CORRECTION OF DIXON PLOTS

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The analysis of algebraic equations for the dependence of the initial velocities of inhibited (seven equations) and activated (seven equations) enzymatic reactions on concentrations of inhibitors (*i*) and activators (*a*) is intended to take into account the sources of errors (Corrections 1–8) in using Dixon plots for calculation of constants of inhibition and characteristics of types of inhibition (and activation) of the enzymes.

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Introduction

Dixon plot analysis (plots of the dependence of the reciprocal of the initial velocities of the inhibited reactions ($1/v_i$) on increasing concentrations of the inhibitor, $1/v_i=f(i)$, for two or more constant concentrations of a substrate), widely used for the calculation of the constants of enzyme inhibition (K_i), is known in two versions.

Version 1

Dixon, M. (1953)[1] used the following equation for calculating K_{IVi} constants of associative (or competitive according to the conventional terminology[2-5]) type enzyme inhibition (Table 1, line 4)

$$v_{IVi} = \frac{V^0}{1+\frac{K^0_m}{S}\left(1+\frac{i}{K_{IVi}}\right)} \qquad (1)$$

where parameters are characterized by the following ratios,

$$K'_m > K^0_m; V' = V_0; i > 0 \qquad (2)$$

and where K'_m and V' are the values of the effective Michaelis constant, determined in the presence of the inhibitor (*i*), and the maximum reaction rates, respectively, whereas K^0_m and V^0 are the values of the same parameters of the initial (uninhibited $i=0$ and nonactivated $a=0$) enzymatic reactions. Dixon showed that K_{IVi} value of the inhibition constant can be determined by plotting the dependencies in following coordinates

$$1/v_i; i \qquad (3)$$

Herewith, the lines of dependencies of the reciprocal initial rates of the inhibited reactions ($1/v_1$) on the increased concentrations of the inhibitor, $1/v_1 = f(i)$, obtained for two (or more) constant concentrations of the cleaved substrate (for instance, in the case where $S_2 < S_1$), intercept in the second quadrant of ($1/v_1$; *i*) coordinates above the -*i*0 semiaxis, where (Fig. 1), and in the case of noncompetitive[2-5] (or catalytic, *III*$_i$ type)[6-11] of enzyme inhibition (Table 1, line 3) intersect on the -*i*0 semiaxis (Fig. 2).

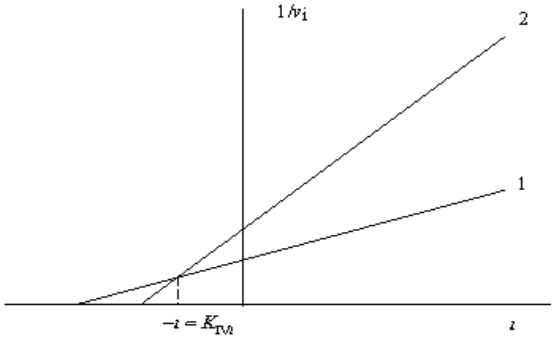


Figure 1. The lines of competitive enzyme inhibition in coordinates ($1/v_i$; *i*). Symbols: line 1 is S_1, line 2 is S_2 concentration of substrates ($S_2 < S_1$)

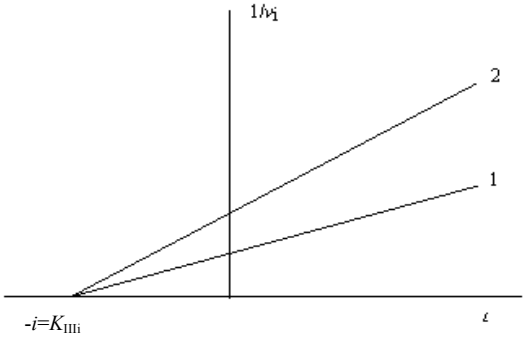


Figure 2. The lines of noncompetitive enzyme inhibition in coordinates ($1/v_i$; *i*). Symbols: line 1 is S_1, line 2 is S_2 concentration of substrates ($S_2 < S_1$).

Version 2

In Version 2 (Dixon, M. M. and Webb, L., 1962),[3] where the same equation was used (Eq. 1, text), it was shown that in the case of competitive enzyme inhibition, two (or more) experimental lines,

$$\frac{1}{v_{i(1)}} = \frac{1}{V_1^0} + \frac{K_{m1}^0}{V_1^0 S_1}\left(1 + \frac{i}{K_{IVi}}\right) = \frac{1}{V_1^0} + \frac{K_{m1}^0}{V_1^0 S_1} + \frac{K_{m1}^0}{V_1^0 S_1 K_{IVi}} i = C_{1(4)} + B_{1(4)} i \quad (4)$$

and

$$\frac{1}{v_{i(2)}} = \frac{1}{V_2^0} + \frac{K_{m2}^0}{V_2^0 S_2}\left(1 + \frac{i}{K_{IVi}}\right) = C_{2(5)} + B_{2(5)} i \quad (5)$$

which are more convenient to analyze in the form of

$$\frac{1}{v_{i(1)}} = C_{1(4)} + B_{1(4)} i \quad (6)$$

and

$$\frac{1}{v_{i(2)}} = C_{2(5)} + B_{2(5)} i \quad (7)$$

when $S_2 < S_1$, will intersect above the -i0 semiaxis where -i =K_{IVi} (Fig. 1). In this case an equality

$$\frac{1}{V_1^0} + \frac{K_{m1}^0}{V_1^0 S_1}\left(1 + \frac{i}{K_{IVi}}\right) = \frac{1}{V_2^0} + \frac{K_{m2}^0}{V_2^0 S_2}\left(1 + \frac{i}{K_{IVi}}\right) \quad (8)$$

will be simplified, to the following, if $V_1^0 = V_2^0$:

$$\frac{K_{m1}^0}{S_1}\left(1 + \frac{i}{K_{IVi}}\right) = \frac{K_{m2}^0}{S_2}\left(1 + \frac{i}{K_{IVi}}\right) \quad (9)$$

or

$$\left(\frac{K_{m2}^0}{S_2} - \frac{K_{m1}^0}{S_1}\right) \cdot \left(1 + \frac{i}{K_{IVi}}\right) = 0 \quad (10)$$

Since multiplier K_m^0 $(1/S_1 - 1/S_2)$ cannot be equal to zero, consequently equations (8 – 10) are correct, if -i = K_{IVi}.

In the second version[3] the same Figures (1) and (2) are given as in the first; (Fig. 1) demonstrates that lines intersect (1) and (2) above the -i0 semiaxis (in the case of competitive type inhibition). In the case of noncompetitive type inhibition lines intersect on the -i0 semiaxis a priori (Fig. 2) without calculations as in Eqs. 4–10.

A simplicity and convenience in calculation of K_{IVi} constants of enzyme inhibition in coordinates $(1/v_I;\ i)$[12-15] made this method to be widely used for demonstration of the type of inhibition and calculation of the constants of inhibition in a number of other cases:

1) calculate K_{IIh} constants of noncompetitive enzyme inhibition,[13-15] type *III*$_i$, (Table 1, line 3),

2) calculate K_{Ii} constant of the mixed-type[16-17] (or biparametrically coordinated, type I_i, inhibition) (Table 1, line 1),

3) calculate K_{Ih} constant of uncompetitive[16,18,19] type II_i, (Table 1, line 2) enzyme inhibition.

The vector method for the representation of enzymatic reactions (Figs. 3, 4)[6-11] showed that $\mathbf{L}_i$ vectors of enzymatic inhibited reactions are symmetrically in the counter direction relative to $\mathbf{L}_a$ vectors of activated enzymatic reactions ($\mathbf{L}_{Ii}$ and $\mathbf{L}_{Ia}$, $\mathbf{L}_{IIIi}$ and $\mathbf{L}_{IIIa}$ etc.,) in the three-dimensional $K'_mV'I$ coordinate system (Fig. 3). The positions of projections of these vectors: L_{Ii} and L_{Ia}, L_{IIIi} and L_{IIIa} etc., in the scalar two-dimensional K'_mV' coordinate system (Fig. 4) are in accord with symmetric anti directivity in the course of change of K'_m and V' parameters in reactions (similar by type) of enzyme inhibition and enzyme activation (Table 1, lines: 1 and 15; 2 and 14; 3 and 13 etc.,) and the positions of projections of these vectors: L_{Ii} and L_{Ia}; L_{IIIi} and L_{IIIa} in the scalar two-dimensional $K'_{m,}V'$ coordinate system (Fig. 4).

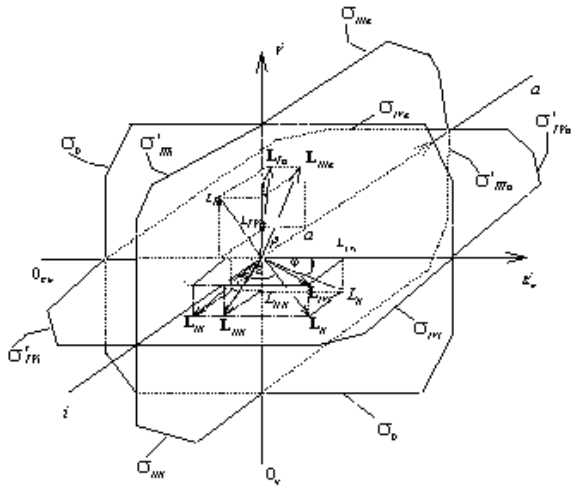


Figure 3. Three-dimensional (branched) $K'_mV'I$ of coordinate system with separate *Pi* and *Pa* semiaxes of molar concentrations of inhibitor *i* and activator *a*. The symbols of kinetic parameters: K'_m, V', K_m^0…, three-dimensional vectors: $\mathbf{L}_{Ii}$, $\mathbf{L}_{IIIi}$… $\mathbf{L}_{Ia,}$ $\mathbf{L}_{IIIa}$, and their projections L_{Ii}, L_{IIIi}... L_{Ia}, L_{IIIa} on the basic σ_0 plane as well as the symbols of projections of directing planes σ_{IVi}, σ_{IIIi}, $\sigma_{IV\alpha}$, $\sigma_{III\alpha}$ on the PK'_m, $P0_{V'}$, $P0_{K'm}$ and PV' coordinate semiaxes are given in the text.

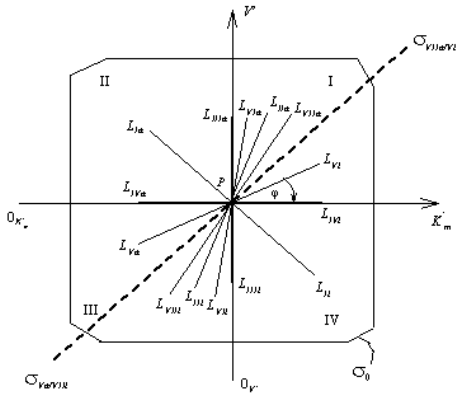


Figure 4. Two-dimensional (scalar) K'_mV' coordinate system. The symbols of kinetic parameters: K'_m, V', $K_m{}^0$ …., the projections L_{Ii}, L_{IIIi}... L_{Ia}, L_{IIIa} of three-dimensional vectors: ($\mathbf{L}_{Ii}$, $\mathbf{L}_{IIIi}$… $\mathbf{L}_{Ia}$, $\mathbf{L}_{IIIa}$) on the basic σ_0 plane (see Fig. 3) and symbols of PK'_m, $P0_{V'}$, $P0_{K'm}$ and PV' coordinate semiaxes the same as in Fig. 3. *I*, *II*, *III* and *IV* – quadrants of coordinate system.

This makes it possible to obtain the equation for calculation of the initial rates of activated ν_a and inhibited ν_i enzymatic reactions where a symmetric opposite of

$$\left(1+\frac{e}{K_э}\right)$$

multiplier (*e* – inhibitor *i* or, activator *a*) was taken into account in these equations (Table 1, lines: 1 and 15; 2 and 14 etc.,) and to propose some examples to practice of the use of the ($1/\nu_i$; *i*) coordinates for calculation of K_i constants of enzyme inhibition and the ($1/\nu_a$; *a*) coordinates for calculation of K_a constants of enzyme activation.

Correction 1. The applicability of the ($1/\nu_{IIIi}$; *i*) coordinates for data processing in noncompetitive, type III_i, enzyme inhibition (Table 1, line 3) can be shown based on the Equation (3) (Table 1) similar by the sequence given above (Eqs. 4 – 10). Namely, equation (3) in (Table 1) shows that the points of intersection ($1/v_{i11} = 1/v_{i12}$) of two experimentally obtained lines plotted by III_i type of enzyme inhibition (when $S_2 < S_1$):

$$\frac{1}{v_{i(11)}}=\frac{1}{V_1^0}+\frac{K_{m1}^0}{V_1^0 S_1}+\left(\frac{1}{V_1^0 K_{IIIi}}+\frac{K_{m1}^0}{V_1^0 S_1 K_{IIIi}}\right)i \quad (11)$$

and

$$\frac{1}{v_{i(12)}}=\left(\frac{1}{V_2^0}+\frac{K_{m2}^0}{V_2^0 S_2}\right)+\left(\frac{1}{V_2^0 K_{IIIi}}+\frac{K_{m2}^0}{V_2^0 S_2 K_{IIIi}}\right)i \quad (12)$$

or, that it is the same

$$\frac{1}{v_{i(11)}}=C_{1(11)}+B_{1(11)}i \quad (13)$$

and

$$\frac{1}{v_{i(12)}}=C_{1(12)}+B_{1(12)}i \quad (14)$$

where

$$C_{1(11)}=\frac{1}{V_1^0}+\frac{K_{m1}^0}{V_1^0 S_1}; \qquad B_{1(11)}=\frac{1}{V_1^0 K_{IIIi}}+\frac{K_{m1}^0}{V_1^0 S_1 K_{IIIi}}$$

analogous for $C_{1(12)}$ and $B_{1(12)}$ determined by the dependencies:

$$i=\frac{\begin{vmatrix}-1 & C_{1(11)}\\ -1 & C_{1(12)}\end{vmatrix}}{\begin{vmatrix}B_{1(11)} & -1\\ B_{1(12)} & -1\end{vmatrix}}; \quad \text{and} \quad \frac{1}{v_i}=\frac{\begin{vmatrix}C_{1(11)} & B_{1(11)}\\ C_{1(12)} & B_{1(12)}\end{vmatrix}}{\begin{vmatrix}B_{1(11)} & -1\\ B_{1(12)} & -1\end{vmatrix}} \quad (15)$$

should not obligatory be on -*i*0 semiaxis of the ($1/\nu_i$; *i*)) coordinates (Fig. 2).

It follows that the position of the points of intersection of experimentally obtained lines Eqs. 4 and 5 (and Eqs. 11 and 12 in the text) of enzyme inhibition in the Dixon plots does not permit to determine competitive and noncompetitive enzyme inhibition.

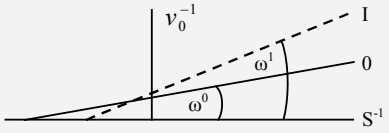
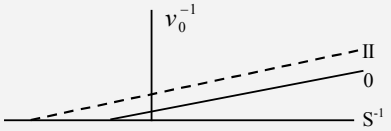
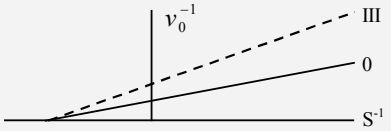
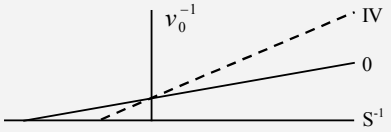
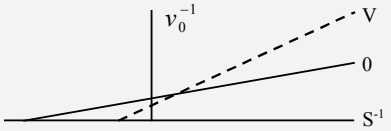
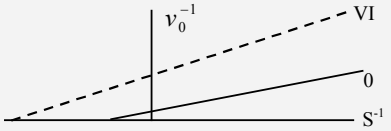
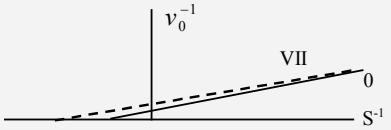
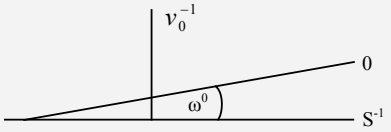
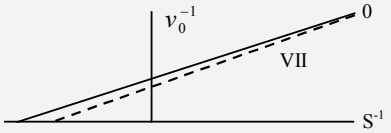
Correction 2. The analysis of Equation (1) (Table 1) shows that the experimentally obtained points (biparametrically coordinated,[6-11] type I_i or mixed-type[2-5] of enzyme inhibition, in the ($1/\nu_i$; *i*) coordinates will belong to a curve of parabolic form:

$$\frac{1}{v_{Ii}}=\left(\frac{1}{V^0}+\frac{K_m^0}{V^0 S}\right)+\left(\frac{1}{V^0 K_{IIIi}}+\frac{K_m^0}{V^0 S K_{IIIi}}+\frac{K_m^0}{V^0 S K_{IVi}}\right)i+\frac{K_m^0}{V^0 S K_{IIIi} K_{IVi}}\cdot i^2 \quad (16)$$

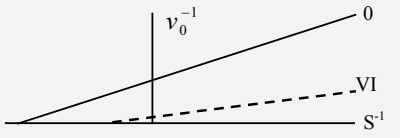
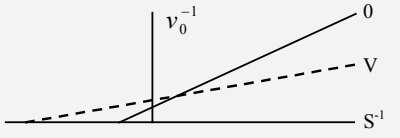
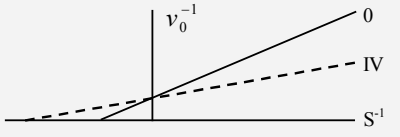
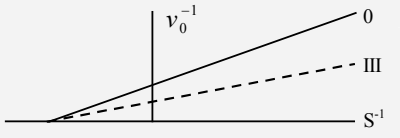
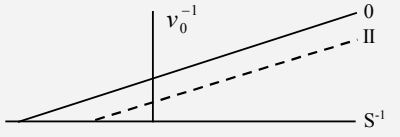
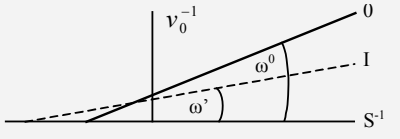
or, that it is the same:

$$\frac{1}{v_{Ii}}=C_{16}+B_{16}\cdot i+A_{16}\cdot i^2 \quad (17)$$

Table 1. Equations for calculation of the v_i and v_a initial rates of enzymic reactions

No	Effect	Type of effect	Correlation between the K'_m and V' parameters	Plots in the (v_0^{-1}; S^{-1}) coordinates	Equations for calculation of v_i and v_a (see. continuation)**
1	Inhibition ($i > 0$)	I_i	$K'_m > K_m^0$; $V' < V^0$		v_{Ii}= (Eq. 1a, in cont.)
2		II_i	$K'_m < K_m^0$; $V' < V^0$ $tg\omega' = tg\omega^0$		v_{IIi}=(Eq. 2a)
3		III_i	$K'_m = K_m^0$; $V' < V^0$		v_{IIIi}= (Eq. 3a)
4		IV_i	$K'_m > K_m^0$; $V' = V^0$		v_{IVi} = (Eq. 4a)
5		V_i	$K'_m > K_m^0$; $V' > V^0$		v_{Vi} = (Eq. 5a)
6		VI_i	$K'_m < K_m^0$; $V' < V^0$ $tg\omega' > tg\omega^0$		v_{VIi} = (Eq. 6a)
7		VII_i	$K'_m < K_m^0$; $V' < V^0$ $tg\omega' < tg\omega^0$		v_{VIIi} = (Eq. 7a)
8	No effect	I_0	$K'_m = K_m^0$; $V' = V^0$		v_0 = (Eq. 8a)
9	Activation ($a > 0$)	VII_a	$K'_m > K_m^0$; $V' > V^0$ $tg\omega' > tg\omega^0$		v_{VIIa} = (Eq. 9a)

Contg. Table 1.

10		VI_a	$K'_m > K_m^0$; $V' > V^0$ $tg\omega' < tg\omega^0$		ν_{VIa}= (Eq. 10a)
11		V_a	$K'_m < K_m^0$; $V' < V^0$		ν_{Va}= (Eq. 11a)
12		IV_a	$K'_m < K_m^0$; $V' = V^0$		ν_{IVa}= (Eq. 12a)
13		III_a	$K'_m = K_m^0$; $V' > V^0$		ν_{IIIa} = (Eq. 13a)
14		II_a	$K'_m > K_m^0$; $V' > V^0$ $tg\omega' = tg\omega^0$		ν_{IIa} = (Eq. 14a)
* 15		I_a	$K'_m < K_m^0$; $V' > V^0$		ν_{Ia} = (Eq. 15a)

*The symbol of a plots in Figs. 1-15 corresponds to the type of reaction under study. For example: line 0 characterizes the position of initial (nonactivated) enzymatic reaction, line I – the position of a plot representing the I_a type of activated enzymatic reaction (Fig. 15) etc.

****Inhibited reactions:**

№ 1. (type I_i, biparametrically coordinated inhibition)

$$v_{Ii} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{yi}}\right)}}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{xi}}\right)} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \qquad (1a)$$

№ 2. (type II_i, unassociative inhibition)

$$v_{IIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{yi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{xa}}\right)}} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \qquad (2a)$$

№ 3. (type III_i, catalytic inhibition)

$$v_{IIIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{yi}}\right)}}{1+\frac{K_m^0}{S}} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}} \qquad (3a)$$

№ 4. (type IV_i, associative inhibition)

$$v_{IVi} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{xi}}\right)} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \qquad (4a)$$

№ 5. (type V_i, pseudoinhibition)

$$v_{Vi} = \frac{V^0 \cdot \left(1+\frac{i}{K_{ya}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1+\frac{i}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \quad (5a)$$

№ 6. (type VI_i, discoordinated inhibition)

$$v_{VIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{yi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{xa}}\right)}} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \quad (6a)$$

№ 7. (type VII_i, transient inhibition)

$$v_{VIIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{yi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{xa}}\right)}} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \quad (7a)$$

№ 8. **Initial** (uninhibited and nonactivated) reaction

$$v_0 = \frac{V^0}{1+\frac{K_m^0}{S}} \quad (8a)$$

Activated reactions:

№ 9. (type VII_a, transient activation)

$$v_{VIIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{ya}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{IVi}}\right)} \quad (9a)$$

№ 10. (type VI_a, discoordinated activation)

$$v_{VIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{ya}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{IVi}}\right)} \quad (10a)$$

№ 11. (type V_a, pseudoactivation)

$$v_{Va} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{a}{K_{ya}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{xa}}\right)}} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{a}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{IVa}}\right)}} \quad (11a)$$

№ 12. (type IV_a, associative activation)

$$v_{IVa} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{xa}}\right)}} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{IVa}}\right)}} \quad (12a)$$

№ 13. (*type* III_a, *catalytic activation*)

$$v_{IIIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{ya}}\right)}{1+\frac{K_m^0}{S}} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}} \quad (13a)$$

№ 14. (type II_a, unassociative activation)

$$v_{IIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{ya}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{IVi}}\right)} \quad (14a)$$

№ 15. (type I_a, biparametrically coordinated activation)

$$v_{Ia} = \frac{V^0 \cdot \left(1+\frac{a}{K_{ya}}\right)}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{xa}}\right)}} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{IVa}}\right)}} \quad (15a)$$

which when $B^2 > 4AC$, will intersect the -i0 semiaxis in two negative points: the nearest (descending curve):

$$i_1 = \frac{-\left(\frac{1}{V^0 K_{\mathrm{IIIi}}} + \frac{K^0_{\mathrm{m}}}{V^0 S K_{\mathrm{IVi}}} + \frac{K^0_{\mathrm{m}}}{V^0 S K_{\mathrm{IIIi}}}\right)}{2\left(\frac{K^0_{\mathrm{m}}}{V^0 S K_{\mathrm{IIIi}} K_{\mathrm{IVi}}}\right)} + \frac{\sqrt{B^2 - 4\left(\frac{K^0_{\mathrm{m}}}{V^0 S K_{\mathrm{IIIi} K_{\mathrm{IVi}}}}\right)\cdot\left(\frac{1}{V^0} + \frac{K^0_{\mathrm{m}}}{V^0 S}\right)}}{2\left(\frac{K^0_{\mathrm{m}}}{V^0 S K_{\mathrm{IIIi}} K_{\mathrm{IVi}}}\right)} \qquad (18)$$

and the far (ascending curve):

$$i_2 = \frac{-B - \sqrt{B^2 - 4AC}}{2A} \qquad (19)$$

As it is seen from Eqs. (16 – 19), neither -i_1 nor -i_2 points of intersection on the -i0 semiaxis have simple relations to the K_{IIIi} and K_{IVi} constants of inhibition, and moreover, the curvature of the plot described by (Eqs. 16, 17) does not permit linear extrapolation dependencies $1/\nu_{\mathrm{Ii}} = \mathrm{f}(i)$ for determination of the K_{Ii} constants in the ($1/\nu_{\mathrm{Ii}}$; i) coordinates.

Examples of processing experimental data, I_{i} type, of enzyme inhibition in the ($1/\nu_{\mathrm{Ii}}$; i) coordinates are available for calculation of the K_{Ii} constants by the point of intersection of the lines over the -i0 semiaxis,[15-17] it is most probably due to a weakly expressed curvature of parabola (Eq. 17) in the intervals which are determined by:

a) the range of i_1-i_{n} concentrations of the inhibitor used and concentrations of S_1 and S_2 substrates in the intervals of curves and

b) the spread in the results of ν_{Ii} determination.

Correction 3. From Eq. (2) (Table 1) it is possible to see that experimentally obtained points of unassociative, type II_{i} enzyme inhibition, in the ($1/\nu_{\mathrm{IIi}}$; i) coordinates will belong to the curve of linear fractional dependence

$$\frac{1}{\nu_{\mathrm{IIi}}} = \frac{1}{V^0} + \frac{i}{V^0 K_{\mathrm{IIIi}}} + \frac{K^0_{\mathrm{m}}}{V^0 S}\frac{\left(1 + \frac{i}{K_{\mathrm{IIIi}}}\right)}{\left(1 + \frac{i}{K_{\mathrm{IVa}}}\right)} \qquad (20)$$

which in case when K_{IIIi}=K_{IVa} will be simplified as a straight line in the form as:

$$\frac{1}{\nu_{\mathrm{IIi}}} = C_{1(20)} + B_{1(20)} i \qquad (21)$$

where

$$C_{1(20)} = \frac{1}{V_1^0} + \frac{K^9_{m1}}{V_1^0 S_1}; \qquad B_{1(20)} = \frac{1}{V_1^0 K_{\mathrm{III}i}}$$

analogously for $C_{1(22)}$ and $B_{1(22)}$ determined by the appropriate dependencies. At another concentration of a substrate (for, instance, when $S_2 < S_1$), the second line:

$$\frac{1}{\nu_{\mathrm{IIi}}} = C_{1(22)} + B_{1(22)} \cdot i \qquad (22)$$

will be plotted above the first one and the intercept will be longer by $(C_{1(22)} + B_{1(22)})/(C_{1(21)} + B_{1(21)})$ times as compared to the previous one, it implies that the lines (Eqs. 21 and 22) have no point of intersection.

In experiments the ($1/\nu_{\mathrm{IIi}}$; i) coordinates are often used for analysis of data of uncompetitive type of enzyme inhibition (Table 1, Line 2) demonstrating the parallelity of the straight lines (Eqs. 21 and 22) and also the points of their intersection[16,18-20] that could be caused by the spread in the experimental $1/\nu_{\mathrm{IIi}}$ points or subjective reasons (Correction 8).

Correction 4. Table 1 shows that algebraic forms of Eqs. 2, 6 and 7 (biparametrically discoordinated: II_{i}, VI_{i}, and VII_{i} types of enzyme inhibition) are identical as the result of coincidence of the positions of $\mathbf{L}_{\mathrm{IIi}}$, $\mathbf{L}_{\mathrm{VIi}}$ and $\mathbf{L}_{\mathrm{VIIi}}$ vectors of these reactions in one octant of the $K'_{\mathrm{m}}V'I$ coordinates system (Fig. 3) and their orthogonal projections on basic σ_0 plane (Fig. 4) characterized by similar ratio of K'_{m} and V' parameters (Table 1). These individual types of enzyme inhibition are different in angles of slopes of the experimentally obtained lines in Lineweaver-Burk plots (Table 1, lines: 2 and 14, 6 and 10, 7 and 9). The analysis of the forms of equations (6 and 7, Table 1) shows the situation as discussed above (Correction 3). Namely,

a) at the second concentration of substrate (S_2), if an equality K_{IIIi}=K_{IVa} becomes K_{IVa}>K_{IIIi}, the experimental points of the second dependence will form the curve without points of intersection with the first line in the II quadrant of the $1/\nu_{\mathrm{VIi}}$; i) (and $1/\nu_{\mathrm{VIIi}}$; i) coordinate (Math & Stat, Queen's University, Canada 1987, by Bell I., Davis J. and Rice S.) permitting of no linear extrapolation of the $1/\nu_{\mathrm{VIi}}$; (and $1/\nu_{\mathrm{VIIi}}$) points.

b) if the equality K_{IIIi}=K_{IVa} becomes K_{IVa}<K_{IIIi}, then the second curved graph (Eq. 22) will intersects the first graph (Eq. 21) left off y semiaxis in the II quadrant of the $1/\nu_{\mathrm{VIi}}$; i) (and $1/\nu_{\mathrm{VIIi}}$; i) coordinate system (Figs. 1 and 2), but the curvature of the second graph allows of no linear extrapolation of the $1/\nu_{\mathrm{VIi}}$; (and $1/\nu_{\mathrm{VIIi}}$) points.

Correction 5. Equations (5 and 11) (Table 1), which are symmetrically opposite by the

$$\left(1+\frac{e}{K_{э}}\right)$$

multiplier, are also symmetrically opposite and in Dixon plots. The position of the multiplier in the denominator (Eq. 23) leads to complication in calculation of the K_{Vi} constants.

$$\frac{1}{v_{Vi}}=\frac{1}{V^0\left(1+\frac{i}{K_{IIIa}}\right)}+\frac{K_m^0}{V^0S}\frac{\left(1+\frac{i}{K_{IVi}}\right)}{\left(1+\frac{i}{K_{IIIa}}\right)} \qquad (23)$$

but in Eq. (24) it is in the numerator:

$$\frac{1}{v_{Va}}=\frac{1}{V^0}\left(1+\frac{a}{K_{IIIi}}\right)+\frac{K_m^0}{V^0S}\frac{\left(1+\frac{a}{K_{IIIi}}\right)}{\left(1+\frac{a}{K_{IVa}}\right)} \qquad (24)$$

From it follows that if an equality K_{IIIa}=K_{IVi} the Equation (23) represents hyperbolic dependence in the form:

$$\frac{1}{v_{Vi}}=\frac{K_m^0}{V^0S}+\frac{1}{V^0}\left(\frac{K_{IIIa}}{i+K_{IIIa}}\right)=C_{23}+B_{23}\left(\frac{K_{IIIa}}{i+K_{IIIa}}\right) \qquad (25)$$

This curve does not intersect the *y* semiaxis in the ($1/v_i$; i) coordinates, but Equation (24) if an equality K_{IIIi}=K_{IVa} will be simplified as a straight line in the form as:

$$\frac{1}{v_{Va}}=C_{24}+B_{24}a \qquad (26)$$

represents linear dependence of experimental points characterizing the position of a series of parallel lines without intersection points (see Correction 3).

Correction 6. Equation (12) (Table 1) similar to Eq. (4) of this table in the ($1/v_{IVa}$; a) coordinates transforms into the equation of hyperbolic dependence:

$$\frac{1}{v_{IVa}}=\frac{1}{V^{0,}}+\frac{K_m^0}{V^0S}\left(\frac{K_{IVa}}{K_{IVa}+a}\right) \qquad (27)$$

or,

$$\frac{1}{v_{IVa}}=C_{27}+B_{27}\left(\frac{K_{IVa}}{K_{IVa}+a}\right) \qquad (28)$$

permitting of no linear extrapolation of the $1/v_{IVa}$ points.

Equation (13) (Table 1) similar to equation (3) (Table 1) in the ($1/v_{IIIa}$; a) coordinates also transforms into the equation:

$$\frac{1}{v_{IIIa}}=\left(\frac{1}{V^0}+\frac{K_m^0}{V^0S}\right)\frac{K_{IIIa}}{K_{IIIa}+a} \qquad (29)$$

of hyperbolic dependence:

$$\frac{1}{v_{IIIa}}=C_{29}\left(\frac{1}{1+a/K_{IIIa}}\right) \qquad (30)$$

permitting of no linear extrapolation of the $1/v_{IIIa}$ points.

Correction 7. As expected equation (15) (Table 1) similar to (Eq. 1) (Table 1) in the ($1/v_{Ia}$; a) coordinates characterizes the position of the $1/v_{Ia}$ points on the curve of a reciprocal quadratic dependence:

$$\frac{1}{v_{Ia}}=\frac{1}{C_{31}}+\frac{1}{B_{31}}\cdot\frac{1}{a}+\frac{1}{A_{31}}\cdot\frac{1}{a^2} \qquad (31)$$

permitting of no linear extrapolation of the $1/v_{Ia}$ points in ($1/v_{Ia}$; a) coordinates.

Correction 8. To represent data on type II_i of enzyme inhibition in the ($1/v_{IIi}$; i) coordinates we use the results of study on the inhibitory effect of the increasing concentrations of isopropanol (i-PrOH) on the initial rates of cleavage of p-nitrophenylphosphate (pNPP) catalyzed by eel alkaline phosphatase,[21] the enzyme (EC 3.1.3.1) – a product of Sigma (USA).

The results of the study (Fig. 5), (SigmaPlot 10, USA) show that the presence of the inhibitor at concentration of 0.0002 M leads to the change in the parameters of pNPP cleavage: V'= 2.927 μmol·min^{-1}μg protein^{-1}, K'_m= 4.47·10^{-5} M (V^0= 3.162·μmol·min^{-1}μg protein^{-1}, K^0_m= 4.824·10^{-5} M), at the inhibitor concentration of 0.0005 M they changed to: V'= 2.66 μmol·min^{-1}μg protein^{-1}) K'_m= 4.071·10^{-5} M and at the inhibitor concentration of 0.001 M they changed to V' = 2.307·10^{-5} μmol·min^{-1}μg protein^{-1} K'_m= = 3.525·10^{-5} M.

Table 2. Equations for calculation of the K_i and K_a constants

Type of effect	New name of the types of enzymic reactions	Traditional name	Equation for calculation of the K_i and K_a constants
I_i	biparametrically coordinated inhibition	mixed inhibition	$K_{Ii} = \frac{i}{\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}}$
II_i	unassociative inhibition	uncompetitive inhibition	$K_{IIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}}$
III_i	catalytic inhibition	noncompetitive inhibition	$K_{IIIi} = \frac{i}{V^0 / V' - 1}$
IV_i	associative inhibition	competitive inhibition	$K_{IVi} = \frac{i}{K_m' / K_m^0 - 1}$
V_i	pseudoinhibition		$K_{Vi} = \frac{i}{\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}}$
VI_i	discoordinated inhibition		$K_{VIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}}$
VII_i	transient inhibition		$K_{VIIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}}$
I_0	initial (uninhibited $i = 0$ and and nonactivated) enzymatic reaction		
VII_a	transient activation		$K_{VIIa} = \frac{a}{\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}}$

contg. Table 2.

VI_a	discoordinated activation		$K_{VIa} = \frac{a}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
V_a	pseudoactivation		$K_{Va} = \frac{a}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
IV_a	associative activation	competitive activation	$K_{IVa} = \frac{a}{K_m^0 / K_m^{'} - 1}$
III_a	catalytic activation	noncompetitive activation	$K_{IIIa} = \frac{a}{V^{'} / V^0 - 1}$
II_a	unassociative activation	uncompetitive activation	$K_{IIa} = \frac{a}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
I_a	biparametrically coordinated activation *	mixed activation	$K_{Ia} = \frac{a}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$

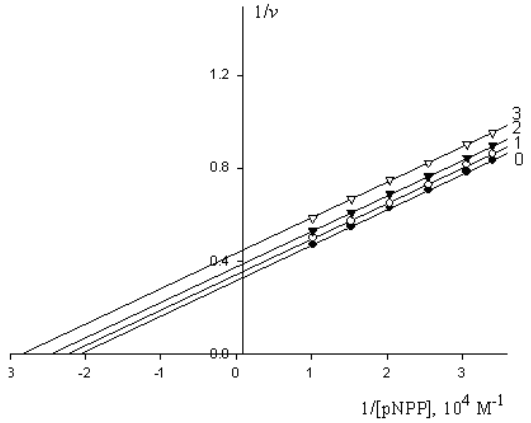


Figure 5. The inhibitory effect of isopropanol (*i*) on the initial rates of pNPP cleavage catalyzed by -eel alkaline phosphatase in the Lineweaver-Burk plot. The concentration of the inhibitor (M); 0.0002; 0.0005 and 0.001 are line 1, 2 and 3, respectively. Line 0 – inhibitor is absent, ν μmol·min^{-1}μg protein^{-1}.

This is in accord with type II_i of unassociative enzyme inhibition (Table 1, line 2).

The vector method of the representation of enzymatic reactions in the $K'_mV'I$ coordinate system[6-11] showed that in order to calculate the K_{IIi} constant of this type enzyme inhibition, the following equation is valid:

$$K_{IIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}} \quad (32)$$

If one substitutes values using data from Fig. 5 in this equation, then it is possible to calculate the following values of the K_{IIi} (10^{-3} M): 1.77; 1.89 and 1.91 at the first, second and third concentration of isopropanol, respectively.

With Equation (32) the other, more desirable possibility of calculating these constants emerges, i.e. plotting the dependencies of alteration to the value of a denominator (A) in this equation in the (A, i) coordinates:

$$A = 1/K_{Iii} \cdot i \quad (33)$$

hence,

$$K_{IIi} = 1/\text{tg a}, \quad (34)$$

where (tg a) is an angle of the slope of the experimentally obtained line (Fig. 6) to 0i semiaxis.

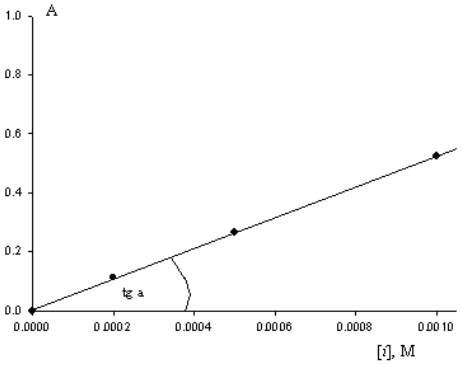


Figure 6. The dependence of alteration to A parameters (of Eq. 32) based on data from Fig. 5 on the increasing concentration of isopropanol.

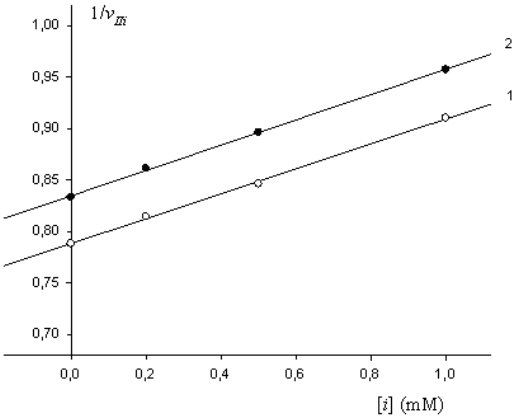


Figure 7. Representation of Fig. 6 data in the $1/v_{IIi}$; i coordinates. Key: line 1 is the concentration of pNPP $0.98 \cdot 10^{-4}$ M, line 2 is the concentration of pNPP $0.49 \cdot 10^{-4}$ M.

This gives the average (best possible) value of the constant of inhibition $K_{IIi} = 1.83\ 10^{-3}$ M. It points to a more than 30-time weaker binding ($K_{IIi}/K_m^0 = 183/4.8 = 31$) of the enzyme to isopropanol as compared to a substrate.

According to data representation (Fig. 5) in the ($1/n_{IIi}$; i) coordinates (Fig. 7, SigmaPlot 10) straight line 2 is over straight line 1 and they are parallel, namely these straight lines have no points of intersection. Hence, there is no possibility to calculate the value of K_{IIi} constant of enzyme inhibition with the help of the ($1/n_{IIi}$; i) coordinates.[17,22,23]

It was shown (Fig. 6) that in this case it is necessary to use equation (2) (Table 2).

Conclusions

1. The results of the analysis (Corrections 1 – 8) show that the presence of the intersection point of straight lines in the Dixon plots is insufficient to refer the mechanism of enzyme inhibition as competitive, noncompetitive, mixed-type or uncompetitive type without the representation of similar data in the Lineweaver-Burk plot.[19, 22-27]

2. Attempts to use parallelism of graphs plotted in the ($1/v_i$; i) coordinates in order to prove the mechanism of enzyme inhibition without referring to the program of plotting these graphs are also unconvincing since the opinion of scientists may be different considering whether the straight lines are parallel or not.

3. To calculate K_i constant of enzyme inhibition (and K_a constant of enzyme activation) taking into account the presence of sources of possible errors (Corrections 2 – 8) it is recommended to plot dependencies in the Lineweaver-Burk plot for which simple methods for determination of reaction types are developed and equations for calculation of the appropriate constants are obtained (Table 2).[7,10,11]

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