

## An EXCEL template for calculation of enzyme kinetic parameters by non-linear regression

Agustín Hernández<sup>1</sup> and María Teresa Ruiz<sup>2</sup>

<sup>1</sup>Katholieke Universiteit Leuven, Laboratorium voor Moleculaire Celbiologie, Kardinaal Mercierlaan 92, Leuven-Heverlee, B-3001 Belgium and <sup>2</sup>The Sainsbury Laboratory, The John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK

Received on August 16, 1997; revised and accepted on September 15, 1997

### Abstract

**Motivation:** An EXCEL template has been developed for the calculation of enzyme kinetic parameters by non-linear regression techniques. The tool is accurate, inexpensive, as well as easy to use and modify.

**Availability:** The program is available from <http://www.ebi.ac.uk/biocat/biocat.html>

**Contact:** [agustin.hernandez@bio.kuleuven.ac.be](mailto:agustin.hernandez@bio.kuleuven.ac.be)

### Introduction

Accurate estimation of the kinetic parameters of enzymes is an important part in the analysis of biochemical problems. The advantages of using non-linear regression instead of linearization of the model have been documented extensively (Suelter, 1985; Tommasini *et al.*, 1985; Henderson, 1992). However, at present, few possibilities exist that allow the calculation of kinetic parameters from raw data by non-linear regression without having to deal with a computer program that is intended for more powerful statistical analysis. Those that have been designed for the specific calculation of kinetic parameters often can only fit Michaelis–Menten models and do not provide high-quality graphics; they tend to be written for DOS operating systems and modification of their models and outputs is not possible in most cases. The objective of the developers was to provide a tool for the statistical analysis of enzyme kinetics that was accurate and inexpensive, but, at the same time, easy to use and modify. EXCEL provided the environment for these characteristics to be implemented, with the added values that the resulting files are Apple/PC compatible and can produce publication-quality graphs if necessary. The template (ANEMONA.XLT) is a collection of mathematical models organized in different worksheets. Each model has all its different intermediate steps on sight, allowing any kind of modification.

### Computer requirements

Apple Macintosh or IBM compatible, running EXCEL version 5.0 or later, and 'Solver Add-In', installed in the EXCEL program.

### Organization of the file

The file is saved as a template. The different worksheets each deal with a different mathematical model. These sheets can be filled, used and saved in the same file or they can be copied and pasted into other EXCEL files, where they will retain the same properties as in the original.

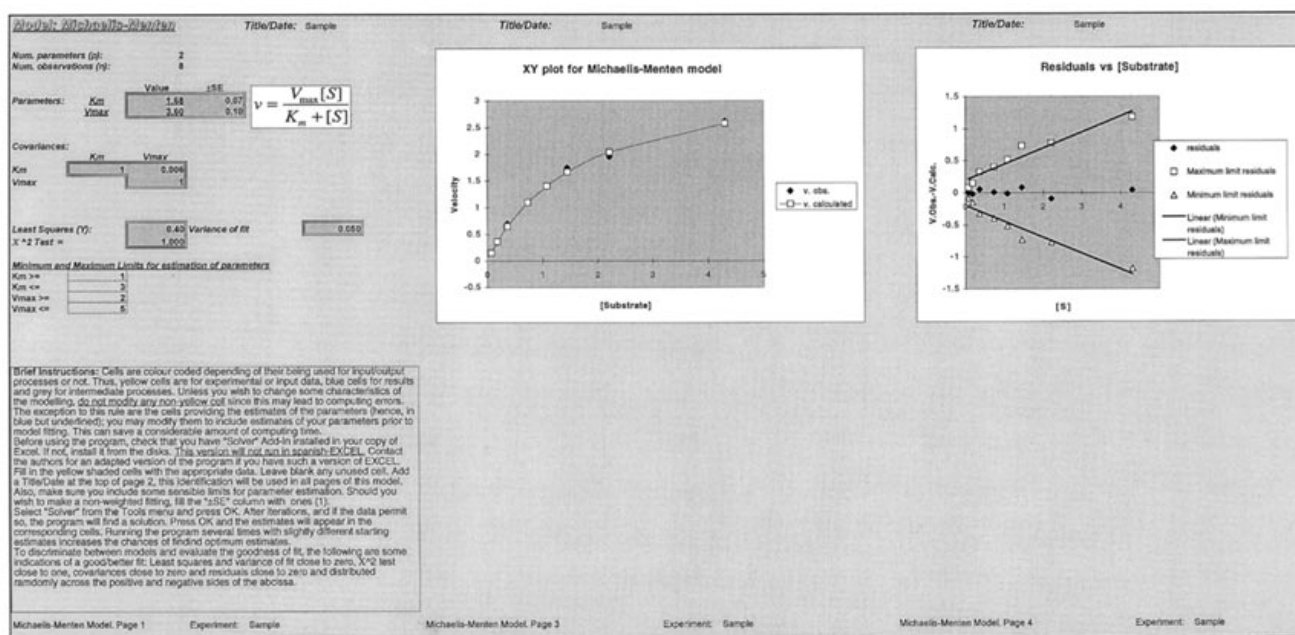
The models implemented are those corresponding to mono- or pseudo-monosubstrate reactions and the most common bisubstrate reactions: Michaelis–Menten, double Michaelis–Menten, Hill model, substrate inhibition, the linear cases of reversible inhibition (competitive, uncompetitive, non-competitive and mixed competitive–uncompetitive inhibition), and ping-pong, ordered and random bisubstrate mechanisms. Also, a weighted linear model has been implemented.

### Mathematical characteristics

Non-linear regression is performed by the least-squares method, where the function

$$Y = \sum w (v_o - v_i)^2$$

is minimized by varying the appropriate parameters on the model (e.g.  $K_m$ ,  $V_{max}$ ,  $K_i$ ). Here,  $v_o$  is the observed velocity,  $v_i$  is the theoretical velocity obtained from the model and  $w$  is a weighting factor. By default, the weighting factor is the squared inverse of the standard error of the data points. Alternative weightings can be implemented by modifying the appropriate cells in the worksheet. The search method for estimates of the parameters that minimize the  $Y$  function can be chosen from the Newton method or the steepest descent method (for a description, see Johnson and Faunt, 1992) by choosing the appropriate option in the Solver menu of EXCEL. Also, the criteria for convergence in the iteration process can be modified in the same menu. The standard error of the fitted parameters is calculated by inversion of the information matrix. Limits for the variation of the kinetic parameters can be set in the appropriate cells of the worksheet.



**Fig. 1.** Partial view of the first printed pages from a typical worksheet showing the results after non-linear fitting of the Michaelis–Menten equation. For clarity, page 2 (input data) has been omitted.

## Output of the file

If convergence is reached, the estimated values of the kinetic parameters are displayed, together with their standard error, covariances, final value of the  $Y$  function, the variance of fit and a  $\chi^2$  test of the fit. At the same time, graphs showing the original data and the fitted function versus the independent variable (concentration of substrate or inhibitor) and the residuals versus the independent variable are also provided (Figure 1). To aid in the analysis of residuals, confidence limits for the residuals are also plotted.

## References

- Henderson, P.J.F. (1992) Statistical analysis of enzyme kinetic data. In Eisenthal, R. and Danson, M.J. (eds), *Enzyme Assays: A Practical Approach*. IRL Press, Oxford, pp. 277–316.
- Johnson, M.L. and Faunt, L.M. (1992) Parameter estimation by least-squares methods. *Methods Enzymol.*, **210**, 1–37.
- Suelter, C.H. (1985) *A Practical Guide to Enzymology*. John Wiley and Sons, New York.
- Tommasini, R., Endrenyi, L., Taylor, P.A., Mahuran, D.J. and Lowden, J.A. (1985) A statistical comparison of parameter estimation for the Michaelis-Menten kinetics of human placental hexosaminidase. *Can. J. Biochem. Cell Biol.*, **63**, 225–230.