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Mathematical inverse function (equation) for enzyme kinetics

Seema Karna Dongare[1], Manali Rameshrao Shinde[1] and Vitthalrao Bhimasha Khyade[2,3]
[1]Department of Microbiology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati Dist. Pune-413115, India
[2]Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No-35, Baramati, Pune 413 115, Maharashtra, India
[3]Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Tal. Baramati, Dist. Pune–413115 India
vbkhyade.2016@gmail.com

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Abstract

The most significant application of Lineweaver–Burk plot lies in the determination of Constant of Michaelis in enzyme kinetics. The constant of Michaelis is well recognized as, "Km". This constant of Michaelis is concentration of substrate [S] and it give the velocity (v) of reaction that correspond to half of it's maximal or Vmax. The Km, the Michaelis constant, for practical purposes, is the concentration of substrate that allows the enzyme velocity to achieve half of it's maximum Vmax ($V_{max} \div 2$). Most of the readings of inverse of enzyme velocity ($1 \div v$) occupy position far to the right of the x-axis. Through the reverse the mathematical steps and get inverse of substrate concentration ($1 \div S$) back from some output value, say inverse of respective velocity ($1 \div v$), it is necessary to carry out the steps exactly in back sequence. That is to say, one should subtract the inverse of maximum velocity ($1 \div Vmax$) from inverse of respective velocity ($1 \div v$) and then multiply the result by $\frac{Vmax}{Km}$. This is going to yield the equation correspond to: $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$. The $1 \div S$ and $1 \div v$ for given enzyme catalyzed biochemical reaction deserve symmetry, that is to say the symmetry between a Lineweaver Burk Plot (the real function) and the inverse function for enzyme kinetics of present attempt. 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: ($\frac{1}{Vmax - Km}$, $\frac{1}{Vmax - Km}$).

Keywords: Baramati Constant, $\frac{1}{Vmax - Km}$, mathematical approach.

Introduction

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 upto 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[1] 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 constnt 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 varies. Generally, the enzyme with a higher Km value has little bit lower affinity for its substrate. According to Keith J. Laidler[2], 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. 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[1] can be written as: $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$

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". 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*[3], 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. Each and every method is with positive and negative points of advantages. According to Hayakawa *et al*[3], 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*[3], 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[4].

Dick[5] explained type of inhibition of enzyme activity or stoping the working of enzymes. Of couse, this explaination 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[6,7]. John E. Dowd and Douglas Briggs[8] 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 (significant) 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 equation for inverse form of enzyme kinetics

According to Hall, Arthur Graham and Frink, Fred Goodrich[9-11], in mathematics, the equation (mathematical function) is represented in two forms: Real form and Inverse form. The inverse function may also called as anti – function. According to Scheinerman, Edward R. [9,12-14], the inverse function or equation is a function or equation that "reverses" real form of equation[14,15]. If the equation or function designated as " *f* " used for the investing "*x*" yields product in the form of result of "*y*", afterward using opposite order equation "*g*" for "*y*" yields product in the form of result of "*x*", and again backward [" $f(x) = y$"] conditionally, "$g(y) = x$". This much part of concept exclusively belong to mathematics[16-20].

For the purpose of fortified understanding of the concept of "Inverse Function", mathematics is citing simple example. Let us have a look on this instance of mathematical calculations. Consider the equation, mathematically termed as "Equation with real values" of "Real variable". Let us for instance, it is represented by "$f(x)$" = 6 x - 8 or y = 6 x - 8 (In "y = m x + c" format[21-24]. This is because, the original Lineweaver Burk plot deserve this format[25-27]. This mathematical equation is guiding for lay hold of *x*. Further, it is saying that, now multiply this *x* by six. Subtract eight from the figure obtained[28-30].

Let us now have directly the equation for "Lineweaver-Burk-Plot": $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$.

The mathematical equation of Lineweaver Burk plot, itself is self explainatory. Reciprocal of substrate concentration (1÷[S]) is to be multiplied with the slope, that is Km÷Vmax. Finally, it is necessary to add the inverse of maximum velocity (1÷Vmax) to get the value of inverse of respective velocity [1÷(v)] obtained from the bioassay of the of enzyme activity. Of course, such type of attempt is not new idea or concept. It has already been expected in the "Lineweaver-Burk-Plot" of kinetics of enzymes. The efforts of present attempt are concerned with getting the inverse function (equation) for "Lineweaver-Burk-Plot" of kinetics of enzymes.

This present mathematical attempt for obtaining the inverse function for "y = m x + c" is concerned with getting the value of "X" back. To reverse the mathematical steps and 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 mathematical calculation steps exactly in backward steps or in reverse sequence or the order.

It means, in very first step, it is essential to subtract the inverse of maximum velocity (1÷Vmax) from inverse of respective velocity (1÷v) and then multiply the result by $\frac{Vmax}{Km}$. This is going to yield the equation correspond to: $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$.

For graphical presentation of equations of regular "Lineweaver-Burk-Plot", it is necessary to take the inverse of substrate concentration (1÷S) on X – axis and inverse of respective velocity (1÷v) of enzyme catalyzed biochemical reaction on Y – axis. This is going to serve the purpose in expectation of Lineweaver Burk plot: $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$.

For graphical presentation of equation: $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$; it is necessary to take $\frac{1}{V}$ on X – axis and $\frac{1}{S}$ on Y – axis. Allotment of take $\frac{1}{V}$ and $\frac{1}{S}$ is going to serve purpose in expectation of Inverse function for Lineweaver Burk plot. That is to say plotting the readings of (1÷S) against (1÷v) and plotting the readings of (1÷v) against (1÷S) (Figure-1).

Mathematical properties of equation for inverse form of enzyme kinetics

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 involveved processes. It's inverse form of equation is making this mathematical associan 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.

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.

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.

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.

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: $(\frac{1}{Vmax-Km}, \frac{1}{Vmax-Km})$.

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.

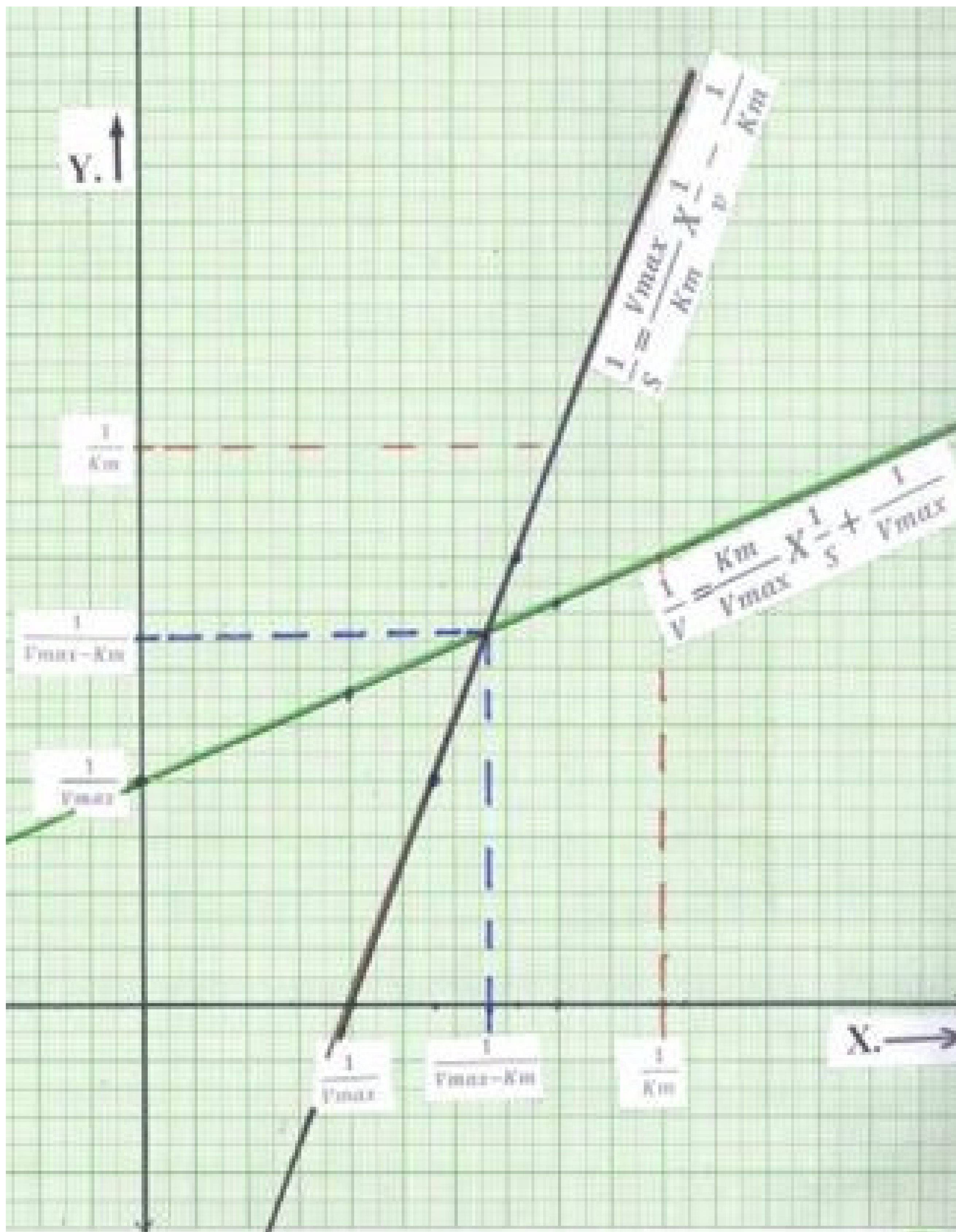

Figure-1: Inverse Form of Lineweaver-Burk Plot for Enzyme Kinetics Alongwith it's Real Form.

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.

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[31-33]. 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 imposibel for practical accessibility of the concept in expectation of Michaelis–Menten equation[34-36].

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. Moreover, the "inverse function or equation for enzyme kinetics" explains "Species Specific Nature of Enzymes"[37-41].

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[35]. 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}÷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 substarte molecules in enzyme catalyzed biochemical reaction.

Conclusion

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 $\frac{Vmax}{Km}$. This is going to yield the equation correspond to: $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$. 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 $\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$ and $\frac{1}{S} = \frac{Vmax}{Km} X \frac{1}{v} - \frac{1}{Km}$ correspond to: ($\frac{1}{Vmax-Km}$, $\frac{1}{Vmax-Km}$). The 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 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 ($\frac{1}{V} = \frac{Km}{Vmax} X \frac{1}{S} + \frac{1}{Vmax}$) 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 substarte 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.

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