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The Indian Square for Enzyme Kinetics Through the Regular Form of Lineweaver-Burk Plot (Double Reciprocal Plot); It's Inverse Form and Other Additional Form of Plots (Equations)

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ABSTRACT

Each enzyme deserves a specific Michaelis-Menten constant (Km), which is determined through the double reciprocal plot, also recognized as Lineweaver-Burk plot. This constant of Michaelis-Menten (Km) is concentration of substrate [S] and it avails the velocity (v) of reaction to proceed up to half of it's maximal or Vmax. The regular Lineweaver-Burk plot and it's inverse form are designated as y_1 and y_2 lines respectively. Present attempt is considering additional plots or the lines keeping the concept in regular Lineweaver-Burk plot constant. These plots include: y_3 and y_4. In slope and intercept form the lines y_3 and y_4 expressed as: $y_3 = -[(Km \div Vmax)(X)] + [(km+1) \div (Vmax)]$ and $y_4 = -[(Vmax \div Km)(X)] + [(Vmax -1) \div (Km)]$. In addition; $y_5 = X + 0$ and $y_6 = - X + 1$ are the two reference lines are also considered in this attempt. The line y_3 intersect the reference line y_5 at the point "A", the x- co-ordinate and y- co-ordinate of which are equal to each other and correspond to: $[(Km+1) \div (Vmax+Km)]$. The line y_1 intersect the reference line y_6 at the point "B", the x- co-ordinate and y- co-ordinate of which respectively correspond to: $[(Vmax - 1) \div (Vmax + Km)]$ and $[(Km + 1) \div (Vmax + Km)]$. The point at which the reference line y_5 attains $[(Vmax - 1) \div (Vmax + Km)]$ is labeled as the point "C". The X – co-ordinate and Y – co-ordinate of the point "C" corresponds to: $[(Vmax - 1) \div (Vmax + Km)]$ $[(Km + 1) \div (Vmax + Km)]$ respectively. The point of intersection of the line y_2 and the reference line y_6 is labeled as the point "D". The X – co-ordinate and Y – co-ordinate of the point "D" corresponds to: $[(Km + 1) \div (Vmax + Km)]$ and $[(Vmax - 1) \div (Vmax + Km)]$ respectively. The length of segment AB=BC=CD=DA and it correspond to: $[(Vmax - Km - 2) \div (Vmax + Km)]$. The point "A"; "B"; "C" and "D" constitute the vertices of square ABCD. The resulting mathematical square, herewith labeled as: "Indian Square For Enzyme Kinetics". Each of the four vertices (corners) have known coordinates in terms of Vmax and Km, the key indices in enzyme kinetics.

Keywords : Indian Square, Enzyme Kinetics, Mathematical Approach

I. INTRODUCTION

In geometry, a square is a regular quadrilateral, which means that it has four equal sides and four equal angles (90-degree angles, or (100-gradian angles or right angles) (Weisstein, Eric). It can also be defined as a rectangle in which two adjacent sides have equal length. A square with vertices ABCD would be denoted □ {\displaystyle \square } as "Square: ABCD". The diagonals of a square bisect each other and meet at 90°. The diagonals of a square bisect its angles. Opposite sides of a square are both parallel and equal in length. All four angles of a square are equal. Each is 360°/4 = 90°, so every angle of a square is a right angle. All four sides of a square are equal. The diagonals of a square are equal (Zalman Usiskin and Jennifer Griffin, 2008).

The velocity (v) of biochemical reaction catalyzed by the enzyme vary according to the status of factors like: concentration of the substrate [S]; hydrogen ion concentration; temperature; concentration of the respective enzyme; activators and inhibitors. There is no linear response of velocity (v) of biocatalyzed reaction to the concentration of the substrate [s]. This may be due to saturable nature of enzyme catalyzed biochemical reactions. If the initial velocity (v) or rate of the enzyme catalyzed biochemical reaction is expressed in terms of substrate-concentration of [S], it appears to increase. That is to say, initial velocity (v) of the enzyme catalyzed biochemical reaction get increase according to the increase in the concentration of substrate [S]. This tendency of increase in initial velocity (v) of the enzyme catalyzed biochemical reaction according to the increase in the concentration of substrate [S] is observed up to certain level of the concentration of substrate [S]. At this substrate concentration [S], the enzyme exhibit saturation and exert the initial velocity (v) of the biocatalyzed reaction to achieve maximum velocity (V_{max}). Hans Lineweaver and Dean Burk (1934) suggested the double reciprocal plot for presenting the information in the form of readings or the data on the concentration of substrate [S] and rate or velocity (v) of the biocatalyzed reaction. In enzyme kinetics, double reciprocal plot suggested by Hans Lineweaver and Dean Burk is well esteemed graphical presentation of the data on concentration of the substrate [S] and velocity (v) of the biocatalyzed reaction recognized as, the "Lineweaver–Burk plot". This plot deserve wide applicability. The most significant application of Lineweaver–Burk plot lies in the determination of concentration of substrate [S] which is responsible for achievement of the half the maximum rate or the velocity (Vmax ÷ 2) of the biochemical reaction catalyzed by the enzymes. The "Km" or Michaelis constant is the concentration of substrate [S] responsible for yield of the reaction rate, which is corresponding to exactly half the rate or velocity of maximal (Vmax ÷ 2) for enzyme involved biochemical reaction. For practical purposes, this "Km" or Constant of Michaelis is the reading pertaining [S] that allows velocity to achieve half with reference to maximum rate or velocity (Vmax). The affinity of enzymes for their substrate vary. Generally, the enzyme with a higher Km value has little bit lower affinity for its substrate. According to Keith J. Laidler (1997), enzymes with lower affinity for their substrate, requires a greater volume of substrate or substrate concentration for the purpose to achieve maximum rate or velocity of enzyme involved biochemical reactions.

The wide range of applicability is the distinguishing feature of Lineweaver–Burk plot. In the past, there was no computer facilities as today. In such a critical situation, the parameters of enzyme kinetics, Km and Vmax served a lot through this Lineweaver-Burk plot for fortified concept of enzyme kinetics. In this Lineweaver-Burk plot, reading the inverse of maximum velocity of biocatalyzed reaction (1÷ Vmax) take the position of y-intercept (Fig.1). The negative value of inverse of Km (1÷Km) take the position of x-intercept. The quick visual impression of the inverse form of substrate concentration and rate or velocity of reaction is one more advantage of Lineweaver-Burk plot. And this feature help for understanding the concept of enzyme inhibition. Accordingly, mathematical equation suggested by Lineweaver and Dean Burk (1934) can be written as: $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$

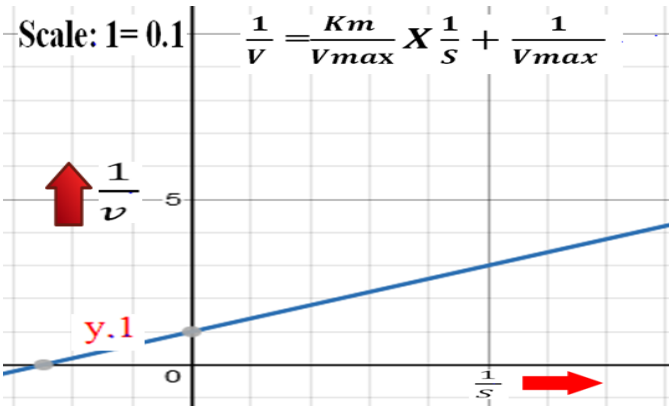

Fig. 1: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) $y_1 = = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$

This Lineweaver–Burk plot deserve wide applicability. It is useful for the determination of K_m , the most significant factor in enzyme kinetics. The intercept on y – axis of "Lineweaver–Burk-Plot" is the reciprocal of V_{max} or (1/ V_{max}). And intercept on X – axis of "Lineweaver–Burk-Plot" is the reciprocal of - K_m or (–1/K_m). Reciprocals of both, [S] and (v) are utilized in the Lineweaver-Burk plot. That is to say, this plot is pertaining $\frac{1}{V}$ and $\frac{1}{S}$. Therefore, "Lineweaver–Burk-Plot" is also termed as a double reciprocal graph. This attempt through the "Lineweaver-Burk-Plot", is giving quick, concept or idea of the biochemical reaction. It also allow to understand the mechanism of activation of enzyme and inhibition of enzyme. Researchers including authors of present attempt designating the double reciprocal plot as a Nobel Plot. Most of researchers entertaining the enzyme kinetics through this double reciprocal plot are non-mathematical academicians. Present attempt is trying it's best to minimize the errors in understanding the concepts in enzyme kinetics through modification in the "Lineweaver-Burk-Plot".

Each and every method is with positive and negative points of advantages. According to Hayakawa, et al (2006), there is distortion of error structure through this double reciprocal plot of "Lineweaver-Burk-Plot". It is therefore, method of graphical presentation of "Lineweaver–Burk-Plot" (double-reciprocal-plot) appears to attempt to minimize errors. This may yield easier method of calculation of constants or parameters of enzyme kinetics. On this line of improvement of method of calculation of constants or parameters of enzyme kinetics, much more work is already exist. According to Hayakawa, et al (2006), methods of improvement in the calculation of constants or parameters of enzyme kinetics are under the title, "non-linear regression or alternative linear forms of equations". And they include: the plot of "Hans-Woolf"; the plot of "Eadie-Hofste"; such and the others (Greco and Hakala, 1979).

Dick (2011) explained type of inhibition of enzyme activity or stoping the working of enzymes. Of course, this discussion is based exclusively on "Lineweaver–Burk-Plot" (reciprocals ob both the axes) is able to group or classify the inhibitors of actions of enzymes. Accordingly, the inhibitors of enzyme can basically be grouped into the types like: The "Competitive Inhibitors"; "Non-competitive inhibitors" and "uncompetitive inhibitors". The inhibitors of enzyme of "Competitive" class deserve one and the same point of intersection on the Y-axis. It clearly means, inhibitors of enzyme of "Competitive" class are not affecting on maximal rate or velocity of reaction (competitive inhibitors provide protection the maximum velocity Vmax. They keep this maximum velocity Vmax non-affected). But, slopes of equations are not same. Slopes are different slopes. The inhibitors of enzyme of "Non-competitive" class deserve one and the same point of intersection on the X-axis. It clearly means, inhibitors of enzyme of "Non-competitive" class are not affecting on the Km, the [S] for half the maximal rate or velocity of reaction (Km is remains unaffected by non-competitive inhibitors. The inverse of Km doesn't change). The non-competitive inhibition produces plots with the same x-intercept (–1/K_m) as uninhibited enzyme (K_m is unaffected) but different slopes and y-intercepts. Uncompetitive inhibition causes different intercepts on both the y- and x-axes (Berg, et al, 2002). John E. Dowd and Douglas Briggs (1965) reviewed the literature on "Estimates of Michaelis – Menten kinetic constants through the use of different linear transformation" and listed some problems with Lineweaver–Burk plot (double reciprocal plot). Accordingly, Lineweaver–Burk plot (double reciprocal graph) is appearing in most of the new as well older books of biochemistry. It seems in having prone to error. There may be mistake in

understanding the expected for researchers. The readings of inverse of "v" are on Y – axis. The readings of inverse of "[S]" are on X – axis. The lower values of both the readings (inverse of "v" and inverse of "[S]") are occupying higher (signifiacant) position in graph. And… and… higher values of both the readings (inverse of "v" and inverse of "[S]") are occupying lower (non-significant) position in graph. Both the conditions may be interpreted wrongly.

MATHEMATICAL PROPERTIES OF EQUATION FOR INVERSE FORM OF ENZYME KINETICS:

(A). The mathematical equation for regular Lineweaver Burk plot is explaining binary relation between the inverse of substrate concentration and $\frac{1}{S}$ and $\frac{1}{V}$ of enzyme involved processes. It's inverse form of equation is making this mathematical association of $\frac{1}{S}$ and $\frac{1}{V}$ more fortified. It means, characters of inverse form of mathematical equation for kinetics of enzyme matches to the characters of converse associations.

(B). Theoretically, mathematical equation for regular Lineweaver Burk plot for a given enzyme is with unique inverse function. That is to say, for each concentration of substrate, there is a unique value for velocity of enzyme catalyzed biochemical reaction. This proceed ahead since the inverse form of equation must be the converse association.

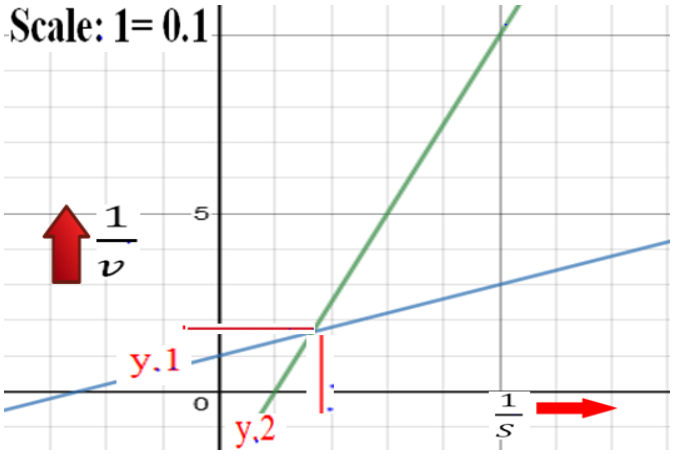

Fig. 2: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) along with it's Inverse form $y_2 = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$

(C). If the 1÷S and 1÷v for given enzyme catalyzed biochemical reaction are inverses of each other, then the domain of 1÷S is equal to the range of 1÷v and the range of 1÷v is equal to the domain of 1÷S.

(D).The 1÷S and 1÷v for given enzyme catalyzed biochemical reaction deserve symmetry, then there is a similar type symmetry in between the real form of equation and it's inverse form of equation.

(E).The co-ordinates of the point of intersection of both the equations $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$ and $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$ correspond to: ($\frac{1}{Vmax-Km}$, $\frac{1}{Vmax-Km}$).

(F). The 1÷S and 1÷v for given enzyme catalyzed biochemical reaction may deserve "one-to-one". Even if a function 1÷S is not one-to-one relation with 1÷v ; it is attainable for the purpose of definition of inverse form of equation through limiting the areas or the domain.

(G). The inverse form of equation in enzyme kinetics is a function that reverses real form of mathematical equation for regular Lineweaver Burk plot. If the equation for (1÷v), applied to input (1÷ S), yields a consequence or result of (1÷v). After appertaining its inverse equation (1÷S) to (1÷v) gives the outcome 1÷S, and contrariwise.

(H). If ($\frac{1}{S}$, $\frac{1}{v}$) is a point on the graph of the original equation of Lineweaver–Burk plot, then the point ($\frac{1}{S}$, $\frac{1}{v}$) must be a point on the graph of the inverse function of enzyme kinetics. The Lineweaver–Burk plot and it's inverse function are mirror images of each other with respect to the line y=x.

APPLICATIONS OF MATHEMATICAL INVERSE FUNCTION (EQUATION) FOR ENZYME KINETICS:

Applications related to enzyme catalyzed processes deserve ubiquitous nature. This ubiquitous nature is both in natural alive condition and in laboratory experimental conditions. Detailed study of kinetics of enzyme catalyzed reactions remains controversial. Michaelis–Menten equation expect reaching a non-changing position of response for further side limit of experimentation. At this condition of experimentation, enzyme concentration far beyond molar concentration of sites that liable for accessibility. It needs large amount of substrate. Substantial study at laboratory level is going to prove the concept in expectation of Michaelis–Menten equation. This situation may be the limiting factor applicability of the concept in expectation of Michaelis–Menten equation. In such situation, it become exclusively impossible for practical accessibility of the concept in expectation of Michaelis–Menten equation.

At the point of non-accessibility of the concept in expectation of Michaelis–Menten equation, "inverse function or equation for enzyme kinetics" deserve applicability. Here it is essential to mention that, "inverse function or equation for enzyme kinetics" is giving contrivance for analysis of kinetics that are involving enzymes. It may establish contrivance for bridging the concept of kinetics of enzyme related reactions reaching the steady or "Non-changing Position" It reveals compactness of attack of enzyme with it's active site corresponding to the site of substrate. More over, the "inverse function or equation for enzyme kinetics" explain "Species Specific Nature of Enzymes".

Intersection of the line y_1 and line y_2 (Fig. 2):

The lines y_1 ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$) and y_2 ($\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$) are the mathematical inverse functions for each other (Seema K. Dongare, et al, 2018). Let us have a attempt to intersect the line y_1 and y_2 for obtaining the x- and y- co-ordinates for the point of intersection.

$y_1 = y_2$

Step – 1: $\frac{Km}{Vmax} X + \frac{1}{Vmax} = \frac{Vmax}{Km} X - \frac{1}{Km}$

Step – 2: $\frac{1}{Vmax} + \frac{1}{Km} = [\frac{Vmax}{Km} - \frac{Km}{Vmax}] X$

Step – 3: $\frac{Vmax + Km}{Vmax.Km} = [\frac{(Vmax-Km)(Vmax+Km)}{Km.Vmax}] X$

Step – 4: $\frac{(Vmax + Km)\ Km\ Vmax}{Vmax.Km\ (Vmax-Km)(Vmax+Km)} = X$

Step – 5: $\frac{1}{(Vmax-Km)} = X$

The regular Michaelis–Menten equation ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$) explains the influence of concentration of substrate [S] on velocity of enzyme catalyzed biochemical reaction. It's inverse function may explain the influence of velocity of enzyme catalyzed biochemical reaction (v) on concentration of substrate [S]. That is to say, inverse function of the regular Michaelis–Menten equation and ($\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$) is going to explain the role of product of enzyme catalyzed biochemical reaction in controlling the rate of reaction. The regular Michaelis–Menten equation ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$) is demonstrating the Km (Michaelis constant), the substrate concentration [S] at which the velocity (v) of the enzyme catalyzed biochemical reaction attain half of it's maximum ($V_{max} \div 2$). And … and … the inverse function is demonstrating the [1÷ (Vmax. – Km)], (Baramati Constant), point on both, the regular Michaelis–Menten equation ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$) and it's inverse function ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$). At this point [1÷ (Vmax. – Km)], (Baramati Constant), both the equations are equal with each other. This point [1÷ (Vmax. – Km)], (Baramati Constant) is going

to serve the saturation of enzyme molecules and the substrate molecules in enzyme catalyzed biochemical reaction. Through the reverse the mathematical steps and to get inverse of substrate concentration (1÷S) back from some output value, say inverse of respective velocity (1÷v), it is necessary to carry out the steps exactly in opposite sequence. It means that, it is prime need to subtract the inverse of maximum velocity (1÷Vmax) from inverse of respective velocity (1÷v) and then multiply the result by $\frac{\mathbf{Vmax}}{\mathbf{Km}}$. This is going to yield the equation correspond to: $\frac{\mathbf{1}}{\mathbf{S}} = \frac{\mathbf{Vmax}}{\mathbf{Km}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{v}} - \frac{\mathbf{1}}{\mathbf{Km}}$ (Fig.2). The 1÷S and 1÷v for given enzyme catalyzed biochemical reaction deserve symmetry. That is to say, the real form of "Lineweaver-Burk-Plot" and it's inverse form derived in the present attempt, are exhibiting the symmetry. The co-ordinates of the point of intersection of both (Real form of Lineweaver Burk plot and it' Inverse form) the equations $\frac{\mathbf{1}}{\mathbf{V}} = \frac{\mathbf{Km}}{\mathbf{Vmax}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{S}} + \frac{\mathbf{1}}{\mathbf{Vmax}}$ and $\frac{\mathbf{1}}{\mathbf{S}} = \frac{\mathbf{Vmax}}{\mathbf{Km}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{v}} - \frac{\mathbf{1}}{\mathbf{Km}}$ correspond to: ($\frac{\mathbf{1}}{\mathbf{Vmax-Km}}$, $\frac{\mathbf{1}}{\mathbf{Vmax-Km}}$). The inverse function of the regular Michaelis–Menten equation and ($\frac{\mathbf{1}}{\mathbf{S}} = \frac{\mathbf{Vmax}}{\mathbf{Km}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{v}} - \frac{\mathbf{1}}{\mathbf{Km}}$) is going to serve to understand the concept on role of product in enzyme involved reaction. The [1÷ (Vmax. – Km)], (Baramati Constant), point on both, the regular Michaelis–Menten equation ($\frac{\mathbf{1}}{\mathbf{V}} = \frac{\mathbf{Km}}{\mathbf{Vmax}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{S}} + \frac{\mathbf{1}}{\mathbf{Vmax}}$) and it's inverse function ($\frac{\mathbf{1}}{\mathbf{V}} = \frac{\mathbf{Km}}{\mathbf{Vmax}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{S}} + \frac{\mathbf{1}}{\mathbf{Vmax}}$). At this point [1÷ (Vmax. – Km)], (Baramati Constant), both the equations are equal with each other. This point [1÷ (Vmax. – Km)], (Baramati Constant) is going to serve the saturation of enzyme molecules and the substrate molecules in enzyme catalyzed biochemical reaction. The attempt on the inverse function for enzyme kinetics of present attempt may open a new chapter to classify the enzymes on the basis of mathematical approach (Seema K. Dongare, et al, 2018).

ONE MORE ATTEMPT ON THE ESTABLISHMENT OF THE EQUATIONS (y_3; y_4 ; y_5 and y_6) FOR THE ENZYME KINETICS:

In this attempt, four new plots or the lines with their respective equations are going to be considered. These new lines include: y_3; y_4 ; y_5 and y_6.

(I). Attempt for the line y_3 (Fig.3):

Let us first consider the line y_3. The slope and intercept on y- axis of this y_3 line are considered as: $\frac{-Km}{Vmax}$ and $\frac{Km+1}{Vmax}$ respectively. Therefore, the mathematical equation in "Slope-intercept" form (in the form of typical y= m x +c) of this line y_3 can be written as:

$y_3 = \frac{-Km}{Vmax}\,x + \frac{Km+1}{Vmax}$

If we replace the "x" by (1÷ S) and substitute the Km = [S (Vmax –v) ÷ v]; the mathematical equation for the line y_3 is going to transform into:

$$= \frac{-S(Vmax-v)}{v\,Vmax\,s} + \frac{S(Vmax-v)+v}{v\,Vmax}$$

Simplification will yields into

$$= \frac{-(Vmax-v)}{v\,Vmax} + \frac{S(Vmax-v)+v}{v\,Vmax}$$

$$= \frac{S(Vmax-v)+v-(Vmax-v)}{v\,Vmax}$$

$$= \frac{(Vmax-v)(s-1)+v}{v\,Vmax}$$

It definitely means for plotting the y_3; it is necessary to consider X = (1÷ S) and

$$Y = \frac{(Vmax-v)(s-1)+v}{v\,Vmax}$$

Through replacing the values of Vmax; V and S, it is possible to calculate the respective values of Y. This is going to serve the purpose of plotting this new line (y_3) along with y_1 and y_2.

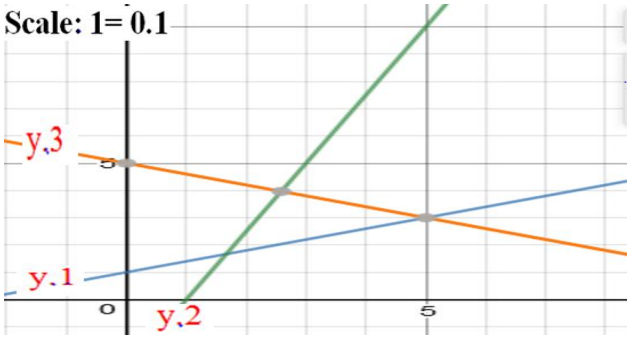

Fig. 3: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) (y_1) along with it's Inverse form (y_2) and (y_3).

Limitations and Significance of the Line (y_3):

Line y_3 is a line with a negative slope. This line is trending downward from left to right. In other words, the line's rise to run ratio is a negative value. Slope of a line explains the pattern of change occurring in a system. It also indicate the direction of change, whether positive or negative. The lines y_1 and y_2 (Fig. 1 and 2) are with a positive slope. Both of them are going up from left to right. While, the line y_3 (Fig. 3) is with a negative slope. It is going down from left to right.

The line y_3 is representing a negative correlation between two variables [X = (1÷ S) and Y = $\frac{(Vmax-v)(s-1)+v}{v\,Vmax}$].

It means, that as X increases, the Y tends to decrease and here in present attempt, it attains to it's minimum, that is ($\frac{Km+1}{Km}$). Negative correlation represents a significant relationship between the variables x and y, which, depending on what they are modeling, can be understood as input and output, or cause and effect.

The ranges for X and Y for the line y_3 are zero to one and (1÷ Vmax) to and $\frac{Km+1}{Vmax}$. Thus, the line y_3 is going to help to imagine the maximum and minimum values, both for X and Y.

Intersection of the line y_3 and line y_1 (Fig. 3):

The line y_1 ($\frac{Km}{Vmax}X\frac{1}{S}+\frac{1}{Vmax}$) and the line y_2 ($\frac{Vmax}{Km}X-\frac{1}{Km}$) and y_3 ($\frac{-Km}{Vmax}X+\frac{Km+1}{Vmax}$) are shown in the figure 3. Let us have a attempt to intersect the line y_2 and y_3 for obtaining the x- and y- co-ordinates for the point of intersection.

$Y_1 = y_3$

Step – 1: $\frac{Km}{Vmax}X\frac{1}{S}+\frac{1}{Vmax}=\frac{-Km}{Vmax}X+\frac{Km+1}{Vmax}$

Step – 2: $\frac{1}{Vmax}-\frac{Km+1}{Vmax}=[\frac{-Km}{Vmax}+\frac{-Km}{Vmax}]X$

Step – 3: $\frac{1-Km-1}{Vmax}=[\frac{(-2\,Km)}{Vmax}]X$

Step – 4: $\frac{-Km}{Vmax}=[\frac{(-2\,Km)}{Vmax}]X$

Step – 5: $\frac{-Km}{Vmax}\,\frac{Vmax}{-2\,Km}=X$

Step – 6: $\frac{1}{2}=X$

Intersection of the line y_3 and line y_2 (Fig. 3):

The lines y_2 ($\frac{Vmax}{Km}X-\frac{1}{Km}$) and y_3 ($\frac{-Km}{Vmax}X+\frac{Km+1}{Vmax}$) are shown in the figure 3. Let us have a attempt to intersect the line y_2 and y_3 for obtaining the x- and y- co-ordinates for the point of intersection.

$Y_2 = y_3$

Step – 1: $\frac{Vmax}{Km}X-\frac{1}{Km}=\frac{-Km}{Vmax}X+\frac{Km+1}{Vmax}$

Step – 2: $\frac{Km+1}{Vmax}+\frac{1}{Km}=[\frac{Vmax}{Km}+\frac{Km}{Vmax}]X$

Step – 3: $\frac{Km(Km+1)+Vmax}{Vmax.Km}=[\frac{(Vmax.Vmax+Km.Km)}{Km.Vmax}]X$

Step – 4: $\frac{Km(Km+1)+Vmax}{Vmax.Km}\,\frac{Vmax.Km}{(Vmax.Vmax+Km.Km)}=X$

Step – 5: $\frac{Km(Km+1)+Vmax}{(Vmax.Vmax+Km.Km)}=X$

(II). Attempt for the line y_4 (Fig. 4):

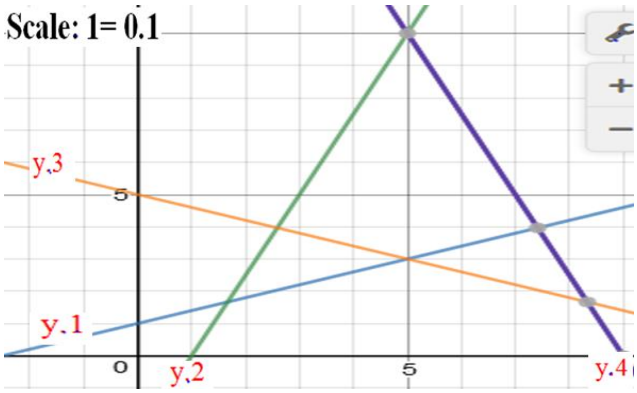

Fig. 4: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) (y_1) along with it's Inverse form (y_2); (y_3) and (y_4).

Let us consider the line y_4. The slope and intercept on y- axis of this y_4 line are considered as: $\frac{-Vmax}{Km}$ and $\frac{Vmax-1}{Km}$ respectively. Therefore, the mathematical equation in "Slope-intercept" form (in the form of typical y= m x +c) of this line y_4 can be written as:

$Y_4 = \frac{-Vmax}{Km} x + \frac{Vmax-1}{Km}$

If we replace the "x" by (1÷ v) and substitute the Km = [S(Vmax –v) ÷ v]; the mathematical equation for the line y_4 is going to transform into:

$$= \frac{-v\,Vmax}{S(Vmax-v)\,v} + \frac{v\,(Vmax-1)}{S(Vmax-v)}$$

Simplification will yields into

$$= \frac{-Vmax}{S(Vmax-v)} + \frac{v\,(Vmax-1)}{S(Vmax-v)}$$

$$= \frac{v\,(Vmax-1) - Vmax}{S(Vmax-v)}$$

$$= \frac{v\,Vmax - v - Vmax}{S(Vmax-v)}$$

$$= \frac{Vmax\,(v-1) - v}{S(Vmax-v)}$$

It definitely means for plotting the y_4; it is necessary to consider X = (1÷ v) and

$Y = \frac{Vmax\,(v-1) - v}{S(Vmax-v)}$

Through replacing the values of Vmax; V and S, it is possible to calculate the respective values of Y. This is going to serve the purpose of plotting this new line (y_4) along with y_1; y_2 and y_3.

Limitations and Significance of the Line (y_4):

Line y_4 is a line with a negative slope. This line is tending downward from left to right. In other words, the line's rise to run ratio is a negative value. Slope of a line explains the pattern of change occurring in a system. It also indicate the direction of change, whether positive or negative. The lines y_1 and y_2 (Fig. 1 and 2) are with a positive slope. Both of them are going up from left to right. While, the line y_3 and y_4 (Fig. 4) are with a negative slope. Both the lines y_3 and y_4 going down from left to right.

The line y_4 is representing a negative correlation between two variables [X = (1÷ v) and $Y = \frac{Vmax\,(v-1) - v}{S(Vmax-v)}$].

It means, that as X increases, the Y tends to decrease and here in present attempt, it attains to it's minimum, that is $\frac{Vmax-1}{Vmax}$.

Negative correlation represents a significant relationship between the variables x and y, which, depending on what they are modeling, can be understood as input and output, or cause and effect.

The range of values for X and Y for the line y_4 are zero to $\frac{Vmax-1}{Vmax}$ and from $\frac{Vmax-1}{Km}$ to zero.

Thus, the line y_4 is going to help to imagine the maximum and minimum values, both for X and Y. More over, the line y_4 is inverse form of the line y_3.

Intersection of the line y_4 with line y_1 (Fig. 4):

The line y_1 ($\frac{\mathbf{Km}}{\mathbf{Vmax}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{S}}+\frac{\mathbf{1}}{\mathbf{Vmax}}$) and y_4 ($\frac{\mathbf{-\ Vmax}}{\mathbf{Km}}\mathbf{x}+\frac{\mathbf{Vmax-1}}{\mathbf{Km}}$) are shown in the figure 4. Let us have a attempt to intersect the line y_1 and y_4 for obtaining the x- and y- co-ordinates for the point of intersection.

$y_4 = y_1$

Step – 1: $\frac{-Vmax}{Km}\text{x}+\frac{Vmax-1}{Km}=\frac{\boldsymbol{Km}}{\boldsymbol{Vmax}}X+\frac{\mathbf{1}}{\boldsymbol{Vmax}}$

Step – 2: $[\frac{-Vmax}{Km}-\frac{\boldsymbol{Km}}{\boldsymbol{Vmax}}]\text{X}=\frac{\mathbf{1}}{\boldsymbol{Vmax}}-\frac{Vmax-1}{Km}$

Step – 3: $[\frac{-Vmax.Vmax-Km.Km}{Km.Vmax}]\text{X}=\frac{\boldsymbol{Km-Vmax(Vmax-1)}}{\boldsymbol{Vmax.Km}}$

Step – 4: $[\frac{-(Vmax.Vmax+Km.Km)}{Km.Vmax}]\text{X}=\frac{-[\boldsymbol{Vmax(Vmax-1)-Km}])}{\boldsymbol{Vmax.Km}}$

Step – 5: $\text{X}=\frac{[\boldsymbol{Vmax(Vmax-1)-Km}])}{(\boldsymbol{Vmax.Vmax+Km.Km})}$

Intersection of the line y_4 with line y_2 (Fig. 4):

The line y_2 ($\frac{\mathbf{Vmax}}{\mathbf{Km}}\mathbf{X}-\frac{\mathbf{1}}{\mathbf{Km}}$) and y_4 ($\frac{\mathbf{-\ Vmax}}{\mathbf{Km}}\mathbf{x}+\frac{\mathbf{Vmax-1}}{\mathbf{Km}}$) are shown in the figure 4. Let us have a attempt to intersect the line y_2 and y_4 for obtaining the x- and y- co-ordinates for the point of intersection.

$y_4 = y_2$

Step – 1: $\frac{-Vmax}{Km}\text{x}+\frac{Vmax-1}{Km}=\frac{\boldsymbol{Vmax}}{\boldsymbol{Km}}\boldsymbol{X}-\frac{\mathbf{1}}{\boldsymbol{Km}}$

Step – 2: $\frac{Vmax-1}{Km}+\frac{\mathbf{1}}{\boldsymbol{Km}}=[\frac{\boldsymbol{Vmax}}{\boldsymbol{Km}}+\frac{\boldsymbol{Vmax}}{\boldsymbol{Km}}]\text{X}$

Step – 3: $\frac{Vmax-1+1}{Km}=[\frac{\boldsymbol{2Vmax}}{\boldsymbol{Km}}]\text{X}$

Step – 4: $\frac{Vmax}{Km}=[\frac{\boldsymbol{2Vmax}}{\boldsymbol{Km}}]\text{X}$

Step – 5: $\frac{Vmax}{Km}\ \frac{Km}{2\,Vmax}=\text{X}$

Step – 6: $\frac{1}{2}=\text{X}$

Intersection of the line y_4 with line y_3 (Fig. 4):

The line y_1 ($\frac{\mathbf{Km}}{\mathbf{Vmax}}\mathbf{X}\frac{\mathbf{1}}{\mathbf{S}}+\frac{\mathbf{1}}{\mathbf{Vmax}}$) and y_4 ($\frac{\mathbf{-\ Vmax}}{\mathbf{Km}}\mathbf{x}+\frac{\mathbf{Vmax-1}}{\mathbf{Km}}$) are shown in the figure 4. Let us have a attempt to intersect the line y_1 and y_4 for obtaining the x- and y- co-ordinates for the point of intersection.

$y_4 = y_3$

Step – 1: $\frac{-Vmax}{Km}\text{x}+\frac{Vmax-1}{Km}=\frac{-Km}{Vmax}\text{x}+\frac{Km+1}{Vmax}$

Step – 2: $[\frac{-Vmax.Vmax+Km.Km}{Km.Vmax}]\text{X}=\frac{Km+1}{Vmax}-\frac{Vmax-1}{Km}$

Step – 3: $[\frac{-(Vmax.Vmax-Km.Km)}{Km.Vmax}]\text{X}=\frac{Km\,(Km+1)-Vmax\,(Vmax-1)}{Vmax.Km}$

Step – 4: $[\frac{-(Vmax.Vmax-Km.Km)}{Km.Vmax}]\text{X}=\frac{-[Vmax\,(Vmax-1)-Km(Km+1)]}{Vmax.Km}$

Step – 5: $\text{X}=\frac{[Vmax\,(Vmax-1)-Km(Km+1)]}{Vmax.Vmax-Km.Km}$

(III). Attempt for the lines y_5 (Fig. 5):

Let us first consider the line y_5. The slope and intercept on y- axis of this y_5 line are considered as: one and zero respectively. Therefore, the mathematical equation in "Slope-intercept" form (in the form of typical y= m x +c) of this line y_5 can be written as:

$Y_5 = x + 0$

This y_5 is the only line in this present attempt allowing to replace the X by any suitable parameter in the enzyme kinetics.

This y_5 is the only line in this present attempt allowing to replace the X by any suitable parameter in the enzyme kinetics. The value for X is directly proportional to the value of Y (With proportional constant is equal to one). The minimum value for X for the line y_5 is zero. There is no upper limit for this line y_5.

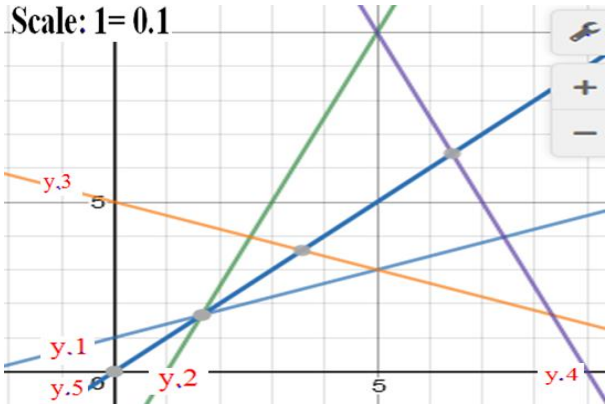

Fig. 5: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) (y_1) along with it's Inverse form (y_2); (y_3); (y_4) and (y_5).

Intersection of the line y_5 with other lines in the attempt (Fig. 5):

The figure – 5 in the present is well explaining the intersection of the line y_5 with the other lines. The point of intersection of line y_5 with the line y_1 and y_2 is one and the same. X – and Y – co-ordinates of the point of intersection of line y_5 with the line y_1 and y_2 are same and correspond to: [(1÷ (Vmax – Km)].

Now let us have a look on the intersection of the line y_5 with the y_3.

$Y_5 = y_3$

Step -1: $X + 0 = \frac{-Km}{Vmax} x + \frac{Km+1}{Vmax}$

Step -2: $[\frac{1}{1} + \frac{Km}{Vmax}] X = \frac{Km+1}{Vmax}$

Step -3: $[\frac{Vmax+Km}{Vmax}] X = \frac{Km+1}{Vmax}$

Step -4: $X = \frac{Km+1}{Vmax} x \frac{Vmax}{Vmax+Km}$

Step -5: $X = \frac{Km+1}{Vmax+Km}$

Let us label this point of the intersection of the line y_5 with the line y_3 as: "A". Both, the X – co-ordinate and Y – co-ordinate of the point correspond to : $X = \frac{Km+1}{Vmax+Km}$

Now let us have a look on the intersection of the line y_5 with the y_4.

$Y_5 = y_4$

Step -1: $X + 0 = \frac{-Vmax}{Km} x + \frac{Vmax-1}{Km}$

Step -2: $[\frac{1}{1} + \frac{Vmax}{Km}] X = \frac{Vmax-1}{Km}$

Step -3: $[\frac{Vmax+Km}{Km}] X = \frac{Vmax-1}{Km}$

Step -4: $X = \frac{Vmax-1}{Km} x \frac{Km}{Vmax+Km}$

Step -5: $X = \frac{Vmax-1}{Vmax+Km}$

Let us label this point of the intersection of the line y_5 with the line y_4 as: "C".

Both, the X – co-ordinate; Y – co-ordinate of this point ("C") are one and same and correspond to: $X = \frac{Vmax-1}{Vmax+Km}$

(IV). Attempt for the lines y_6 (Fig. 6):

Let us first consider the line y_6. The slope and intercept on y- axis of this y_6 line are considered as: one and one respectively. Therefore, the mathematical equation in "Slope-intercept" form (in the form of typical y= m x +c) of this line y_6 can be written as:

$Y_6 = - x + 1$

This y_6 is the line in this present attempt allowing to replace the X by suitable parameter in the enzyme kinetics.

The value for X is directly proportional to the value of Y (With proportional constant is equal to one). The minimum value and maximum value for both, X and Y for the line y_6 correspond to zero and one respectively.

The line y_5 and line y_6; both are the inverse form of each other.

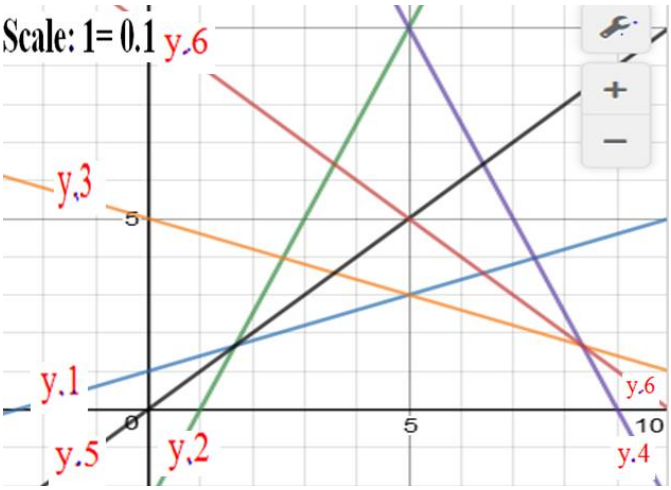

Fig. 6: Regular form of Linrweaver-Burk Plot (Double Reciprocal Plot) (y_1) along with it's Inverse form (y_2); (y_3); (y_4); (y_5) and (y_6).

Intersection of the line y_6 with other lines in the attempt (Fig. 6):

The figure – 6 in the present is well explaining the intersection of the line y_6 with the other lines. The point of intersection of line y_6 with the line y_3 and y_4 is one and the same. X – co-ordinate of the point of intersection of line y_6 with the line y_3 and y_4 correspond to: [1 - (1÷ (Vmax – Km)] = [(Vmax – Km -1) ÷ (Vmax – Km)]. Y – co-ordinate of the point of intersection of line y_6 with the line y_3 and y_4 correspond to: [(1÷ (Vmax – Km)].

Now let us have a look on the intersection of the line y_6 with the y_1.

$Y_1 = y_6$

Step -1: $\frac{Km}{Vmax} x + \frac{1}{Vmax} = - X + 1$

Step -2: $[\frac{Km}{Vmax} + \frac{1}{1}] X = \frac{1}{1} - \frac{1}{Vmax}$

Step -3: $[\frac{Km+Vmax}{Vmax}] X = \frac{Vmax-1}{Vmax}$

Step -4: $X = \frac{Vmax-1}{Vmax} x \frac{Vmax}{Vmax+Km}$

Step -5: $X = \frac{Vmax-1}{Vmax+Km}$

Let us label this point of the intersection of the line y_6 with the line y_1 as: "B". Both, the X – co-ordinate and Y – co-ordinate of the this point "B" correspond to : $X = \frac{Vmax-1}{Vmax+Km}$

Now let us have a look on the intersection of the line y_6 with the y_2.

$Y_2 = y_6$

Step -1: $\frac{Vmax}{Km} x - \frac{1}{Km} = - X + 1$

Step -2: $[\frac{Vmax}{Km} + \frac{1}{1}] X = \frac{1}{1} + \frac{1}{Km}$

Step -3: $[\frac{Km+Vmax}{Km}] X = \frac{Km+1}{Km}$

Step -4: $X = \frac{Km+1}{Km} x \frac{Km}{Vmax+Km}$

Step -5: $X = \frac{Km+1}{Vmax+Km}$

Let us label this point of the intersection of the line y_6 with the line y_1 as: "D". The X – co-ordinate of the this point "D" correspond to : $X = \frac{Km+1}{Vmax+Km}$

The Y – co-ordinate of the this point "D" correspond to :

$Y = \frac{1}{1} - \frac{Km+1}{Vmax+Km} = \frac{Vmax+Km-Km-1}{Vmax+Km} = \frac{Vmax-1}{Vmax+Km}$

(V). Attempt for the Indian Square For Enzyme Kinetics (Fig. 7 and 8):

The present attempt tried it's best to derive and to set some new equations keeping the meaning and concept expected by the well esteemed standard Lineweaver-Burk plot, the double reciprocal plot for the enzyme kinetics.

Let us refer (have a look) on the fig.7. This fig. 7 is nothing but the result of the present attempt on derivation of some new equations keeping the meaning and concept expected by the well esteemed standard Lineweaver-Burk plot, the double reciprocal plot for the enzyme kinetics constant. The equations in this attempt include: y_3; y_4; y_5 and y_6. The line y_1 itself is the original Lineweaver-Burk Plot (Double reciprocal plot). The line y_2 is the inverse form of line y_1 ; the original Lineweaver-Burk Plot (Double reciprocal plot). The point of intersection of the line y_5 and y_6 is here, in the present attempt is labeled as, "O".

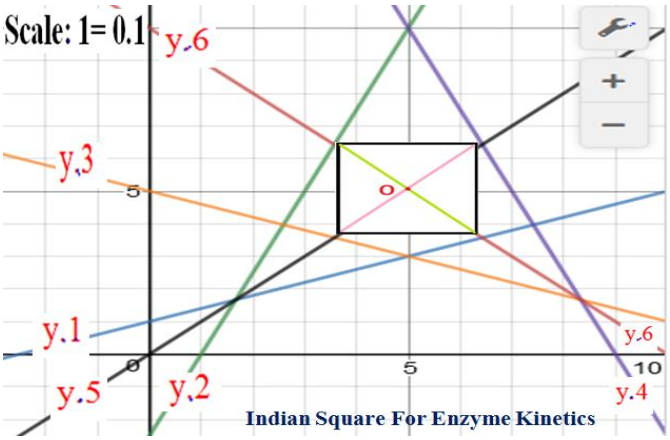

Fig. 7 : Indian Square For Enzyme Kinetics Through The Linrweaver-Burk Plot (Double Reciprocal Plot) (y_1) and The Other Lines In the Present Attempt (y_2; y_3; y_4; y_5 and y_6).

The attempt on the intersection of the line y_5 and y_3 is yielding the point "A" as appearing in fig. 8. The

attempt on the intersection of the line y_6 and y_1 is yielding the point "B" as appearing in fig. 8. The attempt on the intersection of the line y_5 and y_4 is yielding the point "C" as appearing in fig. 8. The attempt on the intersection of the line y_6 and y_6 is yielding the point "D" as appearing in fig. 8.

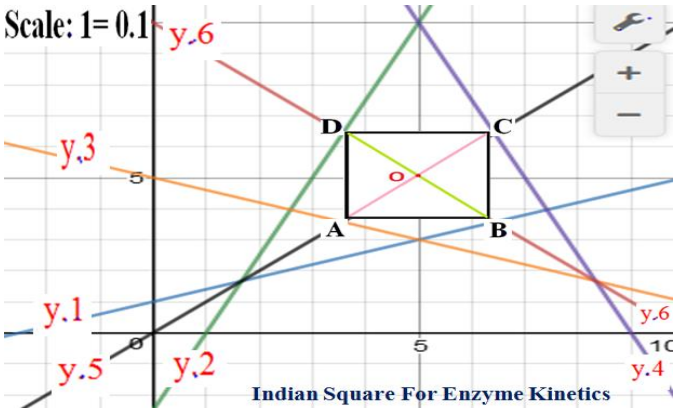

Fig. 8 : Position of Indian Square For Enzyme Kinetics in Linrweaver-Burk Plot (Double Reciprocal Plot).

The X- co-ordinates and Y- co-ordinates of the four points (A,B,C,D) of the square obtained by the intersection of various lines in the present attempt are listed below:

The X – co-ordinate of the point "A" correspond to: $\frac{Km+1}{Vmax+Km}$

The Y – co-ordinate of the point "A" correspond to: $\frac{Km+1}{Vmax+Km}$

The X – co-ordinate of the point "B" correspond to: $\frac{Vmax-1}{Vmax+Km}$

The Y – co-ordinate of the point "B" correspond to: $\frac{Km+1}{Vmax+Km}$

The X – co-ordinate of the point "C" correspond to: $\frac{Vmax-1}{Vmax+Km}$

The Y – co-ordinate of the point "C" correspond to: $\frac{Vmax-1}{Vmax+Km}$

The X – co-ordinate of the point "D" correspond to: $\frac{Km+1}{Vmax+Km}$

The Y – co-ordinate of the point "D" correspond to: $\frac{Vmax-1}{Vmax+Km}$

The distance between the point "A" and point "B" (OR the length of segment AB) can be obtained by subtraction of the X- co-ordinate of the point "A" from the X – co-ordinate of the point "B". Therefore, the length of segment (AB) correspond to:

$$= \frac{Vmax-1}{Vmax+Km} - \frac{Km+1}{Vmax+Km}$$
$$= \frac{Vmax-1-Km-1}{Vmax+Km}$$
$$= \frac{Vmax-Km-2}{Vmax+Km}$$

The distance between the point "B" and point "C" (OR the length of segment BC) can be obtained by subtraction of the Y- co-ordinate of the point "B" from the Y – co-ordinate of the point "C". Therefore, the length of segment (BC) correspond to:

$$= \frac{Vmax-1}{Vmax+Km} - \frac{Km+1}{Vmax+Km}$$
$$= \frac{Vmax-1-Km-1}{Vmax+Km}$$
$$= \frac{Vmax-Km-2}{Vmax+Km}$$

The distance between the point "C" and point "D" (OR the length of segment CD) can be obtained by subtraction of the X- co-ordinate of the point "D" from the X – co-ordinate of the p2oint "C".

Therefore, the length of segment (CD) correspond to:

$$=$$
$$= \frac{Vmax-1}{Vmax+Km} - \frac{Km+1}{Vmax+Km}$$
$$= \frac{Vmax-1-Km-1}{Vmax+Km}$$
$$= \frac{Vmax-Km-2}{Vmax+Km}$$

The distance between the point "D" and point "A" (OR the length of segment DA) can be obtained by subtraction of the Y- co-ordinate of the point "A" from the Y – co-ordinate of the point "D".

Therefore, the length of segment (DA) correspond to:

$$= \frac{Vmax-1}{Vmax+ Km} - \frac{Km+1}{Vmax+ Km}$$

$$= \frac{Vmax- 1-Km-1}{Vmax+ Km}$$

$$= \frac{Vmax- Km-2}{Vmax+ Km}$$

From the above steps of attempts of obtaining the distance between the points of intersection of various lines or length of segment (AB; BC; CD and DA); all the four sides of rectangle ABCD are equal and correspond to:

$$= \frac{Vmax- Km-2}{Vmax+ Km}$$

Therefore, rectangle ABCD is the square.

This square (ABCD) resulted from the intersection of the line y_3 and line y_5; line y_1 and line y_6; line y_4 and y_5; line y_2 and line y_6; here, through this present is designated as "Indian Square For Enzyme Kinetics".

(VI). Properties and Significance of Indian Square For Enzyme Kinetics:

1. The regular form of Lineweaver-Burk plot (the line y_1 [equation: (1÷v) = (Km÷Vmax) (1÷S) + (1÷Vmax)] intersect the line y_6 (equation: - X + 1) at the point "B" of the proposed "Indian Square For Enzyme Kinetics". The X – co-ordinate and Y – co-ordinate of this point "B" correspond to: [(Vmax – 1) ÷ (Vmax + Km)] and [(Km + 1) ÷ (Vmax + Km)] respectively.
2. Subtraction of Y- co-ordinate of the point "B" from the X – co-ordinate of the point "B" is going to yield the length of each side of the proposed "Indian Square For Enzyme Kinetics".
   ∴ [(Vmax – 1) ÷ (Vmax + Km)] - [(Km + 1) ÷ (Vmax + Km)] = [(Vmax – Km – 2) ÷ (Vmax + Km)].
   The length of each side of the proposed "Indian Square For Enzyme Kinetics" correspond to: [(Vmax – Km – 2) ÷ (Vmax + Km)].
3. The length of segment AB; the segment BC; the segment CD and the segment DA of the proposed "Indian Square For Enzyme Kinetics" are equal and correspond to: [(Vmax – Km – 2) ÷ (Vmax + Km)].
4. The point "A" of the proposed "Indian Square For Enzyme Kinetics" is the point of intersection of the line y_3 and the line y_5. The X – co-ordinate and Y – co-ordinate of the point "A" of the proposed "Indian Square For Enzyme Kinetics" correspond to: [(Km +1) ÷ (Vmax + Km)] and [(Km +1) ÷ (Vmax + Km)] respectively. (Both, the X – co-ordinate and Y – co-ordinate of the point "A" of the proposed "Indian Square For Enzyme Kinetics" are equal to each other and correspond to: [(Km +1) ÷ (Vmax + Km)]).
5. The point "C" of the proposed "Indian Square For Enzyme Kinetics" is the point of intersection of the line y_4 and the line y_5. The X – co-ordinate and Y – co-ordinate of the point "C" of the proposed "Indian Square For Enzyme Kinetics" correspond to: [(Vmax - 1) ÷ (Vmax + Km)] and [(Vmax - 1) ÷ (Vmax + Km)] respectively. (Both, the X – co-ordinate and Y – co-ordinate of the point "C" of the proposed "Indian Square For Enzyme Kinetics" are equal to each other and correspond to: [(Vmax - 1) ÷ (Vmax + Km)]).
6. The point "D" of the proposed "Indian Square For Enzyme Kinetics" is the point of intersection of the line y_2 and the line y_6. The X – co-ordinate and Y – co-ordinate of the point "D" of the proposed "Indian Square For Enzyme Kinetics" correspond to: [(Km + 1) ÷ (Vmax + Km)] and [(Vmax - 1) ÷ (Vmax + Km)] respectively.
7. The length of segment AB=BC=CD=DA and it correspond to: [(Vmax - Km - 2) ÷ (Vmax + Km)]. The point "A"; "B"; "C" and "D" constitute the vertices of square ABCD.
8. The resulting mathematical square, herewith labeled as: "Indian Square For Enzyme Kinetics". Each of the four vertices (corners) have known coordinates in terms of Vmax and Km, the key indices in enzyme kinetics.

9. The value of [(Km + 1) ÷ (Vmax + Km)] is greater than [(1÷Vmax)] and less than [(1÷2)]. This range can be written as: [(1÷Vmax)] < [(Km + 1) ÷ (Vmax + Km)] < [(1÷2)].
10. The value of [(Vmax - 1) ÷ (Vmax + Km)] is greater than [(1÷2)] and less than [(Vmax -1) ÷ Vmax)]. This range can be written as: [(1÷2)] < [(Vmax - 1) ÷ (Vmax + Km)] < [(Vmax -1) ÷Vmax)].

II. CONCLUSION

Each enzyme deserve a specific Michaelis-Menten constant (Km), which is determined through the double reciprocal plot, also recognized as Lineweaver-Burk plot. This constant of Michaelis-Menten (Km) is concentration of substrate [S] and it avails the velocity (v) of reaction to proceed up to half of it's maximal or Vmax. The regular Lineweaver-Burk plot and it's inverse form are designated as y_1 and y_2 lines respectively. Present attempt is considering additional plots or the lines keeping the concept in regular Lineweaver-Burk plot constant. These plots include: y_3 and y_4. In slope and intercept form the lines y_3 and y_4 expressed as: y_3= -[(Km÷Vmax)(X)] + [(km+1)÷(Vmax)] and y_4= -[(Vmax÷Km)(X)] + [(Vmax -1)÷(Km)]. In addition; $y_5 = X + 0$ and $y_6 = - X + 1$ are the two reference lines are also considered in this attempt. The line y_3 intersect the reference line y_5 at the point "A", the x- co-ordinate and y- co-ordinate of which are equal to each other and correspond to: [(Km+1)÷(Vmax+Km)]. The line y_1 intersect the reference line y_6 at the point "B", the x- co-ordinate and y- co-ordinate of which respectively correspond to: [(Vmax – 1) ÷ (Vmax + Km)] and [(Km + 1) ÷ (Vmax + Km)]. The point at which the reference line y_5 attains [(Vmax – 1) ÷ (Vmax + Km)] is labeled as the point "C". The X – co-ordinate and Y – co-ordinate of the point "C" correspond to: [(Vmax – 1) ÷ (Vmax + Km)] [(Km + 1) ÷ (Vmax + Km)] respectively. The point of intersection of the line y_2 and the reference line y_6 is labeled as the point "D". The X – co-ordinate and Y – co-ordinate of the point "D" correspond to: [(Km + 1) ÷ (Vmax + Km)] and [(Vmax – 1) ÷ (Vmax + Km)] respectively. The length of segment AB=BC=CD=DA and it correspond to: [(Vmax - Km - 2) ÷ (Vmax + Km)]. The point "A"; "B"; "C" and "D" constitute the vertices of square ABCD. The value of [(Km + 1) ÷ (Vmax + Km)] is greater than [(1÷Vmax)] and less than [(1÷2)]. This range can be written as: [(1÷Vmax)] < [(Km + 1) ÷ (Vmax + Km)] < [(1÷2)]. The value of [(Vmax - 1) ÷ (Vmax + Km)] is greater than [(1÷2)] and less than [(Vmax -1) ÷ Vmax)]. This range can be written as: [(1÷2)] < [(Vmax - 1) ÷ (Vmax + Km)] < [(Vmax -1) ÷Vmax)].
The resulting mathematical square, herewith labeled as: "Indian Square For Enzyme Kinetics". Each of the four vertices (corners) have known coordinates in terms of Vmax and Km, the key indices in enzyme kinetics.

III. ACKNOWLEDGEMENTS

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IV. REFERENCES

[1]. W., Weisstein, Eric. "Square". mathworld. http://mathworld.wolfram.com/Square.html Weisstein, Eric W. "Square." From MathWorld--A Wolfram Web Resource. http://mathworld.wolfram.com/Square.html
[2]. Zalman Usiskin and Jennifer Griffin, "The Classification of Quadrilaterals. A Study of Definition", Information Age Publishing, 2008, p. 59, ISBN 1-59311-695-0.
[3]. "Problem Set 1.3". jwilson.coe.uga.edu. Retrieved 2017-12-12.

[4]. Josefsson, Martin, "Properties of equidiagonal quadrilaterals" Forum Geometricorum, 14 (2014), 129-144.

[5]. "Maths is Fun - Can't Find It (404)". www.mathsisfun.com. Retrieved 2017-12-12.

[6]. Chakerian, G.D. "A Distorted View of Geometry." Ch. 7 in Mathematical Plums (R. Honsberger, editor). Washington, DC: Mathematical Association of America, 1979: 147.

[7]. 1999, Martin Lundsgaard Hansen, thats IT (c). "Vagn Lundsgaard Hansen". www2.mat.dtu.dk. Retrieved 2017-12-12.

[8]. "Geometry classes, Problem 331. Square, Point on the Inscribed Circle, Tangency Points. Math teacher Master Degree. College, SAT Prep. Elearning, Online math tutor, LMS". gogeometry.com. Retrieved 2017-12-12.

[9]. Park, Poo-Sung. "Regular polytope distances", Forum Geometricorum 16, 2016, 227-232. http://forumgeom.fau.edu/FG2016volume16/FG201627.pdf

[10]. John H. Conway, Heidi Burgiel, Chaim Goodman-Strauss, (2008) The Symmetries of Things, ISBN 978-1-56881-220-5 (Chapter 20, Generalized Schaefli symbols, Types of symmetry of a polygon pp. 275-278).

[11]. Wells, Christopher J. "Quadrilaterals". www.technologyuk.net. Retrieved 2017-12-12. Clark, W. E. and Suen, S. "An Inequality Related to Vizing's Conjecture." Electronic J. Combinatorics 7, No. 1, N4, 1-3, 2000. http://www.combinatorics.org/Volume_7/Abstracts/v7i1n4.html.

[12]. Coxeter, H. S. M. and Greitzer, S. L. Geometry Revisited. Washington, DC: Math. Assoc. Amer., p. 84, 1967.

[13]. Detemple, D. and Harold, S. "A Round-Up of Square Problems." Math. Mag. 69, 15-27, 1996.

[14]. Dixon, R. Mathographics. New York: Dover, p. 16, 1991.

[15]. Eppstein, D. "Rectilinear Geometry." http://www.ics.uci.edu/~eppstein/junkyard/rect.html.

[16]. Fischer, G. (Ed.). Plate 1 in Mathematische Modelle aus den Sammlungen von Universitäten und Museen, Bildband. Braunschweig, Germany: Vieweg, p. 2, 1986.

[17]. Fukagawa, H. and Pedoe, D. "One or Two Circles and Squares," "Three Circles and Squares," and "Many Circles and Squares (Casey's Theorem)." §3.1-3.3 in Japanese Temple Geometry Problems. Winnipeg, Manitoba, Canada: Charles Babbage Research Foundation, pp. 37-42 and 117-125, 1989.

[18]. Gardner, M. The Sixth Book of Mathematical Games from Scientific American. Chicago, IL: University of Chicago Press, pp. 165 and 167, 1984.

[19]. Guy, R. K. "Rational Distances from the Corners of a Square." §D19 in Unsolved Problems in Number Theory, 2nd ed. New York: Springer-Verlag, pp. 181-185, 1994.

[20]. Harris, J. W. and Stocker, H. "Square." §3.6.6 in Handbook of Mathematics and Computational Science. New York: Springer-Verlag, pp. 84-85, 1998.

[21]. Kern, W. F. and Bland, J. R. Solid Mensuration with Proofs, 2nd ed. New York: Wiley, p. 2, 1948.

[22]. Yaglom, I. M. Geometric Transformations I. New York: Random House, pp. 96-97, 1962.

[23]. Lineweaver, H; Burk, D. (1934). "The determination of enzyme dissociation constants". Journal of the American Chemical Society. 56 (3): 658–666. doi:10.1021/ja01318a036.

[24]. Hayakawa, K.; Guo, L.; Terentyeva, E.A.; Li, X.K.; Kimura, H.; Hirano, M.; Yoshikawa, K.; Nagamine, T.; et al. (2006). "Determination of specific activities and kinetic constants of biotinidase and lipoamidase in LEW rat and Lactobacillus casei (Shirota)". Journal of Chromatography B. 844 (2): 240–50.

doi:10.1016/j.jchromb.2006.07.006. PMID 16876490.

[25]. Greco, W.R.; Hakala, M.T. (1979). "Evaluation of methods for estimating the dissociation constant of tight binding enzyme inhibitors" (PDF). Journal of Biological Chemistry. 254 (23): 12104–12109. PMID 500698.

[26]. Dowd, John E.; Riggs, Douglas S. (1965). "A comparison of estimates of Michaelis–Menten kinetic constants from various linear transformations" (pdf). Journal of Biological Chemistry. 240 (2): 863–869.

[27]. Keith J. Laidler (1997). New Beer in an Old Bottle: Eduard Buchner and the Growth of Biochemical Knowledge, pp. 127–133, ed. A. Cornish-Bowden, Universitat de València, Valencia, Spain, 1997 http://bip.cnrs-mrs.fr/bip10/newbeer/laidler.pdf

[28]. Dick RM (2011). "Chapter 2. Pharmacodynamics: The Study of Drug Action". In Ouellette R, Joyce JA. Pharmacology for Nurse Anesthesiology. Jones & Bartlett Learning. ISBN 978-0-7637-8607-6

[29]. Berg, Jeremy M.; Tymoczko, John L.; Stryer, Lubert (2002). "The Michaelis-Menten Model Accounts for the Kinetic Properties of Many Enzymes". Biochemistry. 5th edition. W H Freeman.

[30]. John E. Dowd and Douglas Briggs (1965). Estimates of Michaelis – Menten kinetic constants from various linear transformation. The Journal of Biological Chemistry Vol. 240 No. 2 February, 1965. http://www.jbc.org/content/240/2/863.full.pdf

[31]. Hall, Arthur Graham; Frink, Fred Goodrich (January 1909). "Chapter II. The Acute Angle [14] Inverse trigonometric functions". Written at Ann Arbor, Michigan, USA. Trigonometry. Part I: Plane Trigonometry. New York, USA: Henry Holt and Company / Norwood Press / J. S. Cushing Co. - Berwick & Smith Co., Norwood, Massachusetts, USA. p. 15. Retrieved 2017-08-12. https://en.wikipedia.org/wiki/Inverse_function

[32]. Scheinerman, Edward R. (2013). Mathematics: A Discrete Introduction. Brooks/Cole. p. 173. ISBN 978-0840049421.

[33]. Keisler, Howard Jerome. "Differentiation" (PDF). Retrieved 2015-01-24. §2.4

[34]. Scheinerman, Edward R. (2013). Mathematics: A Discrete Introduction. Brooks/Cole. p. 173. ISBN 978-0840049421.

[35]. Korn, Grandino Arthur; Korn, Theresa M. (2000) [1961]. "21.2.-4. Inverse Trigonometric Functions". Mathematical handbook for scientists and engineers: Definitions, theorems, and formulars for reference and review (3 ed.). Mineola, New York, USA: Dover Publications, Inc. p. 811;. ISBN 978-0-486-41147-7.

[36]. Oldham, Keith B.; Myland, Jan C.; Spanier, Jerome (2009) [1987]. An Atlas of Functions: with Equator, the Atlas Function Calculator (2 ed.). Springer Science+Business Media, LLC. doi:10.1007/978-0-387-48807-3. ISBN 978-0-387-48806-6. LCCN 2008937525.

[37]. Briggs, William; Cochran, Lyle (2011). Calculus / Early Transcendentals Single Variable. Addison-Wesley. ISBN 978-0-321-66414-3.

[38]. Devlin, Keith J. (2004). Sets, Functions, and Logic / An Introduction to Abstract Mathematics (3rd ed.). Chapman & Hall / CRC Mathematics. ISBN 978-1-58488-449-1.

[39]. Fletcher, Peter; Patty, C. Wayne (1988). Foundations of Higher Mathematics. PWS-Kent. ISBN 0-87150-164-3.

[40]. Lay, Steven R. (2006). Analysis / With an Introduction to Proof (4 ed.). Pearson / Prentice Hall. ISBN 978-0-13-148101-5.

[41]. Smith, Douglas; Eggen, Maurice; St. Andre, Richard (2006). A Transition to Advanced Mathematics (6 ed.). Thompson Brooks/Cole. ISBN 978-0-534-39900-9.

[42]. Thomas, Jr., George Brinton (1972). Calculus and Analytic Geometry Part 1: Functions of One

Variable and Analytic Geometry (Alternate ed.). Addison-Wesley.

[43]. Wolf, Robert S. (1998). Proof, Logic, and Conjecture / The Mathematician's Toolbox. W. H. Freeman and Co. ISBN 978-0-7167-3050-7.

[44]. Stryer L, Berg JM, Tymoczko JL (2002). Biochemistry (5th ed.). San Francisco: W.H.Freeman. ISBN 0-7167-4955-6.

[45]. Schomburg I, Chang A, Placzek S, Söhngen C, Rother M, Lang M, Munaretto C, Ulas S, Stelzer M, Grote A, Scheer M, Schomburg D (January 2013). "BRENDA in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA". Nucleic Acids Research. 41 (Database issue): D764–72. doi:10.1093/nar/gks1049. PMC 3531171. PMID 23203881

[46]. Radzicka A, Wolfenden R (January 1995). "A proficient enzyme". Science. 267 (5194): 90–931. Bibcode:1995Sci...267...90R. doi:10.1126/science.7809611. PMID 7809611.

[47]. Callahan BP, Miller BG (December 2007). "OMP decarboxylase—An enigma persists". Bioorganic Chemistry. 35 (6): 465–9. doi:10.1016/j.bioorg.2007.07.004. PMID 17889251.

[48]. Michaelis L, Menten M (1913). "Die Kinetik der Invertinwirkung" [The Kinetics of Invertase Action]. Biochem. Z. (in German). 49: 333–369.; Michaelis L, Menten ML, Johnson KA, Goody RS (2011). "The original Michaelis constant: translation of the 1913 Michaelis-Menten paper". Biochemistry. 50 (39): 8264– 9. doi:10.1021/bi201284u. PMC 3381512. PMID 21888353.

[49]. Briggs GE, Haldane JB (1925). "A Note on the Kinetics of Enzyme Action". The Biochemical Journal. 19 (2): 339–339. doi:10.1042/bj0190338. PMC 1259181. PMID 16743508.

[50]. Ellis RJ (October 2001). "Macromolecular crowding: obvious but underappreciated". Trends in Biochemical Sciences. 26 (10): 597–604. doi:10.1016/S0968- 0004(01)01938-7. PMID 11590012.

[51]. Kopelman R (September 1988). "Fractal reaction kinetics". Science. 241 (4873): 1620

[52]. Bibcode:1988Sci...241.1620K. doi:10.1126/science.241.4873.1620. PMID 17820893.

[53]. Segel, L.A.; Slemrod, M. (1989). "The quasi-steady-state assumption: A case study in perturbation". ThermochimActa31 (3): 446–477. doi:10.1137/1031091.

[54]. Leskovac, V. (2003). Comprehensive enzyme kinetics. New York: Kluwer Academic/Plenum Pub. ISBN 978-0-306-46712-7.

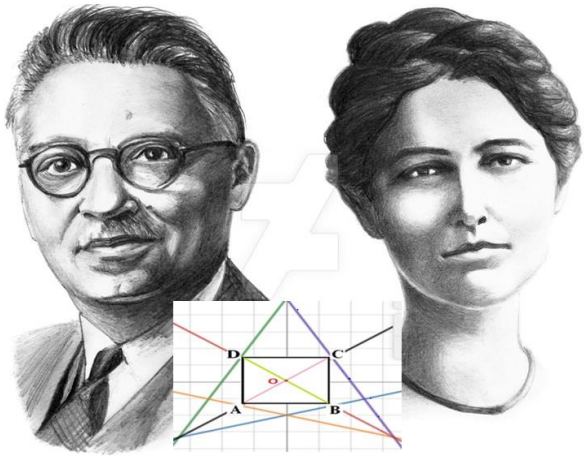
[55]. Greco, W.R.; Hakala, M.T. (1979). "Evaluation of methods for estimating the dissociation constant of tight binding enzyme inhibitors,".J BiolChem254 (23): 12104–12109. PMID 500698.

[56]. Hayakawa, K.; Guo, L.; Terentyeva, E.A.; Li, X.K.; Kimura, H.; Hirano, M.; Yoshikawa,K.; Nagamine, T. et al. (2006). "Determination of specific activities and kinetic constants of biotinidase and lipoamidase in LEW rat and Lactobacillus casei (Shirota)". J Chromatogr B AnalytTechnol Biomed Life Sci844 (2): 240–50.doi:10.1016/j.jchromb.2006.07.006. PMID 16876490.

[57]. Vitthalrao B. Khyade; Vivekanand V. Khyade and Sunanda V. Khyade (2017). The Baramati Quotient for the accuracy in calculation of Km value for Enzyme Kinetics. International Academic Journal of Innovative Research Vol. 4, No. 1, 2017, pp. 1-22.ISSN 2454-390X www.iaiest.com

[58]. Seema Karna Dongare, Manali Rameshrao Shinde, Vitthalrao Bhimasha Khyade (2018). Mathematical Inverse Function (Equation) For Enzyme Kinetics. Research Journal of Recent Sciences. Res. J. Recent Sci. Vol. 7(12), 9-15, December (2018). www.isca.in, www.isca.me

[59]. Seema Karna Dongare, Manali Rameshrao Shinde, Vitthalrao Bhimasha Khyade (2018). Mathematical Inverse Function (Equation) For Enzyme Kinetics. International Journal of Scientific Research in Chemistry (IJSRCH) | Online ISSN: 2456-8457 © 2018 IJSRCH | Volume 3 | Issue 4: 35– 42. http://ijsrch.com/archive.php?v=3&i=6&pyear=2018



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