# Graphical spreadsheet tools for learning enzyme kinetics

Simon Brown and David C. Simcock

***Abstract*—Many students lack sufficient mathematical skill or confidence to be able to visualise a process just by looking at an equation. This is particularly apparent when students are working on the dynamics of enzyme action. Understanding the basic concepts of enzyme kinetics is essential to any appreciation of the chemical processes that occur in living organisms. Fortunately, learning can be enhanced by direct manipulation of the parameters while students observe the behaviour of the graphs of the functions. Spreadsheets are described that enable students to analyse the significance of the parameters at several levels, to connect concepts between levels of treatment and to use the basic expression in the analysis of their own experiments. Methods for constructing these spreadsheets in Excel and the essentials of the relevant theory are also summarised.**

***Keywords*—dynamics, enzyme kinetics, nonlinear regression, spreadsheet.**

## I. Introduction

Undergraduate students in the biological sciences often lack confidence in their own mathematical skills which may not be strong [1-3]. Some even find it difficult to understand simple fractions. For example, interpretation of the effects on the magnitude of a fraction of changing the denominator can seem mysterious [4]. This means that something as apparently simple as concentration (the quantity of a solute per litre of solution) is not understood [5-7]. Such problems have been reported often [8-10], but their implication is that it can be difficult to ensure that students really understand even relatively simple concepts if they are described mathematically. This is exacerbated by the reliance of biochemistry on a very wide range of physical, chemical and biological concepts [11], some of which may themselves be expressed mathematically. This underpins the search for practical means to help students comprehend ideas of this sort while bridging the gap to their formal expression. Analogies can be helpful [12], but they require careful consideration [13]. One approach that has proven to be useful is simulation using Microsoft Excel [14]. While this software has various problems [15], it is available on almost every computer and has some attractive capabilities.

In an attempt to provide alternative means of describing such ideas, a suite of Excel spreadsheets has been developed in which the student can (a) manipulate the parameters and observe the consequences, (b) plot their own data and superimpose the appropriate curve and (c) fit the curve to their data using nonlinear least squares regression, if appropriate. Other capabilities could easily be incorporated into the modules should the need arise. The two main advantages of these tools are that both student and teacher can use them at any time, in or out of class, and the teacher does not need very much time to develop each new tool.

Here we describe an example of the use of Excel in this way to help students appreciate the dynamics of enzyme action. Enzymes are proteins that catalyse the chemical reactions on which life depends. Each enzyme is essentially a molecular machine that converts specific raw materials (usually called substrates) into products [12]. The dynamics of the network of reactions occurring in every cell are largely determined by the kinetics of the enzymes in the network. Given this, an understanding of enzyme kinetics is central to an appreciation of the dynamics of the processes that occur within every cell.

Naturally, this is not the first time that spreadsheets have been used to assist students with this. For example Bruist [16] outlined the use of spreadsheets to teach the process of simulating enzyme kinetics to advanced students, but his approach was more mathematically demanding and more limited in scope than that employed here, and did not employ the graphical interface tools available in Excel. Here, we describe (a) the background theory, (b) the steps needed to develop a module and (c) examples of the sorts of modules we have developed to enhance the learning of basic enzyme kinetics.

## II. Background

### A. Michaelis-Menten kinetics

Enzymes are proteins that catalyse the conversion of one or more chemical compounds (the substrates) to one or more others (the products) according to a specific chemical mechanism. Based on this mechanism an expression can be derived for the steady-state rate at which substrate is converted to product [17]. The simplest example is the Michaelis-Menten mechanism

$$\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \qquad (1)$$

[18] in which the enzyme (E) catalyses the conversion of one substrate (S) to one product (P). In the process, the substrate

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binds to the enzyme to form an enzyme-substrate complex (ES). The double-headed arrow indicates that the binding of S to E is reversible, whereas the single-headed arrow indicates that the release of P is not reversible. The $k_i$s are the rate constants for the individual reactions in the mechanism: $k_1$ for S + E → ES and $k_{-1}$ for the reverse of this step, and $k_2$ (which is often designated $k_{cat}$ in the literature, where 'cat' stands for 'catalytic') for ES → E + P. The rate of the reaction ($v$) is the rate at which P accumulates, which is proportional to the concentration of ES.

The Michaelis-Menten mechanism (1) implies that as the concentration of S ($s$) declines, the concentration of P ($p$) increases and the concentration of ES ($c$) increases and then falls. The dynamics of the model are captured by

$$\frac{ds}{dt} = \left(k_1 s + k_{-1}\right)c - k_1 \mathrm{E_t} s \tag{2a}$$

$$\frac{dc}{dt} = k_1 \mathrm{E_t} s - \left(k_1 s + k_{-1} + k_2\right)c, \tag{2b}$$

where $\mathrm{E_t}$ is the total enzyme concentration, $s(0) = s_0$ and we assume that $c(0) = 0$. The usual quasi-steady state assumption is that $dc/dt = 0$ [19], so $c$ can be obtained from (2b) and then substituted into $v = k_2 c$ to yield

$$v = \frac{V_{\max}\, s}{K_{\mathrm{m}} + s} \tag{3}$$

where

$$V_{\max} = k_2 \mathrm{E_t} \tag{4}$$

is the highest value of $v$ which would be reached if all of the enzyme were in the form of ES, and the Michaelis constant

$$K_{\mathrm{m}} = \frac{k_{-1} + k_2}{k_1} \tag{5}$$

is the concentration of S at which $v = ½V_{\max}$.

The quasi-steady state assumption (that $c$ is constant) is valid if the initial substrate concentration ($s_0$) is much greater than $\mathrm{E_t}$, but it becomes less appropriate as $s_0$ approaches $\mathrm{E_t}$ [20]. Experimentally, $s_0$ and $\mathrm{E_t}$ are chosen so that (3) applies and estimates of $K_m$ and $V_{\max}$ are used to characterise each substrate and enzyme pair. However, both $K_m$ and $V_{\max}$ depend on the conditions used in the measurement of $v$. For example, both $v$ and $V_{\max}$ are proportional to $\mathrm{E_t}$, but $K_m$ is a property of individual enzymes and does not change with $\mathrm{E_t}$, but may change with pH, ionic strength or temperature.

The dynamics of S, P, E and ES can be calculated by numerical integration of (2) or by approximation [21]. Alternatively, (3) can be integrated to calculate the approximate substrate concentration $s(t)$ at a specific time ($t$) during the course of a reaction. This yields the well known integrated rate expression

$$s(t) - s_0 + K_m \ln\frac{s(t)}{s_0} = -V_{\max} t \tag{7}$$

which can be written as

$$s(t) = K_m \mathrm{W}\left[\frac{s_0}{K_m}\exp\left(\frac{s_0 - V_{\max} t}{K_m}\right)\right], \tag{8}$$

where $\mathrm{W}(\cdot)$ is the Lambert W function which is the solution to $\mathrm{W}\exp(\mathrm{W}) = x$ [22]. The Lambert W function is not an intrinsic function in Excel, although it has been argued that it should be [23] and certainly it has considerable potential in (bio-)chemistry. There is a reliable numerical algorithm [24] that can be implemented in VBA with little difficulty.

### *B. Enzyme inhibition*

Enzyme activity can be decreased by specific compounds that may or may not share some of the molecular features of S. A compound of this type is known as an inhibitor (I) because it can bind to the enzyme, but cannot be converted to P. In the presence of an inhibitor either or both of $K_m$ and $V_{\max}$ may change depending on how I interacts with the enzyme. The inhibitor may compete with S for the enzyme (forming the enzyme-inhibitor complex IE, competitive inhibition), bind only to ES (forming IES, uncompetitive inhibition) or either (mixed inhibition) as shown in Figure 1. Since neither IE nor IES is converted to P, the rate of the reaction is reduced compared to that in the absence of inhibitor at any $s$ (Figure 2).

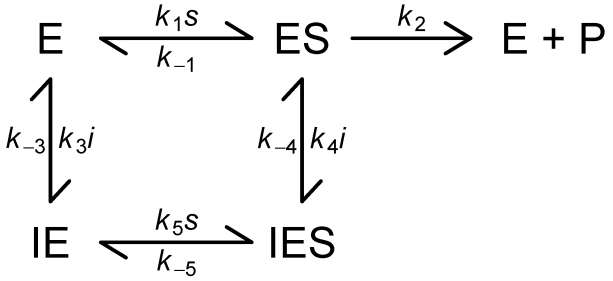


Figure 1. A model of the inhibition of a standard Michaelis-Menten enzyme (note that the top line is the same as (1)). The $k_i$s are the rate constants for each reaction and $i$ is the inhibitor concentration. In some representations of this mechanism, P can be formed from IES, but this is omitted here. The direct interconversion of IE and IES need not occur, but, if it does, $K_5 = k_{-5}/k_5 = K_1K_4/K_3$.

The model shown in Figure 1 yields three modified Michaelis-Menten equations

$$v = \frac{V_{\max}\, s}{\left(1 + i/K_3\right)K_{\mathrm{m}} + s}, \tag{9}$$

$$v = \frac{V_{\max}\, s}{K_{\mathrm{m}} + (1 + i/K_4)s} \tag{10}$$

and

$$v = \frac{V_{\max}\, s}{(1 + i/K_3)K_{\mathrm{m}} + (1 + i/K_4)s} \tag{11}$$

corresponding to competitive, uncompetitive[1] and mixed inhibition, respectively [26, 27]. In (9-11) $i$ is the concentration of the inhibitor (I), $K_3 = k_{-3}/k_3$ and $K_4 = k_{-4}/k_4$ are the dissociation constants of IE and IES, respectively, and $V_{\max}$ and $K_m$ are defined in (4) and (5), respectively.

The effect of an inhibitor on the relationship between $v$ and $s$ depends on how that inhibitor interacts with the enzyme (Figure 1). Competitive inhibitors increase the apparent $K_m$, but do not alter the $V_{\max}$, whereas uncompetitive and mixed inhibitors may alter the apparent $K_m$ and decrease the $V_{\max}$ (Figure 2).

[1] While uncompetitive inhibition is rare [25], it is logically inescapable and is usually included.

### C. Allosteric kinetics

While (1) is a good model for the kinetics of many enzymes, others require quite different treatment. For these, without repeating the underlying theory [28], a plot of $v(s)$ is not the rectangular hyperbola described by (3) and plotted in Figure 2, but a sigmoidal curve. This is often approximated by

$$v = \frac{V_m s^n}{K_{0.5} + s^n}, \qquad (12)$$

where $K_{0.5}$ and $V_m$ are constants analogous to $K_m$ and $V_{max}$, respectively, in (3), and $n$ (which need not be an integer) is the Hill coefficient that is a measure of interaction between those sites on the enzyme at which S binds. The effect of increasing $n$ is to render the curve more sigmoidal, such that $v$ remains very low even in the presence of appreciable $s$, but rises steeply above a specific value and then saturates as $s$ increases further (Figure 3). This property is characteristic of those enzymes that are important control points in the metabolic network or in the cascades of reactions commonly found in signal transduction pathways.

### D. Other models

The models described above are those usually considered in undergraduate courses, but it will be apparent that many others are possible [17]. To take one very simple example, in (1) there is no explicit step in which S is converted to P. The introduction of this step (which might be ES → EP or ES ↔ EP) would further complicate (2). We have also ignored those enzymes that have multiple substrates, in part we feel that this decision is justified by the common practice of using (3) to analyse the kinetics of enzymes of this type [29, 30], even when other models are appropriate [31]. As the models become more elaborate, the likelihood of complex behaviour increases [32, 33] and it is rarely necessary to expose undergraduate students to such intricacies. However, it would not be difficult to develop specific examples of these using the spreadsheet approach described here.

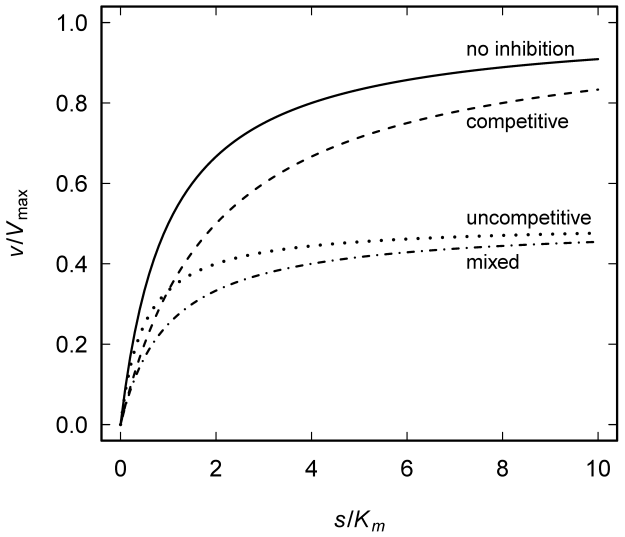


Figure 2. The dependence of the relative enzyme activity ($v/V_{max}$) on the relative substrate concentration ($s/K_m$) in the absence (———, (3)) or presence of a competitive (– – –, (9)), uncompetitive (· · · ·, (10)) or mixed (– · – · –, (11)) inhibitor (I). Where appropriate, it was assumed that $i/K_2$ = 1 and $i/K_3$ = 1.

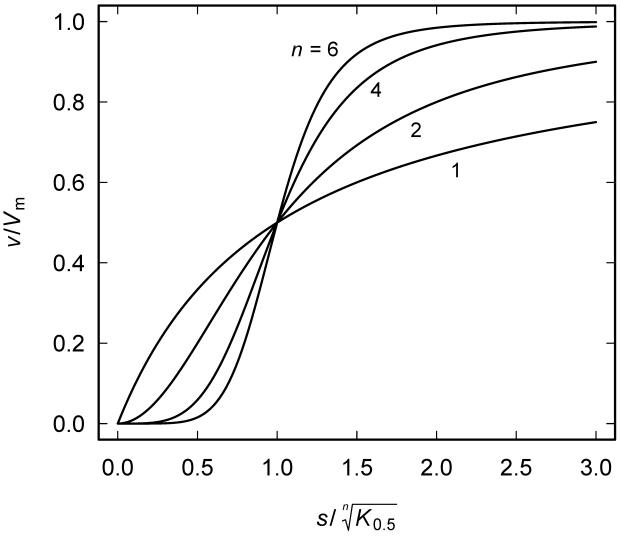


Figure 3. The dependence of the relative enzyme activity ($v/V_m$) of an allosteric enzyme on the relative substrate concentration (12) for $n$ = 1, 2, 4 or 6.

## III. Spreadsheet development

The first step in each of these examples is the preparation of the spreadsheets. This involved the use of the scroll bar tool that is available in Excel to change dynamically the value of various parameters and simultanously alter the theoretical curve (sometimes superimposed on experimental data) plotted on a graph. To make the structure of the spreadsheets entirely accessible to those who are interested, the calculations can usually be seen to the right of the intial view (starting in the 27th column of each spreadsheet).

### A. Preparation of the spreadsheets

There is a growing literature on the animation of functions in Excel [34, 35], but the precise instructions depend on which version of Excel is used. For example, a scroll bar can be inserted using (i) the control toolbox which can be selected under the View/Toolbars menu in Excel 2003 or (ii) the Developer/insert menu in Excel 2010 or Excel 2016. To insert the various controls in Excel 2003 it is necessary to be in 'design mode', which can be achieved by clicking on the setsquare on the control toobox (this is automatically activated when a scroll bar is inserted in Excel 2010). The scroll bar icon on the control toolbox is a pair of opposed carets (∧ and ∨) or triangles (▲ and ▼) in grey squares one above the other, separated by a white space. Press this icon and use the mouse to drag out a scroll bar directly on the worksheet (this can be resized and moved easily). The scroll bar has an integer value, corresponding to the position of the grey scroll box along the length of the scroll bar, that can be written into a linked cell on the spreadsheet. The linked cell can be specified on the properties menu (available by right clicking on the scroll bar), as can the maximum (max) and minimum (min) values. The value in the linked cell is updated as the scroll bar is manipulated (depending on the version of Excel, you may need to turn off 'design mode' to do this) and can be used to calculate a particular parameter

(such as the $V_{max}$ or $K_m$) which then changes dynamically with the movement of the scroll bar.

### B. Nonlinear regression

It is a relatively simple matter to implement the VBA code for nonlinear least squares regression [36] to provide a reasonable fit of the theoretical expression ((3), (7) or (8)) to data inserted into the spreadsheet. Several sources provide code [37-40] that can be adapted to the constraints of Excel, however Duggleby's [37] implementation was used in this instance. This is especially simple and clear code and any interested student could easily work out what is happening.

The software requires an initial estimate of each of the parameters from which the search for the best fit starts. These initial estimates are obtained from the values determined by the user's manipulation of the scroll bars on the spreadsheet. The estimated best fit parameters are use to reset the settings of the scroll bars, which changes the values of the parameters and the graph is updated accordingly. The maximum number of iterations is limited arbitrarily to 50 which is usually sufficient, but, of course, the user can use the output of one regression cycle as the input to the next.

No values of the uncertainties of the parameter estimates are given, although it is a very simple matter to calculate them and write them to the spreadsheet. The comments towards the end of the VBA code in the spreadsheet provide the necessary instructions. From these, it is possible to estimate a confidence band for $v(s)$ [41].

### C. Data transformation

It is well known that (3) and (9-11) can be transformed so that a graph of $1/v$ against $1/s$, among others [42], is linear. These forms are often included in textbooks and taught to students. The transformations date from early last century [43] before the computers and software needed to carry out nonlinear regression became commonplace. As we have argued previously [41], linearising (3) yields biased parameter estimates that are disproportionately influenced by data at high and low $s$ [44-46]. For these reasons, we have not implemented any of the transformations in these spreadsheets, but it would not be difficult to do so were there justification for it.

## IV. Module descriptions

These modules were prompted by the inability of some students to carry out even the most basic analysis the equations of enzyme kinetics. For example, many students have to remember the significance of $K_m$ and $V_{max}$ rather than simply being able to look at (3) and deduce their significance. If this is the case, then it is inevitable that even fewer realise that a change in the catalytic rate constant ($k_2$) necessarily yields some change in $K_m$, but that changes in $k_1$ or $k_{-1}$ do not affect $V_{max}$. Therefore, they find it difficult to see that the two steps are only partly independent.

The suite of spreadsheets[2] developed (i) deal with the behaviour of the Michaelis-Menten model at three different levels (spreadsheets MM1-MM3), (ii) illustrate the three different forms of inhibition ((9-11), spreadsheets named according to the inhibition), (iii) demonstrate the behaviour of allosteric enzymes (spreadsheet 'Allosteric kinetics') and (iv) provide a simple means using nonlinear regression to fit (3) to data obtained in practical classes (spreadsheet 'MM fit'). These spreadsheets are provided in a single file, but, in practice, subsets of these are usually deployed as the need arises.

### A. Phenomenology of Michaelis-Menten kinetics

The simplest level at which the behaviour of (1) can be analysed is based on $V_{max}$ (4) and $K_m$ (5) which can be modified using the scroll bars on the left of the MM1 spreadsheet. As $s$ increases, $v$ rises roughly linearly, but eventually, when $s$ gets larger than $K_m$, $v$ increases less rapidly and approaches $V_{max}$ asymptotically (Figure 2). Increasing the $V_{max}$ (by increasing $E_t$) increases $v$ at any $s$, but $v = ½V_{max}$ when $s = K_m$, irrespective of the enzyme concentration. Increasing the $K_m$ decreases $v$ at any $s$ because more substrate is required to half-saturate the enzyme (3). In effect, this corresponds to (i) reducing the rate at which ES is formed or (ii) increasing the rate at which either S or P is released. These phenomena are easily deduced from (3), but this is rarely easy for students and the spreadsheet provides an illustration.

Manipulation of the rate constants using the scroll bars on the left of the MM2 spreadsheet illustrates some of the subtleties of the model. In essence, this allows students to manipulate (i) the balance between the two steps in the reaction (1) and (ii) the balance between the rates of formation and degradation of the enzyme-substrate complex (ES). For example, a change in the catalytic rate constant ($k_2$) changes both the $K_m$ and the $V_{max}$, but a change in either of the rate constants involved in the binding step ($k_1$ or $k_{-1}$) has no effect on $V_{max}$ but does alter $K_m$.

Underlying the $v(s)$ plots is the variation in the concentration of the enzyme-substrate complex ($c$), substrate ($s$) and product ($p$) during the reaction. The steady-state approximation, from which (3) is derived, assumes that $c$ is constant. However, it can be inferred from the model (1-2) that $c$ increases and then decreases as $s$ declines, and that there is a delay in the appearance of $p$ after the onset of the reaction (as shown in the graphs in the screenshot of spreadsheet MM3 in Figure 4). These details of the model are implicit in the equations, but numerical integration of (2) allows the estimation of the timecourse of $s$, $c$ and $p$ (Figure 4) and the $v(s)$ plots (Figure 2) illustrate the behaviour of the Michaelis-Menten reaction that are clearly reminiscent of those obtained by experimentally by Chance [20].

### B. Inhibition of Michaelis-Menten enzymes

Separate spreadsheets were constructed for each of the three standard modes of inhibition of Michaelis-Menten enzymes (9-11). In each case it is possible to vary $K_3$ and/or $K_4$, depending on the type of inhibition, as well as $V_m$ and $K_m$ using the scollbars. Below the scroll bars on the left of each sheet is a list from which the number of inhibitor concentrations can be selected and these are then specified

[2] Available in the supplementary material as a single Excel file. These spreadsheets have been tested with Excel 2003, 2010 and 2016. The security settings may have to be adjusted and macros enabled before they run properly.

below this. A plot of $v(s)$ is generated for each of the specified inhibitor concentrations as well as that in the absence of inhibitor.

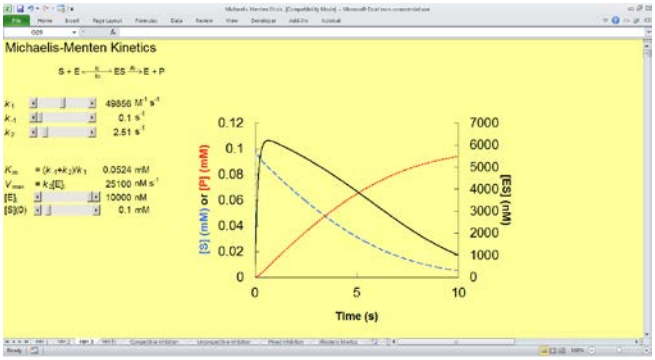


Figure 4. The dynamics of the Michaelis-Menten mechanism. This is a screenshot of the spreadsheet (MM3) in which (1) is analysed at the most detailed level. Manipulation of scroll bars on the left of the sheet change the rate constants ($k_1$, $k_{-1}$ or $k_2$), $E_t$ or $s_0$ and this is reflected in the plots of $s$ ([S], — — —), $c$ ([ES], ———) and $p$ ([P], – – –)[3]. To assist the students (who do not usually see (9-11)), $K_3$ and $K_4$ are replaced in the spreadsheet with $K_{IE}$ and $K_{IES}$, respectively, to emphasise the dissociation of I from IE and IES, respectively.

### C. Allosteric enzyme kinetics

The analysis of the behaviour of (12) is based on $V_m$ (more correctly $E_t$), $K_{0.5}$ and $n$ which can be manipulated using the scroll bars on the left of the spreadsheet. If $n = 1$, (12) is identical to (3). As $n$ increases the gradient of $v(s)$ at low $s$ decreases and that at the steepest part of the curve (where $s^n = (n - 1)K_{0.5}/(n + 1)$, $n > 1$), but the maximum velocity is unaltered (Figure 3).

### D. Nonlinear regression

To fit (3) to experimental data using a VBA implementation of Duggleby's [37] code the data need to be entered onto the 'MM fit' spreadsheet and initial estimates of $K_m$ and $V_{max}$ are required. The data should be entered in the first two columns ($s$, $v$) in the white region (as indicated) and these are plotted automatically on the graph. Estimates of $K_m$ and $V_{max}$ can be adjusted using the scroll bars below the graph until the curve (3) conforms approximately to the data. Once suitable estimates have been obtained, the nonlinear regression routine is initiated by clicking on the 'Improve the fit' button and the estimates and the curve on the graph are then updated.

## V. Discussion

Students in the biological sciences often find enzyme kinetics challenging because of the combination of mathematics and quite complicated dynamics. Few of them find it easy to work out what might happen in a given situation just by looking at the equations. A significant part of the problem is that the subject is expressed mathematically. The use of this simple technique to illustrate the dynamics inherent in the equations overcomes some of the students' problems.

The advantage of these spreadsheets compared with those of Bruist [16], for example, is that no mathematics is required of the user. Instead, we take advantage of the graphical interface of Excel to facilitate a more intuitive interaction. This is especially important for those students who do not have strong mathematical skills. While the functions used here ((2-3), (9-12)) are relatively simple, the same technique could be applied to more complex functions [14, 32-35] for those who have more developed mathematical skills.

These spreadsheets have been helpful to students and have been used in conjunction with a carefully devised analogy [12]. While no formal assessment has been carried out, students were asked to respond to the statement that the spreadsheets were useful and more than 60% of respondents agreed or strongly agreed that they were and none disagreed with the statement. More persuasive to us are (i) cases in which students have been prompted to ask questions by their use of the spreadsheets, (ii) their use in preparing practical reports and (iii) that the spreadsheets have been useful in discussions with students in and out of class.

## VI. Conclusion

We have described (i) a very simple approach that provides a graphical means of illustrating the behaviour of functions and (ii) its application to enzyme kinetics. This has proved useful to students for whom a mathematical expression may be an obstacle to learning. It takes relatively little time for a teacher to develop each spreadsheet. Functions that are not available within Excel (such as the Lambert W function or nonlinear regression) can, with care, be implemented using VBA if required. Given this, while the enzyme kinetics spreadsheets we describe represent a simple example, more demanding applications can be envisaged.

[3] As this might be a potential source of confusion, the use of both [X] and $x$ to designate the concentration of X is standard usage in chemistry. The choice depends on the context: [X] is inconvenient in mathematical expressions where it is often replaced by $x$.

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