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STATISTICAL ANALYSES OF ENZYME KINETICS: INHIBITION

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Using multivariate linear regression approach, open-source scripts have been developed in Gauss 4.0. for the calculation of the kinetic parameters and their dispersions for the cases of competitive, none competitive, mixed and substrate inhibitions. The methods of derivation of corresponding equations of stationary kinetics, their linearization and derivation of analytic forms of kinetic parameters and their dispersions are presented.

Stationary kinetics – inhibition – multivariate linear regression – kinetic parameters – calculation

Կիրառելով բազմաչափ գծային ռեգրեսիայի մոտեցումը՝ Գաուս 4.0. լեզվով հանրամատչելի ծրագրեր են մշակվել մրցակցային, անմրցակից, խառը և սուբստրատային արգելակումների դեպքերում կինետիկական պարամետրերի և դրանց դիսպերսիաների հաշվարկման համար: Ներկայացվել են ստացիոնար կինետիկայի համապատասխան հավասարումների դուրս բերման, դրանց գծայնացման և կինետիկական պարամետրերի և դրանց դիսպերսիաների հավասարումների դուրս բերման մեթոդները:

Ստացիոնար կինետիկա – արգելակում – բազմաչափ գծային ռեգրեսիա – կինետիկական պարամետրեր – հաշվարկում

Используя подход многомерной линейной регрессии, были разработаны публично доступные программы на Gauss 4.0. для расчета кинетических параметров и их дисперсий для случаев конкурентного, бесконкурентного, смешанного и субстратного типов ингибирования. Представлены методы вывода соответствующих уравнений стационарной кинетики, их линеаризации и вывода аналитических форм кинетических параметров и их дисперсий.

Стационарная кинетика – ингибирование – многомерная линейная регрессия – кинетические параметры – расчет

In biochemical researches, mostly in enzyme kinetics, the data set concerning stationary (steady state) kinetics is usually analyzed graphically. In studies of types of enzyme inhibition, the dependence of $1/\nu$ and s/ν on i for different substrate concentrations are used [4, 14]. But this approach causes the strongest criticism from statisticians and gives very unreliable values for K_M, V_{max} and K_I. This approach did not allow estimating the weight of the experimental point when tracing the line through the set of points, and also it did not allow estimating the mistakes in determination of the kinetic parameters. Therefore, the best approach in solving the problems of enzyme inhibition is the statistical approach, namely statistical approaches based on linear regression analysis. This approach enables to estimate the kinetic parameters and their errors taking into account the weights of each experimental point [4].

Kinetic parameters of analysis of enzymatic inhibition in the case of stationary kinetics are easily calculated solving systems of linear differential equations. However, the method of King and Altman, based on graph theory, provides visual and familiar formulas for a biochemist to calculate the initial rates of enzymatic reactions, in which there are clearly distinguished parameters (maximum speed of reaction, the Michaelis constant, inhibition constants, etc.) that are interesting to us [12].

In previous paper using multivariate linear regression approach, open-source scripts have been developed in Gauss 4.0. for the calculation of the kinetic parameters and their dispersions for the cases of simple Michaelis-Menten type and the bi-bi ping-pong type enzyme kinetics [10].

Now we intend to present to the scientific community the statistical data processing methods and scripts calculating the relevant kinetic parameters of enzymatic inhibition in the case of stationary kinetics. These approaches have been used in our works concerning the enzymatic inhibition [1-3, 6-9, 11, 13].

The aim of this work is to derive equations that allow the calculation of the parameters of competitive, none-competitive, mixed and substrate type inhibitions in the case of stationary kinetics, to develop an algorithm that distinguishes among competitive, none-competitive and mixed type inhibitions, as well as to present the corresponding scripts in Gauss 4.0.

Materials and methods. The terms and symbols used by Cornish-Bowden in Chapter 10 [4] were used in the present work.

The equations for reaction velocities of competitive, none-competitive, mixed and substrate type inhibitions were derived using Kings and Altmans graph theory [12].

Equations for kinetic parameters and their variances were derived using multivariate linear regression analyses [5].

The scripts for calculation of kinetic parameters and their variances were written on the matrix language – Gauss 4.0. (Aptech Systems, Inc.).

Results and Discussion. Firstly, we are going to represent the equations and scripts for calculating the steady state kinetic parameters in the case of competitive, none-competitive and mixed type enzyme inhibitions and afterward we will discuss the kinetic parameters for substrate type enzyme inhibitions.

The equation for initial velocity of *competitive type enzyme inhibition* derived using Kings and Altmans graph theory [12] is presented in (1).

$$\nu = \frac{V_{max} S}{K_M + \frac{K_M}{K_1} I + S} \qquad (1)$$

Where: ν is initial velocity, V_{max} – maximal velocity, S – substrate concentration, K_M – Michaelis constant and K_1 – inhibition constant for competitive type enzyme inhibition.

This equation is linearized as follows:

$$\frac{s}{\nu} = \frac{K_M}{V_{max}} + \frac{K_M}{V_{max} K_1} 1 + \frac{1}{V_{max}} S \qquad (2)$$

Denoting:

$$\frac{S}{\nu} \to y\,, \quad \mathbf{1} \to x_1\,, I \to x_2, S \to x_3\,, \frac{K_M}{V_{max}} \to \beta_1\,, \quad \frac{K_M}{V_{max} K_1} \to \beta_2\,, \quad \frac{1}{V_{max}} \to \beta_3\,,$$

we can describe the linearized equation for velocity of the enzymatic reaction (2) together with measurement errors (ε) in vector form:

$$\vec{y} = \vec{X}\vec{\beta} + \vec{\in} \qquad (3)$$

β-s and their variances can be calculated according the Gauss-Markov theorem [5] by following equations:

$$\beta = (X'X)^{-1}X'y\,, \quad V_{(\beta)} = \sigma_0^2(X'X)^{-1} \qquad (4)$$

where: X' is the transposed matrix X, $(X'X)^{-1}$ – inverse matrix $(X'X)$, $V_{(\beta)}$ – variances of β, and σ_0^2 – experimental variance.

When the measurements are given weights, the equations (4) are converted to (5).

$$\beta = ((wX)'X)^{-1}(wX)'y\,, \quad V_{(\beta)} = \sigma_0^2((wX)'X)^{-1} = \sigma_0^2 Z^{-1} = \sigma_0^2 U \qquad (5)$$

The experimental variance is calculated from equations (6).

$$\sigma_0^2 = \frac{SS}{N-p}\,, \quad SS = \Sigma we^2 = \Sigma w_i(y_i - x_i\beta_i)^2 = \Sigma ud^2 \qquad (6)$$

where: SS is the sum of the weighted squares, N – number of measurements, P – number of degrees of freedom (for competitive type enzyme inhibition P=3), e – estimate of ε deviation of y, d – estimate of δ deviation of v, u – weight of d.

$w \approx \frac{v^4}{s^2}$ were calculated by us (derivations are not presented).

The equations for calculating V_{max}, K_M and K_1 and their dispersions (variances) are the followings:

$$V_{max} = \frac{1}{\beta_3}\,, \quad K_M = \frac{\beta_1}{\beta_3}\,, \quad K_1 = \frac{\beta_2}{\beta_3} \qquad (7)$$

$$V_{(V_{max})} = V_{(V_{\frac{1}{\beta_3}})} = \sigma_0^2\frac{M_{33}}{\beta_3^4}\,, \quad V_{(K_M)} = V_{(V_{\frac{\beta_1}{\beta_3}})} = \sigma_0^2\left(\frac{M_{11}}{\beta_3^2} - 2\frac{\beta_1 M_{13}}{\beta_3^3} + \frac{\beta_1^2 M_{33}}{\beta_3^4}\right), \qquad (8)$$

$$V_{(K_1)} = V_{(\frac{\beta_2}{\beta_3})} = \sigma_0^2\left(\frac{M_{22}}{\beta_3^2} - 2\frac{\beta_2 M_{23}}{\beta_3^3} + \frac{\beta_2^2 M_{33}}{\beta_3^4}\right)$$

where: β_1, β_2 and β_3 can be calculated by equations (5), σ_0^2 – by equations (6), and M_{ij} – is the (i, j) element of matrix U (5).

The equation for initial velocity of *none-competitive type enzyme inhibition* derived using Kings and Altmans graph theory [12] is presented in (9).

$$v = \frac{V_{max}S}{K_M + S + \frac{1}{K_2}IS} \qquad (9)$$

where: v is initial velocity, V_{max} – maximal velocity, S – substrate concentration, K_M – Michaelis constant and K_2 – inhibition constant for none-competitive type enzyme inhibition.

This equation is linearized as follows:

$$\frac{S}{v} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}}S + \frac{1}{V_{max}K_2}IS \qquad (10)$$

Denoting:

$$\frac{S}{v} \to y\,, \quad 1 \to x_1\,, \quad S \to x_2\,, \quad IS \to x_3\,, \quad \frac{K_M}{V_{max}} \to \beta_1\,, \quad \frac{1}{V_{max}} \to \beta_2\,, \quad \frac{1}{V_{max}K_2} \to \beta_3\,,$$

we can describe the linearized equation for velocity of the enzymatic reaction (10) together with measurement errors (ε) in vector form (3).

Here again β-s and their variances can be calculated according the Gauss-Markov theorem [5] by equations (4).

When the measurements are given weights, the equations (4) are converted to (5).

And again experimental variance is calculated from equations (6), where in the case of none-competitive inhibition: *SS* is the sum of the weighted squares, N – number of measurements, P – number of degrees of freedom (for none-competitive type enzyme inhibition P=3), e – estimate of ε deviation of y, d – estimate of δ deviation of v, u – weight of d.

For none-competitive type inhibition $w \approx \frac{v^4}{s^2}$ were calculated by us (derivations are not presented).

The equations for calculating V_{max}, K_M and K_2 and their dispersions (variances) are the followings:

$$v_{max} = \frac{1}{\beta_2}\,, \quad K_M = \frac{\beta_1}{\beta_2}\,, \quad K_2 = \frac{\beta_2}{\beta_3} \tag{11},$$

$$V_{(V_{max})} = V_{(V_{\frac{1}{\beta_2}})} = \sigma_0^2 \frac{M_{22}}{\beta_2^4}\,, \quad V_{(K_M)} = V_{(V_{\frac{\beta_1}{\beta_2}})} = \sigma_0^2 \left(\frac{M_{11}}{\beta_2^2} - 2\frac{\beta_1 M_{12}}{\beta_2^3} + \frac{\beta_1^2 M_{22}}{\beta_2^4}\right), \tag{12}$$

$$V_{(K_2)} = V_{(\frac{\beta_2}{\beta_3})} = \sigma_0^2 \left(\frac{M_{22}}{\beta_3^2} - 2\frac{\beta_2 M_{23}}{\beta_3^3} + \frac{\beta_2^2 M_{33}}{\beta_3^4}\right)$$

where: β_1, β_2 and β_3 can be calculated by equations (5), σ_0^2 – by equations (6), and M_{ij} – is the (i, j) element of matrix U (5).

The equation for initial velocity of *mixed type enzyme inhibition* derived using Kings and Altmans graph theory [12] is presented in (13).

$$v = \frac{V_{max}S}{K_M + \frac{K_M}{K_1}I + S + \frac{1}{K_2}IS} \tag{13}$$

where: v is initial velocity, V_{max} – maximal velocity, S – substrate concentration, K_M – Michaelis constant, K_1 – inhibition constant for competitive type enzyme inhibition and K_2 – inhibition constant for none-competitive type enzyme inhibition.

This equation is linearized as follows:

$$\frac{S}{v} = \frac{K_M}{V_{max}} + \frac{K_M}{V_{max}K_1}I + \frac{1}{V_{max}}S + \frac{1}{V_{max}K_2}IS \tag{14}$$

Denoting: $\frac{s}{v} \to y\,,\quad \mathbf{1} \to x_1,\ I \to x_2,\ S \to x_3,\ IS \to x_4,\ \frac{K_M}{V_{max}} \to \beta_1,\ \frac{K_M}{V_{max}K_1} \to \beta_2,$

$\frac{1}{V_{max}} \to \beta_3,\ \frac{1}{V_{max}K_2} \to \beta_4,$

we can describe the linearized equation for velocity of the enzymatic reaction (14) together with measurement errors (ε) in vector form (3).

Here again β-s and their variances can be calculated according the Gauss-Markov theorem [5] by equations (4).

When the measurements are given weights, the equations (4) are converted to (5).

And again experimental variance is calculated from equations (6), where in the case of mixed inhibition: *SS* is the sum of the weighted squares, N – number of measurements, P – number of degrees of freedom (for mixed type enzyme inhibition P=4), e – estimate of ε deviation of y, d – estimate of δ deviation of v, u – weight of d.

For mixed type inhibition $w \approx \frac{v^4}{s^2}$ were calculated by us (derivations are not presented).

The equations for calculating V_{max}, K_M, K_1 and K_2 and their dispersions (variances) are the followings:

$$V_{max} = \frac{1}{\beta_3},\quad K_M = \frac{\beta_1}{\beta_3},\quad K_1 = \frac{\beta_1}{\beta_2},\quad K_2 = \frac{\beta_3}{\beta_4} \qquad (15),$$

$$V_{(V_{max})} = V_{\left(\frac{1}{\beta_3}\right)} = \sigma_0^2 \frac{M_{33}}{\beta_3^4},\quad V_{(K_M)} = V_{\left(\frac{\beta_1}{\beta_3}\right)} = \sigma_0^2\left(\frac{M_{11}}{\beta_3^2} - 2\frac{\beta_1 M_{13}}{\beta_3^3} + \frac{\beta_1^2 M_{33}}{\beta_3^4}\right),$$

$$V_{(K_1)} = V_{\left(\frac{\beta_1}{\beta_2}\right)} = \sigma_0^2\left(\frac{M_{11}}{\beta_2^2} - 2\frac{\beta_1 M_{12}}{\beta_2^3} + \frac{\beta_2^2 M_{22}}{\beta_2^4}\right),\quad V_{(K_2)} = V_{\left(\frac{\beta_3}{\beta_4}\right)} = \sigma_0^2\left(\frac{M_{33}}{\beta_4^2} - 2\frac{\beta_3 M_{34}}{\beta_4^3} + \frac{\beta_3^2 M_{44}}{\beta_4^4}\right)$$

(16)

where: $\beta_1, \beta_2, \beta_3$ and β_4 can be calculated by equations (5), σ_0^2 – by equations (6), and M_{ij} – is the (i, j) element of matrix U (5).

The script with the algorithm that distinguishes among competitive, none-competitive and mixed type inhibitions, as well as calculates the parameters of guessed competitive, none-competitive or mixed type inhibitions and their variances, written in Gauss 4.0, is presented in fig. 1.

The developed logical block distinguishes among the inhibition mechanisms on the base of minimization of the mean of variances of the parameter (i) divided on the parameter for each mechanism (i=1-4 for mixed type inhibition and i=1-3 for competitive and none-competitive type inhibitions). We cannot provide the strong mathematical evidence for this phenomenon, but in every subsequently presented experimental example the inhibition mechanism obtained from the form of curves $1/v - I$ and $S/v - I$ [4] coincided with the mechanism predicted by provided script code.

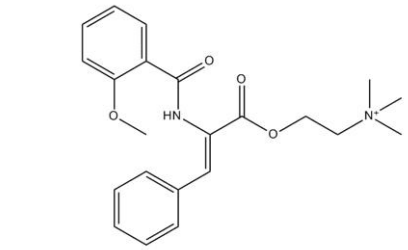
We used the presented multivariate linear regression approach and corresponding script for study the mechanism of inhibition of horse acetylcholinesterase (AChE) and human butyrylcholinesterase (BChE) and corresponding kinetic parameters and their variances with newly synthesized derivative of α,β-dehydrophenylalanine (fig. 2) [1-2, 7-8].

```
A
print "Results";
n=16; k=4; l=3;
fname="D:\\My Documents_D\\Inhib.xls";
{dat1,names}=import(fname,"a1:c17",1);

B
y=dat1[1:n,1]./dat1[1:n,3];
w=dat1[1:n,3].^4 ./dat1[1:n,1].^2;
x1=(dat1[1:n,1]./dat1[1:n,1])~(dat1[1:n,2])~(dat1[1:n,1])~(dat1[1:n,1].*dat1[1:n,2]);
x2=(dat1[1:n,1]./dat1[1:n,1])~(dat1[1:n,2])~(dat1[1:n,1]);
x3=(dat1[1:n,1]./dat1[1:n,1])~(dat1[1:n,1])~(dat1[1:n,1].*dat1[1:n,2]);
z1=(w.*x1)'*x1; z2=(w.*x2)'*x2; z3=(w.*x3)'*x3;
u1=inv(z1); u2=inv(z2); u3=inv(z3);
b1=inv(z1)*((w.*x1)'*y); b2=inv(z2)*((w.*x2)'*y); b3=inv(z3)*((w.*x3)'*y);
Kma=b1[1,1]/b1[3,1]; Vmaxa=1/b1[3,1]; K1a=b1[1,1]/b1[2,1]; K2a=b1[3,1]/b1[4,1];
Kmb=b2[1,1]/b2[3,1]; Vmaxb=1/b2[3,1]; K1b=b2[1,1]/b2[2,1];
Kmc=b3[1,1]/b3[2,1]; Vmaxc=1/b3[2,1]; K2c=b3[2,1]/b3[3,1];
sigma01=(w'*((y-(b1'*x1')).*(y-(b1'*x1'))))/(n-k);
sigma02=(w'*((y-(b2'*x2')).*(y-(b2'*x2'))))/(n-l);
sigma03=(w'*((y-(b3'*x3')).*(y-(b3'*x3'))))/(n-l);
s_Kma=(sigma01*(u1[1,1]/b1[3]^2-2*b1[1]*u1[3,1]/b1[3]^3+b1[1]^2*u1[3,3]/b1[3]^4))^0.5;
s_Kmb=(sigma02*(u2[1,1]/b2[3]^2-2*b2[1]*u2[3,1]/b2[3]^3+b2[1]^2*u2[3,3]/b2[3]^4))^0.5;
s_Kmc=(sigma03*(u3[1,1]/b3[2]^2-2*b3[1]*u3[2,1]/b3[2]^3+b3[1]^2*u3[2,2]/b3[2]^4))^0.5;
s_Vmaxa=(sigma01*u1[3,3]/b1[3]^4)^0.5;
s_Vmaxb=(sigma02*u2[3,3]/b2[3]^4)^0.5;
s_Vmaxc=(sigma03*u3[2,2]/b3[2]^4)^0.5;
s_K1a=(sigma01*(u1[1,1]/b1[2]^2-2*b1[1]*u1[2,1]/b1[2]^3+b1[1]^2*u1[2,2]/b1[2]^4))^0.5;
s_K1b=(sigma02*(u2[1,1]/b2[2]^2-2*b2[1]*u2[2,1]/b2[2]^3+b2[1]^2*u2[2,2]/b2[2]^4))^0.5;
s_K2a=(sigma01*(u1[3,3]/b1[4]^2-2*b1[3]*u1[4,3]/b1[4]^3+b1[3]^2*u1[4,4]/b1[4]^4))^0.5;
s_K2c=(sigma03*(u3[2,2]/b3[3]^2-2*b3[2]*u3[3,2]/b3[3]^3+b3[2]^2*u3[3,3]/b3[3]^4))^0.5;

C
mix=(s_Kma/Kma+s_Vmaxa/Vmaxa+s_K1a/abs(K1a)+s_K2a/abs(K2a))/4;
comp=(s_Kmb/Kmb+s_Vmaxb/Vmaxb+s_K1b/abs(K1b))/3;
ncomp=(s_Kmc/Kmc+s_Vmaxc/Vmaxc+s_K2c/abs(K2c))/3;
if mix<comp;
goto mark;
elseif comp<ncomp;
print "competitive type inhibition";
print "Km" Kmb~s_Kmb;
print "Vmax" Vmaxb~s_Vmaxb;
print "K1" K1b~s_K1b;
else;
print "nonecompetitive type inhibition";
print "Km"  Kmc~s_Kmc;
print "Vmax" Vmaxc~s_Vmaxc;
print "K2"  K2c~s_K2c;
endif;
print "criteria";
print "mix/comp/ncomp" mix~comp~ncomp;
end;
mark:
if mix<ncomp;
print "mixed type inhibition";
print "Km"  Kma~s_Kma;
print "Vmax" Vmaxa~s_Vmaxa;
print "K1"  K1a~s_K1a;
print "K2"  K2a~s_K2a;
else;
print "nonecompetitive type inhibition";
```

Fig. 1. The script for calculating the parameters of Michaelis Menten kinetics and their variances. A – data input block in the form of Excel spreadsheet for n=16, k=4 and l=3, B – block for calculating kinetic parameters and their standard deviations, C – data output logical block. In this script all expressions with indices (a) concerns to mixed type inhibition, expressions with indices (b) – to competitive type inhibition and expressions with indices (c) – to none-competitive type inhibition.

The studied compound was found to be a competitive inhibitor of AchE (K_1 = 11.48 ± 0.96 μM), but a mixed type inhibitor of BChE (K_1 = 0.127 ± 0.046 μM; K_2 = 0.24 ± 0.08 μM).

We used this approach in studying the mechanism of inhibition of L-aspartate-β-decarboxylase of *Pseudomonas dacunhae* by D-aspartic acid and for calculation of corresponding parameters of inhibition [3]. In this case the competitive mechanism of inhibition was shown with following parameters: K_M = 1.14 ± 0.39 mM, V_{max} = 3210.06 ± 171.39 mmol/(g.h), K_1 = 118.44 ±5.98 mM.



(Z)-2-((2-(2-methoxybenzamido)-3-phenylacryloyl)oxy)-
N,N,N-trimethylethanaminium

Fig. 2. The structure of α,β-dehydrophenylalanine choline ester – (Z)-2-((2-(2-methoxybenzamido)-3-phenylacryloyl)oxy)-N,N,N-trimethylethanaminium

Using the presented above statistical approch in studying the inhibition of bovine trypsin by newly synthesized noneproteineous amino acids and peptides on their base showed, that (2*R*,3*S*)-β-hydroxyleucine and N-formyl-(S)-methionyl-(2*R*,3*S*)-β-hydroxyleucine inhibited the enzyme by competitive mechanism with K_1=0.842±0.203 and K_1 = 0.312 ± 0.085 mM, correspondingly [11].

In the case of *Rhodotorulla aurantiaca* KM-1 phenylalanine ammonia-lyase we also demonstrated the competitive inhibition of enzyme by D-phenylalanine with K_1 = 3.38 ± 0.32 mM [6], using the presented above statistical approach.

We used this approach in studying the mechanism of inhibition of aminotransferases of *Erwinia carotovora* by L-β-(N-benzylamino)alanine and L-β-(N-methylamino)alanine in saturating concentrations of cosubstrate – keto acid (10 mM) [13]. It was shown that L-β-(N-benzylamino)alanine was a competitive inhibitor with respect to L-phenylalanine for PAT1 (K_1 = 0.32 ± 0.07 mM, K_M = 0.45 ± 0.10 mM, V_{max} = 11.6 ± 0.4 U/mg). L-β-(N-methylamino)alanine is a noncompetitive inhibitor with respect to L-phenylalanine for PAT3 (K_2 = 138.4 ± 95.4 mM, K_M = 13.7 ±3.9 mM, V_{max} = 18.6 ± 4.1 U/mg).

We also used this approach in studying the mechanism of inhibition of L-aminoacylase of *Rhodococcus armeniensis* AM6.1 by the reaction product – acetate [9]. The results confirmed a competitive type of enzyme inhibition with the following kinetic parameters: K_1 = 104.7 ± 21.6 mM, K_M = 2.5 ± 0.4 mM, V_{max} = 25.1 ± 1.5 U/mg.

The equation for initial velocity of *substrate inhibition type reaction kinetics*, derived using King and Altman approach [12], is presented in (17).

$$v = \frac{V_{max} S}{K_M + S + \frac{S^2}{K_S}} \qquad (17)$$

where: v is initial velocity, V_{max} – maximal velocity, S – concentration of substrate and K_S – constant of substrate inhibition.

This equation is linearized as follows:

$$\frac{S}{v} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}} S + \frac{1}{V_{max} K_S} S^2 \qquad (18)$$

Denoting:

$$\frac{s}{v} \to y\,,\quad 1 \to x_1\,,\quad S \to x_2\,, S^2 \to x_3\,, \frac{K_M}{V_{max}} \to \beta_1,\quad \frac{1}{V_{max}} \to \beta_2\,,\quad \frac{1}{V_{max}K_S} \to \beta_3\,,$$

we can describe the linearized equation for velocity of the enzymatic reaction (18) together with measurement errors (ε) in vector form (3) as in the case of simple Michaelis-Menten kinetics. Here also β-s and their variances can be calculated according the Gauss-Markov theorem [5] by equations (4). When the measurements are given weights, the equations (4) are converted to (5). Here also the experimental variance is calculated from equations (6).

$$\sigma_0^2 = \frac{SS}{N-p},\quad SS = \sum we^2 = \sum w_i(y_i - x_i\beta_i)^2 = \sum ud^2 \qquad (6)$$

Where: *SS* is the sum of the weighted squares, N – number of measurements, P – number of degrees of freedom (for substrate inhibition type reaction kinetics P=3), e – estimate of ε deviation of y, d – estimate of δ deviation of v, u – weight of d.

$w \approx \frac{v^4}{s^2}$ were calculated by us (derivations are not presented).

The equations for calculating K_M, V_{max} and K_S and their variances are the followings:

$$K_M = \frac{\beta_1}{\beta_2},\quad K_S = \frac{\beta_2}{\beta_3},\quad V_{max} = \frac{1}{\beta_2} \qquad (19)$$

$$V_{(K_M)} = V_{\left(\frac{\beta_1}{\beta_2}\right)} = \sigma_0^2\left(\frac{M_{11}}{\beta_2^2} - 2\frac{\beta_1 M_{12}}{\beta_2^3} + \frac{\beta_1^2 M_{22}}{\beta_2^4}\right),\quad V_{(K_S)} = V_{\left(\frac{\beta_2}{\beta_3}\right)} = \sigma_0^2\left(\frac{M_{22}}{\beta_3^2} - 2\frac{\beta_2 M_{23}}{\beta_3^3} + \frac{\beta_2^2 M_{33}}{\beta_3^4}\right),\quad V_{(V_{max})} = V_{\left(\frac{1}{\beta_2}\right)} = \sigma_0^2\frac{M_{22}}{\beta_2^4} \qquad (20)$$

where: β_1, β_2 and β_3 can be calculated by equations (5), σ_0^2 – by equations (6), and M_{ij} – is the (i, j) minor of matrix U (5).

The script code for calculating the parameters of substrate inhibition type reactionkinetics and their variances, written in Gauss 4.0, is presented in fig. 3.

We used the presented multivariate linear regression analyses and corresponding script for studying the substrate inhibition of aspartate aminotransferase of *Erwinia carotovora* by oxaloacetate and 2-ketoglutarate [13]. The calculated K_S for oxaloacetate and 2-ketogltarate was 3.73 ± 1.99 and 10.23 ± 3.20 mM, respectively, in saturating concentrations of cosubstrate – L-phenylalanine.

We also used the multivariate linear regression analyses approach for studying the substrate inhibition in *Rhodococcus armeniensis* AM6.1 [9]. We have shown that among studied substrates N-acetyl-D-leucine and N-acetyl-DL-tyrosine inhibited the rate of their own deacetylation with the inhibition constants of 35.5±28.3 and 15.8±4.5 mM, respectively.

```
A | print "Results";
  | n=7; k=3;
  | fname="D:\\My Documents D\\Data.xlsx";
  | {dat1,names}=import(fname,"a14:b21",3);

B | y=dat1[1:n,1]./dat1[1:n,2];
  | w=dat1[1:n,2].^4 ./dat1[1:n,1].^2;
  | x=(dat1[1:n,1]./dat1[1:n,1])~(dat1[1:n,1])~(dat1[1:n,1]).^2;
  | z=(w.*x)'*x;
  | u=inv(z);
  | b=inv(z)*((w.*x)'*y);
  | Km=b[1,1]/b[2,1]; Vmax=1/b[2,1]; Ks=b[2,1]/b[3,1];
  | sigma0=(w'*((y-(b'*x')').*(y-(b'*x')')))/(n-k);
  | s_Km=(sigma0*(u[1,1]/b[2]^2-2*b[1]*u[1,2]/b[2]^3+b[1]^2*u[2,2]/b[2]^4))^0.5;
  | s_Vmax=(sigma0*u[2,2]/b[2]^4)^0.5;
  | s_Ks=(sigma0*(u[2,2]/b[3]^2-2*b[2]*u[2,3]/b[3]^3+b[2]^2*u[3,3]/b[3]^4))^0.5;

C | print "substrate inhibition";
  | print "Km" Km~s_Km;
  | print "Vmax" Vmax~s_Vmax;
  | print "Ks" Ks~s_Ks;
  | end;
```

Fig. 3. The script code for calculating the parameters of substrate inhibition type reaction kinetics and their variances. A – data input block in the form of Excel spreadsheet for n=7 and k=3, B – block for calculating kinetic parameters and their standard deviations, C – data output block.

Thus, using multivariate linear regression approach, open-source scripts have been developed for the calculation of the kinetic parameters and their dispersions for the different cases of enzyme catalyzed reactions inhibition, including the cases of competitive, none competitive, mixed and substrate inhibitions.

REFERENCES

1. *Григорян А.А., Амбарцумян А.А., Мкртчян М.В., Топузян В.О., Алебян Г.П., Асатрян Р.С.* Синтез и антихолинэстеразная активность 2-(диметиламино) этиловых и холиновых эфиров N-замещенных α,β-дегидроаминокислот. Химико-фармацевтический журнал, *40*, 3, 18-23, 2006.
2. *Григорян А.А., Амбарцумян А.А., Мкртчян М.В., Топузян В.О., Алебян Г.П.* Новые обратимые ингибиторы холинэстераз на основе аминоэтиловых эфиров α,β-дегидроаминокислот. Биолог. журн. Армении, *57*, 1-2, 3-7, 2005.
3. *Дюкова К.Г., Агаджанян А.Е., Амбарцумян А.А., Алебян Г.П.* Усовершенствованная технология получения D-аспарагиновой кислоты и L-аланинна путем биотрансформации DL-аспарагиновой кислоты. Биотехнология, 4, 69-77, 2009.
4. *Корниш-Боуден Э.* Основы ферментативной кинетики, "Мир", М., 282 С., 1979.
5. *Магнус Я.Р., Катышев П.К., Пересецкий А.А.* Эконометрика, "Дело", М., 399 С., 2000.
6. *Bazukyan I.L., Vardanyan A.E., Ambartsumyan A.A., Tozalakyan P.V., Popov Yu. G.* Catalytic Properties of *Rhodotorula aurantiaca* KM-1 Phenylalanine Ammonia-Lyase Applied Biochemistry and Microbiology, *45*, 1, 17-21, 2009.
7. *Grigoryan H.A., Hambardzumyan A.A., Mkrtchyan M.V., Topuzyan V.O., Halebyan G.P., Asatryan R.S.* α,β-Dehydrophenylalanine choline esters: potent reversible inhibitors of human acetylcholinesterase and butyrylcholinesterase. Electronic Journal of Natural Sciences, Biotechnology, NAS of RA, *6*, 1, 14-20, 2006.
8. *Grigoryan H.A., Hambardzumyan A.A., Mkrtchyan M.V., Topuzyan V.O., Halebyan G.P., Asatryan R.S.* α,β-Dehydrophenylalanine choline esters, a new class of reversible inhibitors of human acetylcholinesterase and butyrylcholinesterase. Chem. Biol. Interact., *171*, 108-116, 2008.

9. *Hambardzumyan A.A., Mkhitaryan A.V., Paloyan A.M., Dadayan S.A., Saghyan A.S.* Catalytic Properties of Aminoacylase of Strain *Rhodococcus Armeniensis* AM6.1 Applied Biochemistry and Microbiology, *52*, 3, 250-255, 2016.
10. *Hambardzumyan A.A.* Statistical analyses of enzyme kinetics: simple Michaelis-Menten and bi-bi ping-pong, Biolog. Journal of Armenia, *69*, 2, 6-12, 2017.
11. *Hovhannisyan N, Harutyunyan Sh, Hovhannisyan A, Hambardzumyan A, Chitchyan M, Melkumyan M, Oganezova G, Avetisyan N.* The novel inhibitors of serine proteases, Amino Acids, *37*, 3, 531-536, 2009.
12. *King E.L., Altman C.A.* Schematic method of deriving the rate laws for enzyme-catalyzed reactions. J. Phys. Chem., *60*, 1375-1378, 1956.
13. *Paloyan A.M., Stepanyan L.A., Dadayan S.A., Hambardzumyan A.A., Halebyan Gh.P. Saghiyan A.S.* Catalytic properties of enzymes from *Erwinia carotovora* involved in trans-amination of phenylpyruvate Applied Biochemistry and Microbiology, *49*, 2, 106-112, 2013.
14. *Velick S.F. and Vavra J.* A kinetic and equilibrium analysis of the glutamic oxaloacetate transaminase mechanism. J. Biol. Chem., *237*, 7, 20109-20122, 1962.

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