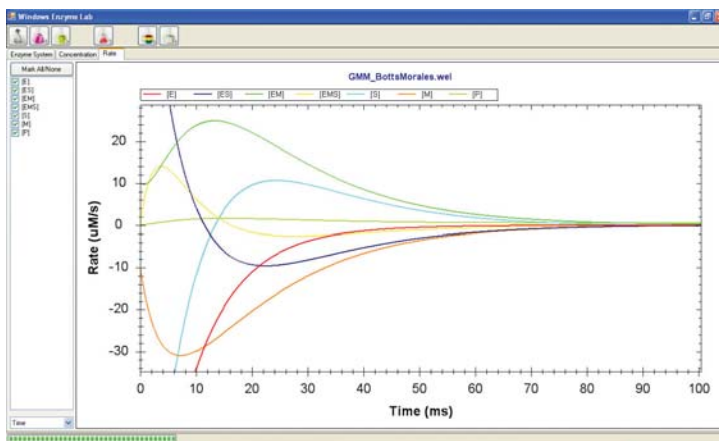


FIG. 2. Example of rate-time curves of progress. The axis scale has been changed to assess the evolution of the species velocities involved with small rate values. Related to the mechanism of Scheme 1.

The enlargement option (*zoom*) of a particular area of the graph has been implemented without using any button or menu option. To change the axis scales, the user simply has to use the mouse to select the area of the graph to be expanded by pressing the principal mouse button on any of the corners of the region of interest by dragging diagonally to the opposite corner. Having

enlarged the axis scale of the desired value, it is also possible to shift the visible area of the curves by simultaneously pressing the main mouse button and the “CTRL” key to locate the area of the curves of interest. The rest of the graphic features are accessed via a pop-up menu (Figure 3).

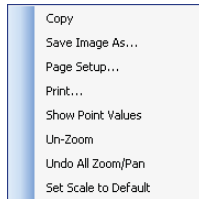


FIG. 3. *Options menu in the graphic panel.*

The pop-up menu appears on the screen by pressing the secondary button of the mouse when it is located within the graphic panel. The options available, which appear in the same order as they do in the menu, are as follows:

- Copy the graph displayed on the screen at the time to the Windows clipboard.
- Save the image shown as a graphic file. The user can choose from among the following graphic formats: EMF, PNG, GIF, JPEG, TIFF or BMP.
- Configure the page size, guidance and margins to be used by the printer.
- Print the curves as they appear on the screen at the time.
- Show the values (x, y) of the points calculated. Curves are drawn with a continuous line but, if the user wishes to ascertain the value of one of the points calculated in the integration process, this option can be enabled by placing the mouse over them, and a small window opens with their coordinates values. Note also that when the mouse cursor is within the area bound by the axis of the abscissas and ordinates, the program shows the coordinates (x, y) from that point in the status bar.
- Undo the last zoom. The display returns to the situation which it was in before being enlarged.

- Eliminate all the enlargements (*zoom*) and screen movements (*pan*) performed until that time.
- Adjust the axis scale to show all the points calculated in all the progress curves selected in the left-hand panel at the same time.

By default, the concentrations and rates obtained after integrating the differential equations are represented initially vs. time. However, the user can change the variable and, in addition to time, may choose to represent the concentration of any of the species involved in the mechanism under study on the horizontal axis. This task is done by the drop-down list box at the bottom of the panel in which the curves to be represented are selected. Figure 4 depicts the rate curves in which the S concentration has been used for the horizontal axis.

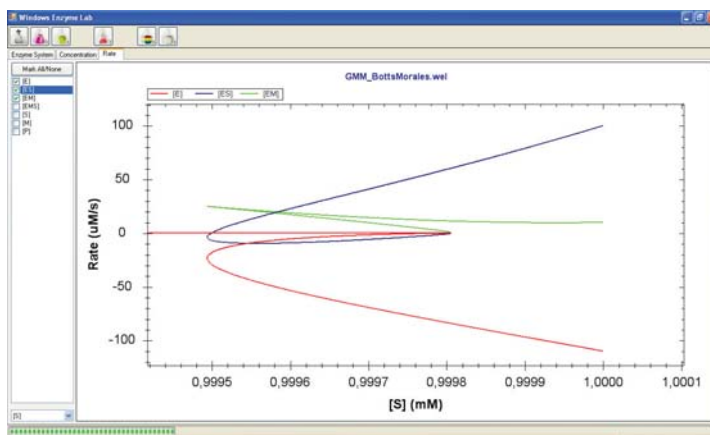


FIG. 4. Example of rate curves. Only three curves have been chosen for a better view: $[E]$, $[ES]$ and $[EM]$. Related to the mechanism of Scheme 1.

4.5. Results in a table form

The application also implements the possibility of exporting the numerical values of the concentrations and rates achieved to a plain text file (ASCII) by integrating differential equations numerically. For this reason, once they have been selected in the tabs for the concentration and/or

rate curves of the desired species (not necessarily the same in both tabs), press the “*Export to ASCII*” button. Then, introduce a name to identify the file where the progress curves numerical values are to be saved. The desired data are saved in columns and this implementation also adds a first column with the time values to this table.

4.6. Graphics of new concentrations that are functions of others

One of the most important features is the ability to graphically represent any mathematical function of the concentrations obtained after the integration process. This characteristic is implemented in the application by means of the quick button in the upper bar labeled “*Add Curves*”.

The syntax and mathematical operations available are the same as those described in Paragraph 4.2 for differential system equations input; that is:

[name] = function of the concentrations

where [name] identifies the concentration of the new expression introduced. To describe the function of the concentrations, the operators sum (+), rest (-), product (*), quotient (/) and power (^) can be used, and the expression will be evaluated in the usual order of priority, in addition to the brackets required to modify priority. It should be noted that once new identifiers are embodied in the graphic panels, they are treated as the species listed in the differential equations system mechanism; therefore, they may be subsequently used to export their values to an ASCII table and/or to create other new identifiers from them. As previously indicated, the application handles these new identifiers in the same way as the species involved in the reaction mechanism; therefore, it allows to also view in the “*Rate*” tab the derivative of the expressions introduced in relation to the time.

4.7. Effect of varying the kinetic parameters

One very interest aspect for researchers in enzymology is this software’s ability to compare the results for the same mechanism described by the same differential equations system for the different values of the initial concentrations and/or rate constants. For instance, this allows us to confirm or reject hypotheses that predict the kinetics behavior of the system being studied under

different conditions. For this purpose, we have added the possibility of varying any system parameter by sequentially taking all the values containing a list of the parameters which were previously introduced by the user. This will facilitate the study of the different values of rate constants or of the initial concentrations of any species in the mechanism. For example, to carry out a study of three different values of constant k_1 :

```
k1 = {10; 200; 1000}
```

where, as shown, the different values of k_1 have been written between brackets $\{\}$ and separated by a semicolon (;). If, instead of a rate constant, the user wishes to study the effect of varying an initial concentration, proceed in the same way: e.g., the notation

```
[S] = {1e-3; 2e-3; 5e-3}
```

indicates that the application must be done in three consecutive studies in which the initial concentration of species [S] takes the values 10^{-3} , $2 \cdot 10^{-3}$ and $5 \cdot 10^{-3}$, respectively. To distinguish which study is the rate or the concentration being represented, the program adds an underscore to each species' name, followed by a number of order that indicates the study to which it belongs: 1 for the first study, that is, the first value on the list; 2 for the second, etc.

5. Conclusion. PM-w2011 is a new computing environment which greatly facilitates many tasks performed when studying an enzyme system. We emphasize its powerful graphic qualities and the possibility of carrying out different simulations by simply varying the kinetic parameters. We also highlight its easy installation and availability since the program can be downloaded without charge from the following Internet address: <http://oretano.iele-ab.uclm.es/~fgarcia/PM-w2011/>.

In our view, this program improves and completes previous computer applications for obtaining simulated progress time curves and other graphic representations. In the near future, we hope to incorporate into the program the possibility of obtaining concentration-time symbolic equations through the analytical integration of the corresponding system of previously linearized differential equation from the enzymatic system of interest. This will enable us to compare the goodness of the approximate analytical equations obtained by comparing their numerical

integration. Our research group is currently working in this direction in an attempt to draw the right conclusions.

The authors would appreciate the opinions and suggestions made by the PM-w2011 program users as they may be taken into account in new versions.

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