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A TRIDIMENSIONAL REPRESENTATION OF ENZYME INHIBITION USEFUL FOR DIAGNOSTIC PURPOSES

RUI FONTES[#], JOÃO MEIRELES RIBEIRO[†] AND ANTONIO SILLERO[*]

[#]*Serviço de Química Fisiológica, Faculdade de Medicina, Hospital de São João, Universidade do Porto, Porto, Portugal*

[†]*Departamento de Bioquímica y Biología Molecular Genética, Facultad de Medicina, Universidad de Extremadura, E-06080 Badajoz, Spain*

[*]*Departamento de Bioquímica, Instituto de Investigaciones Biomédicas del Consejo Superior de Investigaciones Científicas, Facultad de Medicina, Universidad Autónoma de Madrid, E-28029 Madrid, Spain*

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A new three dimensional representation of enzyme inhibition, applied to Lineweaver-Burk, Hanes and Eadie-Hofstee plots is presented. This type of representation has advantages for enzyme inhibition diagnosis, showing graphic characteristics that pass unnoticed in linear plots.

KEY WORDS: Diagnosis of enzyme inhibition, graphic techniques, three dimensional representation

INTRODUCTION

The diagnosis between the different types of enzyme inhibition can be made using linear plots: Lineweaver-Burk, Hanes or Eadie-Hofstee plots, eventually requiring secondary representations of parameters obtained from these plots, usually named replots[1–3]. However, those plots are bidimensional representations of phenomena implying three variables: substrate concentration ([S]), initial velocity (v), and inhibitor concentration ([I]), and as we shall emphasize here conceal some properties of enzyme inhibition that can be unmasked when the plots are modified to introduce the inhibitor concentration as a third axis. The outcome is a three dimensional representation which may has advantages for the diagnosis of the inhibition type.

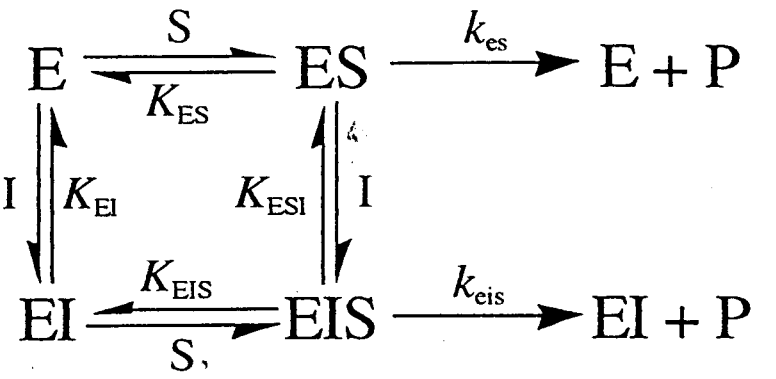
[*]Correspondence: Departamento de Bioquímica, Facultad de Medicina, UAM, Arzobispo Morcillo 4, E-28029 Madrid, Spain.

A BRIEF PRESENTATION OF ENZYME INHIBITION

An exhaustive description of each type of enzyme inhibition and of the mathematical procedures followed to get the corresponding velocity equations can be found elsewhere[1–5]. In this paper, discussion will be limited, as it is usual, to enzymes that bind only one molecule of substrate and/or inhibitor. We will distinguish the total (complete or linear) and partial (non-linear or hyperbolic) inhibitions, depending on whether saturating inhibitor concentrations can completely or partially inhibit the enzyme reaction, respectively[1–3,6]. The tridimensional model will be applied to the following eight cases of reversible enzyme inhibition.

Mixed Partial Inhibitions	MPI
Mixed Total Inhibitions	MPI
Non-competitive Partial Inhibitions	NCPI
Non-competitive Total Inhibitions	NCTI
Competitive Partial Inhibitions	CPI
Competitive Total Inhibitions	CTI
Uncompetitive Partial Inhibitions	UCPI
Uncompetitive Total Inhibitions	UCTI

A plausible general mechanism for enzyme inhibition would be represented by the following scheme:



where $V_{EIS} = k_{eis}[E_T]$, $V_{ES} = k_{es}[E_T]$; K_{ES}, K_{EI}, K_{ESI} and K_{EIS} are the equilibrium constants for, respectively the reactions $ES \rightarrow E + S$, $EI \rightarrow E + I$, $EIS \rightarrow ES + I$, and $EIS \rightarrow EI + S$.

In general this mechanism assumes two binding sites in the enzyme: one active site for the substrate and another site for the inhibitor. However in the cases (CTI) where K_{ESI} and K_{EIS} are infinite it can also represent an enzyme with one area that can bind the substrate and the inhibitor. In the cases where K_{EI} is infinite (UCPI and UCTI) the site for the inhibitor becomes accessible only after the binding of the substrate.

The more general case for this mechanism is mixed partial inhibition (MPI). The Michaelis-Menten equation describing the velocity of the reaction is:

$$v = \frac{[S]V_{ES}\alpha}{[S] + K_{ES}\beta} \tag{1}$$

with,

$$\alpha = 1 + \frac{\frac{V_{EIS} - V_{ES}}{V_{ES}}[I]}{K_{ESI} + [I]} \tag{2}$$

$$\beta = 1 + \frac{\frac{K_{ESI} - K_{EI}}{K_{EI}}[I]}{K_{ESI} + [I]} \tag{3}$$

The other types of enzyme inhibition arise when: (a) the affinity of the substrate is the same for E and EI (NCPI and NCTI); (b) the reaction characteristics of the bound substrate are nor altered by the binding of inhibitor (CPI); (c) the inhibitor can only bind to ES (UCPI and UCTI); and (d) the inhibitor can only bind to E (CTI). Enzyme inhibition is total or partial, depending on whether or not the complex EIS can generate product.

The equations for α and β in the different types of enzymes inhibition are shown in Figure 1.

THE TRIDIMENSIONAL (OR TENNIS COURT) REPRESENTATION OF THE ENZYME INHIBITION APPLIED TO LINEWEAVER-BURK PLOT

The more general equation (that for MPI) describing $1/v$ *versus* $1/[S]$ is

$$\frac{1}{v} = \frac{K_{ES}\beta}{V_{ES}\alpha}\frac{1}{[S]} + \frac{1}{V_{ES}\alpha} \tag{4}$$

α and β have already been defined and both vary with [I] and the values of K_{ESI}, K_{EI}, V_{ES} and V_{EIS}.

In a Lineweaver-Burk plot only two axes are considered and the influence of the inhibitor on the velocity of the reaction is inferred from both the slopes and the intercepts of the linear plots with the y axis. The tridimensional representation considers three axes: x, y and z, representing, $1/[S]$, $1/v$ and $[I]$, respectively. It may be simulated by a *tennis court*, and the straight lines describing $1/v$ *versus* $1/[S]$ by the trajectory of a ball (although, in this representation, passing through the net).

Inhibition type	α	β
NCPI	$1+\frac{\frac{V_{EIS}-V_{ES}}{V_{ES}}[I]}{K_{ESI}+[I]}$	1
NCTI	$1+\frac{-1*[I]}{K_{ESI}+[I]}$	1
UCPI	$1+\frac{\frac{V_{EIS}-V_{ES}}{V_{ES}}[I]}{K_{ESI}+[I]}$	$1+\frac{-1*[I]}{K_{ESI}+[I]}$
UCTI	$1+\frac{-1*[I]}{K_{ESI}+[I]}$	$1+\frac{-1*[I]}{K_{ESI}+[I]}$
CPI	1	$1+\frac{\frac{K_{ESI}-K_{EI}}{K_{EI}}[I]}{K_{ESI}+[I]}$
CTI	1	$1+\frac{1}{K_{EI}}[I]$
MPI	$1+\frac{\frac{V_{EIS}-V_{ES}}{V_{ES}}[I]}{K_{ESI}+[I]}$	$1+\frac{\frac{K_{ESI}-K_{EI}}{K_{EI}}[I]}{K_{ESI}+[I]}$
MTI	$1+\frac{-1*[I]}{K_{ESI}+[I]}$	$1+\frac{\frac{K_{ESI}-K_{EI}}{K_{EI}}[I]}{K_{ESI}+[I]}$

FIGURE 1 Coefficients α and β for the different types of enzyme inhibition. $V_{ES}\alpha$ and $K_{ES}\beta$ are, respectively, the equation for the functions $V_{max}^{app}=f([I])$ and $K_m^{app}=f([I])$. When $V_{EIS}>0$ and $K_{ESI}<\infty$ the inhibition is called partial; in the opposite condition, that is, $V_{EIS}=0$ or $K_{ESI}=\infty$, it is called total. In non-competitive types $K_{EI}=K_{ESI}$; in uncompetitive ones $K_{EI}=\infty$; in competitive total type $K_{ESI}=\infty$, and in competitive partial $V_{ES}=V_{EIS}$.

The Lineweaver-Burk representation corresponds, exclusively, to the view of a spectator looking to the *tennis court* from the lateral edge of the net (as a net judge). On the contrary, the tridimensional representation allows different view points. For instance, the plot of $1/V_{max}^{app}$ (the Lineweaver-Burk intercept with the $1/v$ axis) *versus* $[I]$ can be observed from the positive side of the x axis, and the influence of $[I]$ on V_{max}^{app} can be appreciated; the plot $1/K_m^{app}$ *versus* $[I]$ can be observed from above of the left side field, and the influence of $[I]$ on K_m^{app} visualized.

The model can be represented with PC or Macintosh programs which produce three dimensional plots; for example, the program Mathematica[7] may be useful for these purposes.

For the more general case (MPI), the plot in the vertical plane ($1/V_{max}^{app}$ *versus* [I]) is described by equation (5); the one in the horizontal plane ($1/K_m^{app}$ *versus* [I]), by equation (6).

$$\frac{1}{V_{\max}^{\text{app}}} = \frac{1}{V_{ES}} \left(1 + \frac{\dfrac{V_{ES} - V_{EIS}}{V_{EIS}}[I]}{\dfrac{K_{ESI}V_{ES}}{V_{EIS}} + [I]} \right) \tag{5}$$

$$\frac{1}{K_m^{\text{app}}} = \frac{1}{K_{ES}} \left(1 + \frac{\dfrac{K_{EI} - K_{ESI}}{K_{ESI}}[I]}{K_{EI} + [I]} \right) \tag{6}$$

Examples of total ($V_{EIS} = 0$) and partial ($V_{EIS} > 0$) inhibition types are represented in Figures 2–5. They were drawn according to the following characteristics: K_{ES} and V_{ES} values are always 1; K_{ESI} value is 1.5 except in CTI (Figure 4), where it is ∞; K_{EI} value is 1 (Figures 4 and 5), and 1.5 (Figure 2); V_{ESI} value is 0.25 in all types of partial inhibitions except in CPI (Figure 3) where $V_{ES} = V_{ESI} = 1$. The concentrations of inhibitors were 0, 1, 2, 3 and 4. The continuous line in the Lineweaver-Burk plots and the line closer to the observer in the tridimensional graphics represent the plot $1/v$ *versus* $1/[S]$] for $[I] = 0$. Note that the plot of the Lineweaver-Burk intercept with the y axis $1/v$ *versus* $[I]$, drawn in the vertical plane (the *net*), is a straight line in all cases of total inhibition, and a hyperbola in all cases of partial inhibition (except in CPI, Figure 3).

Non-competitive inhibition (Figure 2)

One single condition defines non-competitive cases: $K_{ESI} = K_{EI}$. The K_m^{app} (and $1/K_m^{app}$) does not change with the inhibitor concentration; the points where the plots intercept the horizontal plane define a straight line parallel to the $[I]$ axis. As stated above, the plot in the vertical plane ($1/V_{max}^{app}$ *versus* $[I]$) is a hyperbola in NCPI and a straight line in NCTI.

Uncompetitive inhibition (Figure 3)

In these cases K_{EI} is infinite and the tridimensional representation can clearly show the fingerprint of uncompetitive inhibition: the plots $1/K_m^{app}$ *versus* $[I]$ (in the horizontal plane) are always straight lines whose slope is $1/(K_{ES}K_{ESI})$. Once again, the plot in the vertical plane ($1/V_{max}^{app}$ *versus* $[I]$) is a hyperbola in the partial inhibition (UCPI) and a straight line in the total inhibition (UCTI). In UTI, $V_{EIS} = 0$ and inhibition is observed for all concentrations of substrate. In UPI, $V_{EIS} < V_{ES}$ and inhibition occurs only if $[S] > K_{ES}V_{EIS}/(V_{ES} - V_{EIS})$.

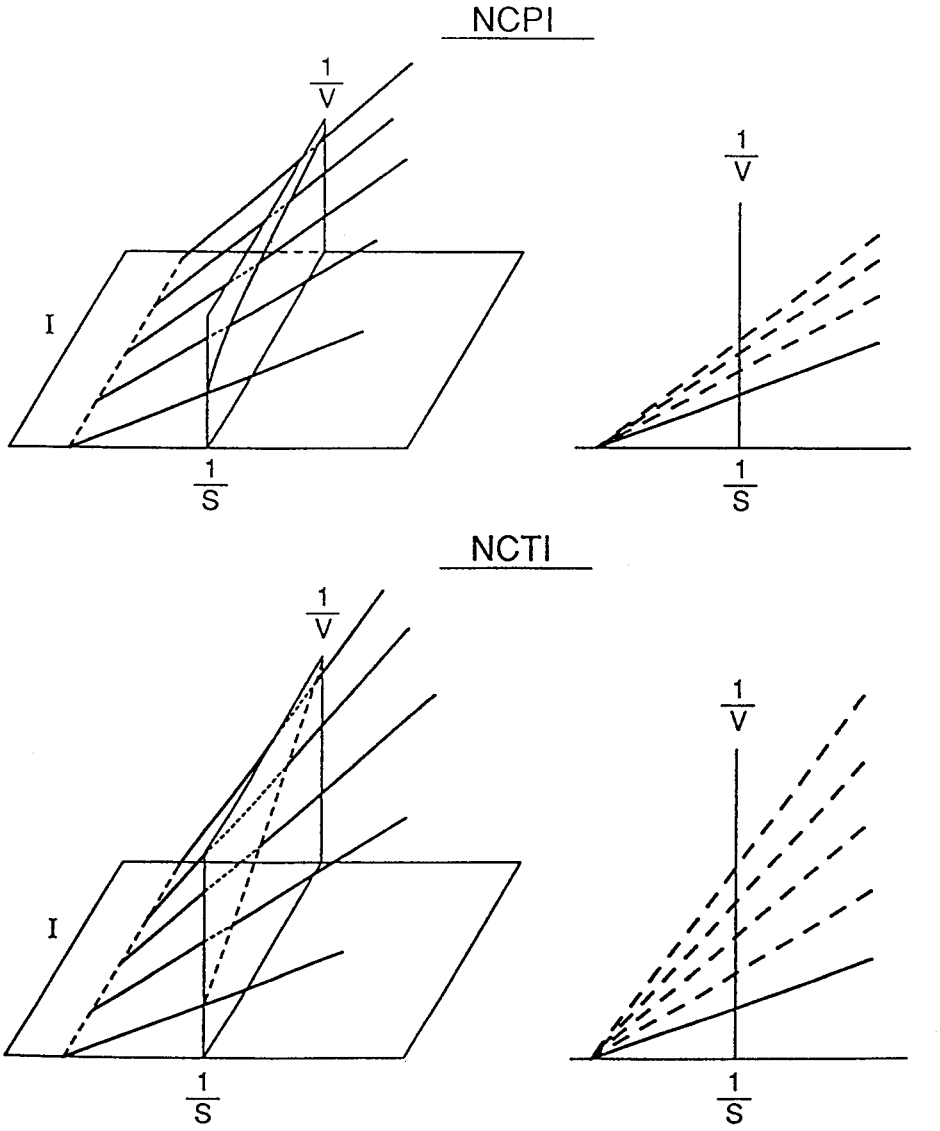


FIGURE 2 Tridimensional representation of the Lineweaver-Burk plot (left side) with the classical bidimensional Lineweaver-Burk plot (right side), for the case of non-competitive partial inhibition (NCPI) and non-competitive total inhibition (NCTI). Kinetic parameters are as indicated in the text. The concentrations of inhibitor used to draw the figures were 0 (the line closer to the observer in the tridimensional model, and the continous line in the Lineweaver-Burk plot), 1, 2, 3, and 4. In the tridimensional model, the points of interception of the linear plots with the vertical or horizontal plane are joined by a dotted line when the intercepts represent a straight line and by a continuous line when the intercept represent a hyperbola.

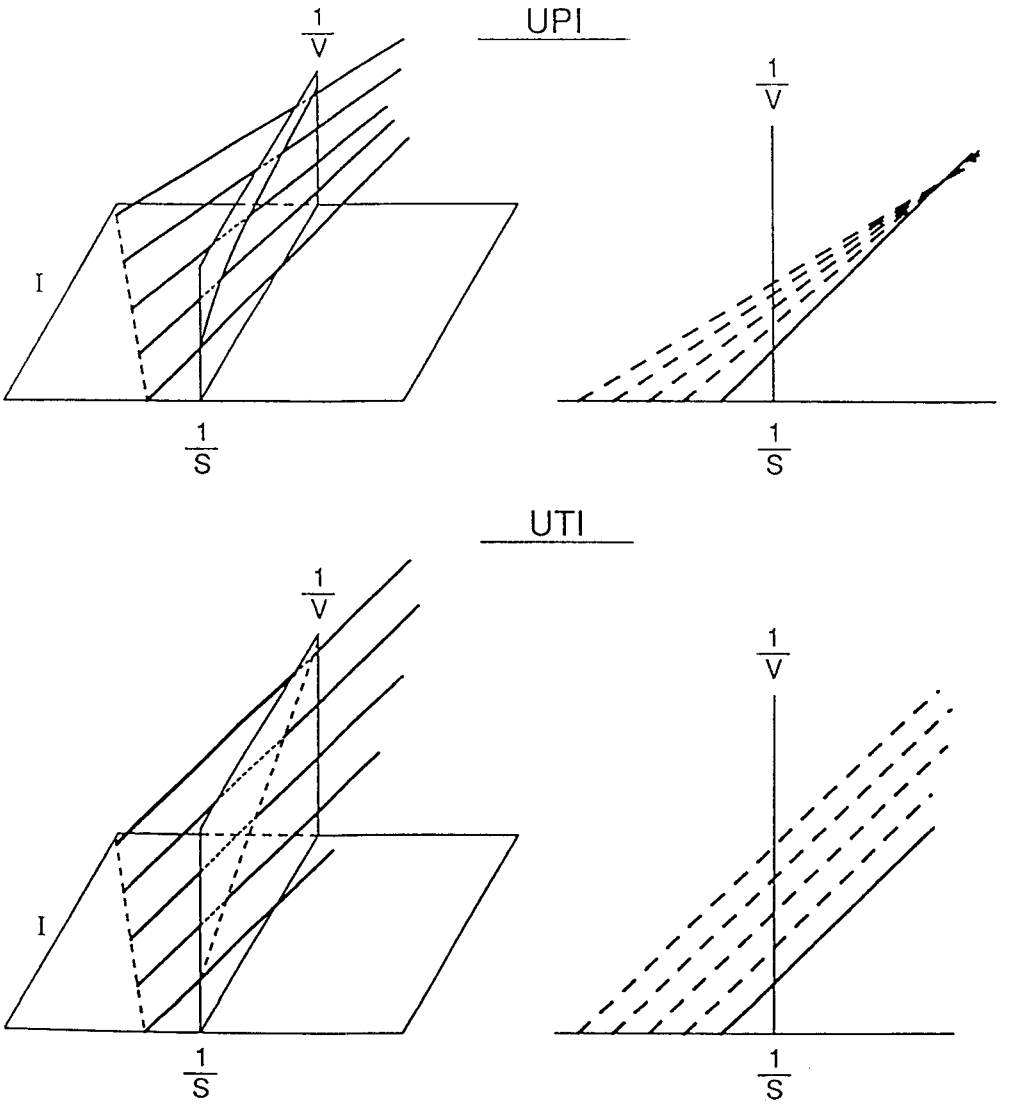


FIGURE 3 Tridimensional representation of the Lineweaver-Burk plot (left side), compared with the classical bidimensional Lineweaver-Burk plot (right side), for the case of uncompetitive partial inhibition (UPI) and uncompetitive total inhibition (UTI). Legend as in Figure 2.

Competitive inhibition (Figure 4)

In these cases the vertical-plane plots are lines parallel to the $[I]$ axis. In the horizontal plane the plots are hyperbolas; when K_{ESI} is infinite (CTI), $1/K_m^{app}$ approaches zero when $[I]$ approaches infinite, i.e., the horizontal asymptote is the $[I]$ axis. In the case of CPI the horizontal asymptote has a non-zero value equal to $1/K_{EIS}$.

Mixed inhibition (Figure 5)

Mixed cases are the ones that can not be included in the situations discussed above. The horizontal plane plots are hyperbolas and, in the examples chosen, as $K_{ES} < K_{ESI}$, they are actually decreasing hyperbolas. The vertical plane plot is a hyperbola in MPI and a straight line in MTI. So, in MPI, both vertical and horizontal planes plots are hyperbolas.

APPLICATION OF THE TRIDIMENSIONAL REPRESENTATION TO OTHER LINEAR PLOTS

The tridimensional representation could be applied to Hanes and to Eadie-Hofstee plots. The general equations describing $[S]/v$ *versus* $[S]$ (Hanes plot; equation (7)) and v *versus* $v/[S]$ (Eadie-Hofstee plot; equation (8)) are

$$\frac{[S]}{v} = \frac{1}{V_{ES}\alpha}[S] + \frac{K_{ES}\beta}{V_{ES}\alpha} \tag{7}$$

$$v = -K_{ES}\beta\frac{v}{[S]} + V_{ES}\alpha \tag{8}$$

where α and β are the coefficients previously defined in equations (2) and (3) (see also Figure 1).

As an example, only MTI is represented with the tridimensional view of the Hanes and Eadie-Hofstee plots (Figure 6).

Hanes plot (Figure 6A)

A view from above of the left side of the *tennis court* shows the plot K_m^{app} *versus* $[I]$; the vertical plot (the *net*) is the plot K_m^{app}/V_{max}^{app} *versus*, $[I]$ which is, actually, the same as the Lineweaver-Burk slope *versus* $[I]$. The plot K_m^{app} *versus* $[I]$ is described by $K_m^{app} = K_{ES}\beta$ (see equation 3 and Figure 1); the more general equation for the vertical-plane plot is

$$\frac{K_m^{app}}{V_{max}^{app}} = \frac{K_{ES}}{V_{ES}}\left(1 + \frac{\dfrac{K_{ESI}V_{ES} - K_{EI}V_{EIS}}{K_{EI}V_{EIS}}[I]}{\dfrac{K_{ESI}V_{ES}}{V_{EIS}} + [I]}\right) \tag{9}$$

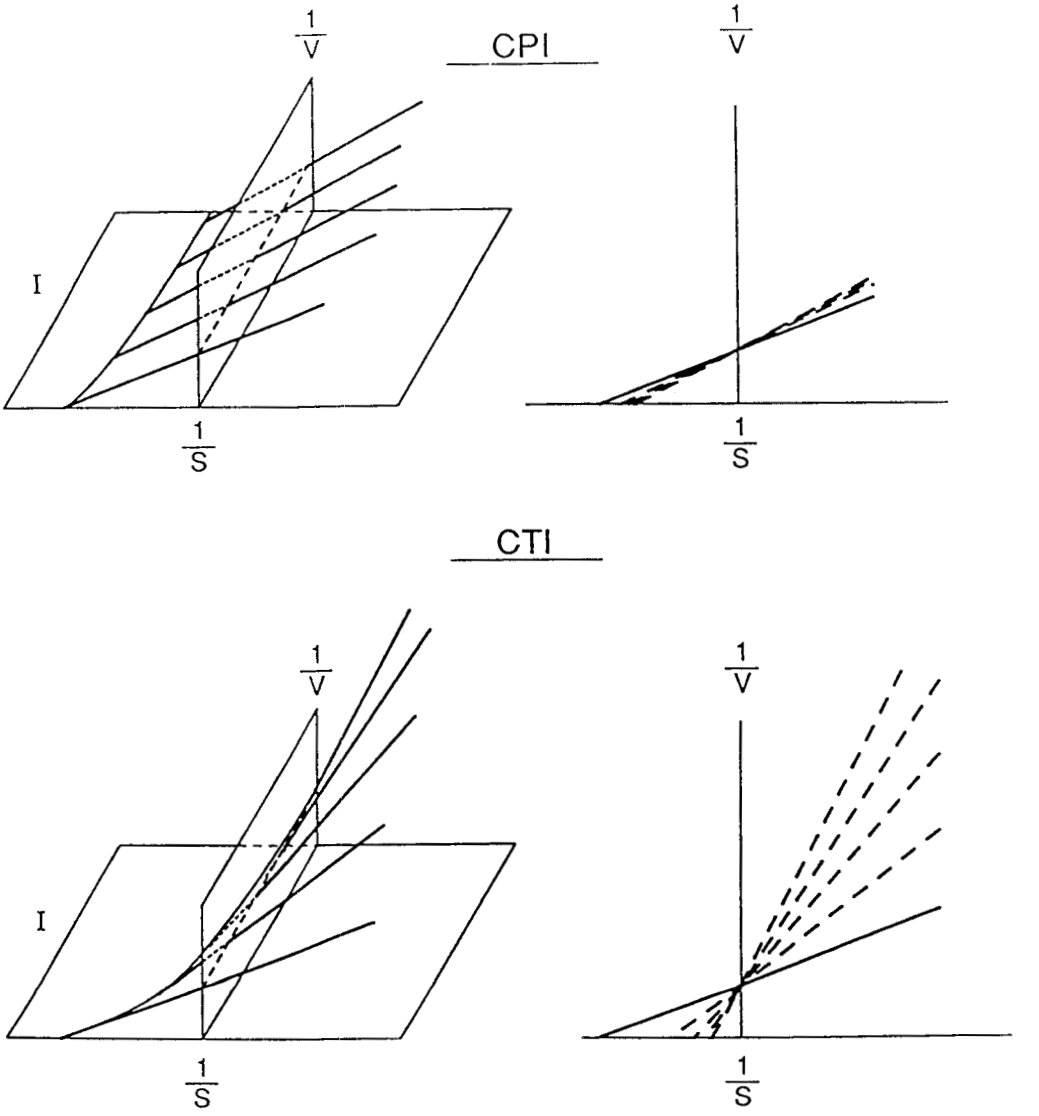


FIGURE 4 Tridimensional representation of the Lineweaver-Burk plot (left side), compared with the classical bidimensional Lineweaver-Burk plot (right side) for the case of competitive partial inhibition (CPI) and competitive total inhibition (CTI). Legend as in Figure 2.

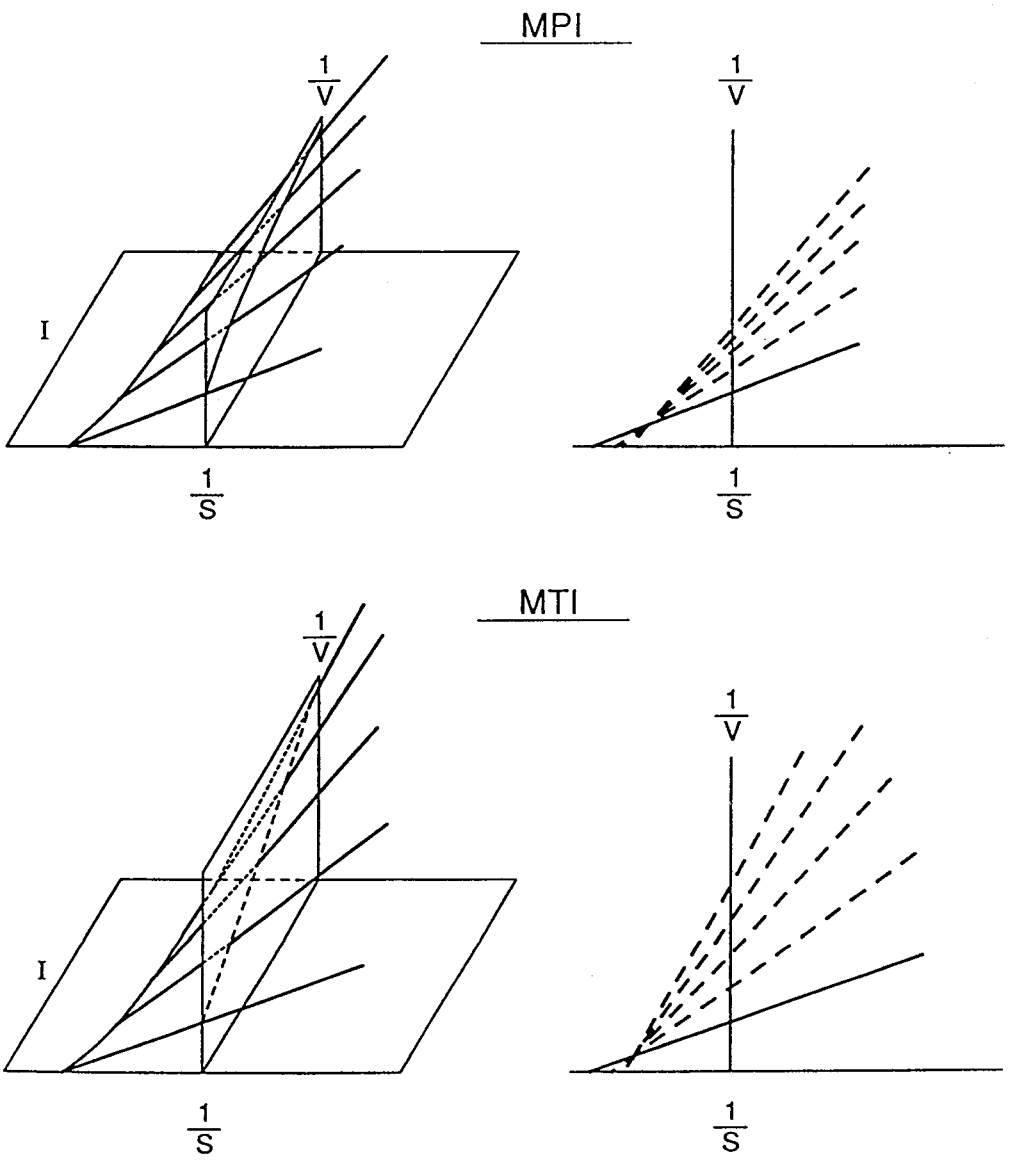


FIGURE 5 Tridimensional representation of the Lineweaver-Burk plot (left side), compared with the classical bidimensional Lineweaver-Burk plot (right side), for the case of mixed partial inhibition (MPI) and mixed total inhibition (MTI). Legend as in Figure 2.

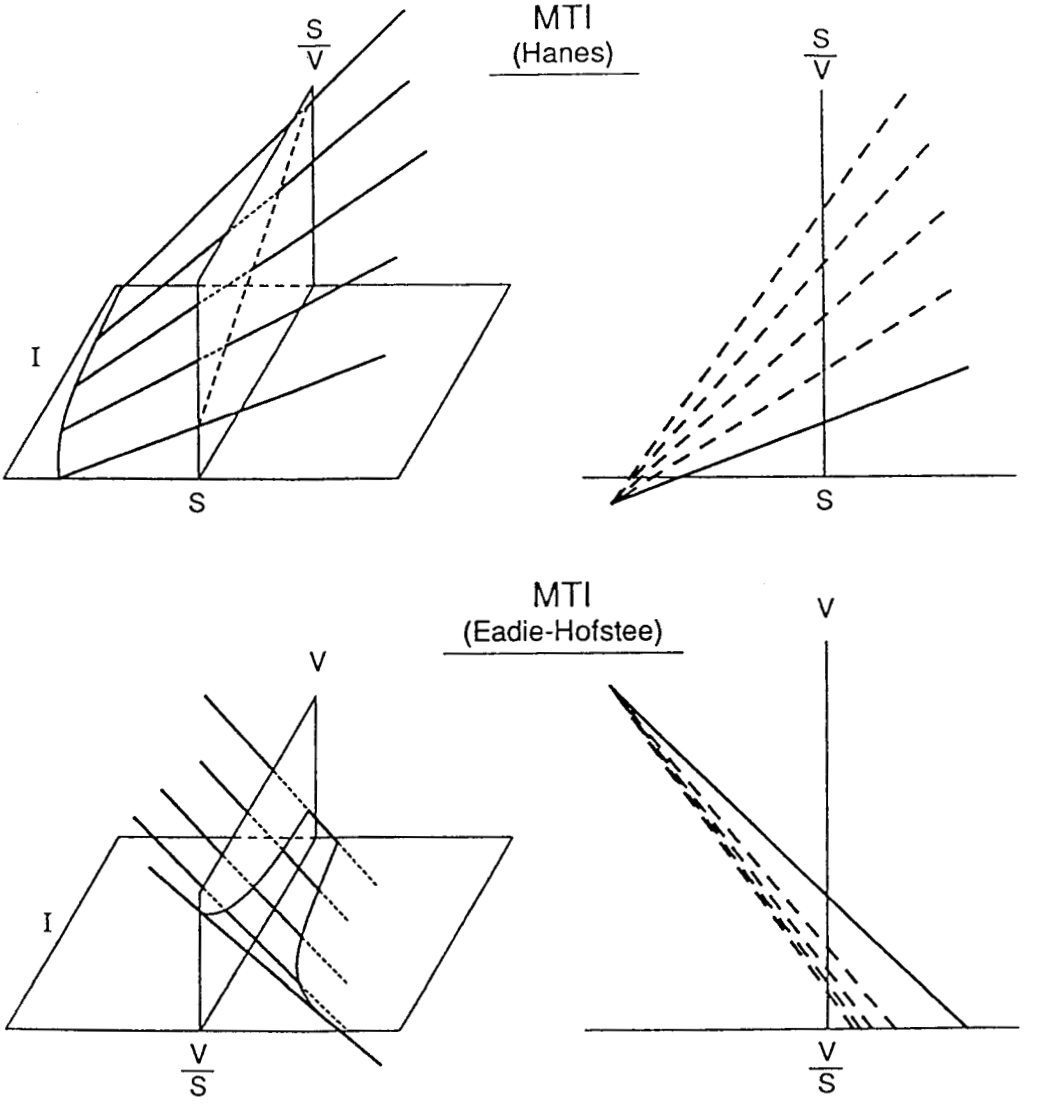


FIGURE 6 Tridimensional representation of the Hanes plot (A; left side) and of the Eadie-Hofstee plot (B; left side), compared with the respective classical bidimensional plots (right side), for the case of mixed total inhibition (MTI). Legend as in Figure 2.

The vertical-plane plots are straight lines in all total types of inhibition; their slopes are positive except in UTI, where the plot is parallel to the $[I]$ axis. In almost all partial inhibition cases the vertical plane plot is hyperbolic; the exceptions are the mixed partial cases where $K_{ESI}V_{ES} = K_{EI}V_{EIS}$. In these cases the plot is a straight line parallel to the $[I]$ axis. The uncompetitive partial vertical plane hyperbolas approach zero when $[I]$ approaches infinite, i.e., their horizontal asymptote is the $[I]$ axis.

The horizontal-plane plots can be a straight line parallel to the $[I]$ axis (non-competitive cases), a positive-slope straight line (competitive total cases) or a hyperbola (all other cases). In all uncompetitive cases the hyperbolas approach zero when $[I]$ approaches infinite.

Eadie-Hofstee plot (Figure 6B)

The vertical-plane plot, $V_{\max}^{app}$ *versus* $[I]$ is described by $V_{\max}^{app} = V_{ES}\alpha$ (see equation 2 and Figure 1). The horizontal-plane plot ($V_{\max}^{app}/K_m^{app}$ *versus* $[I]$) is on the right side field: one has to imagine the right side field of the *tennis court* observed from underground. The more general equation for this plot is:

$$\frac{V_{\max}^{app}}{K_m^{app}} = \frac{V_{ES}}{K_{ES}}\left(1 + \frac{\dfrac{K_{EI}V_{EIS} - K_{ESI}V_{ES}}{K_{ESI}V_{ES}}[I]}{K_{EI} + [I]}\right) \tag{10}$$

The vertical-plane plot can be a straight line parallel to the $[I]$ axis (in CPI and CTI) or a hyperbola (in all other cases).

The horizontal-plane plot can be a straight line parallel to the $[I]$ axis (in UTI and in the MPI where $K_{ESI}V_{ES} = K_{EI}V_{EIS}$), a positive-slope straight line (in UPI), or a hyperbola (in all other cases). The horizontal asymptote for the hyperbolas generated in cases of total inhibition, both in the vertical and horizontal planes, is always the $[I]$ axis.

In this work we present a novel three dimensional representation, useful for enzyme inhibition studies. Although it is outside the scope of this paper to discuss the way to calculate the parameters K_{ES}, K_{EI}, K_{ESI}, V_{ES} and V_{ESI} and the errors associated with them, when experimental data are given, the following approach would be followed: (i) after representation of the three dimensional plot, decisions must be taken (based on the sensibleness of the researcher and, may be, on statistical methods) whether the intersects with the vertical and horizontal planes are straight lines or hyperbolas; (ii) after these decisions have been taken, linear or non linear regression analysis can be applied to calculate the parameters and the errors associated with them[8,9]. In our view, the inclusion of the errors associated with $1/v$, (Lineweaver-Burk); $[S]/v$, (Hanes) and v, $v/[S]$ (Eadie-Hofstee), would overload the three dimensional representations.

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References

1. Dixon, M. and Webb, E.C. (1979), *Enzymes*, 3rd. Edn. Longman, London.
2. Cornish-Bowden, A. (1979), *Fundamentals of Enzyme Kinetics*, Butterworths, London.
3. Palmer, T. (1991), *Understanding Enzymes*, 3rd. Edn, Ellis Horwood, New York.
4. Botts, J. and Morales, M. (1953), *Trans Faraday Soc.*, **49**, 696–707.
5. Frieden, C. (1964), *J. Biol. Chem.*, **239**, 3522–3531.
6. Nomenclature Committee of the International Union of Biochemistry (NC-IUB) (1982), *Eur. J. Biochem.*, **128**, 281–291.
7. Wolfram, S. (1991) *Mathematica*, 2nd Edn. Addison-Wesley Publishing Co. Redwood City, California.
8. Wilkinson, G.N. (1961) *Biochem. J.*, **80**, 324–332.
9. Duggleby, R.G. (1981) *Anal. Biochem.*, **110**, 9–18.