The “Baramati Quotient” for the accuracy in calculation of Km value for Enzyme Kinetics

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Abstract
The attempt deals with the derivation of mathematical formula for the calculation of Michaelis-Menten constant ($K_m$). Mathematically, the Baramati Quotient (BQ) correspond to the $X – co-ordinate$ of the point, for the line $Y_3 (Y_3 = - X + 1)$ when it attains the point which is equal to:

$$X = \frac{1}{V_{max}} \left( \frac{K_m V_{max}^2 (V_{max} + 1) - (V_{max} - 1)}{K_m V_{max} (V_{max} + 2) - 1} \right)$$

The method of Calculation of Km through Baramati Quotient (BQ) is easy to understand to the non-mathematical students; the parameter $[S (V_{max} – v)]$ allows to screen the data on enzyme bioassay in the form of substrate concentration [$S$] and velocity ($v$) of enzyme catalyzed biochemical reaction. This is because, mostly, all the $[S]$ and corresponding ($v$) in the Bioassay attempt are not obeying the modulation of Lineweaver–Burk plot. The method is keeping the concept of response of ($v$) to the [$S$]. The $x – co-ordinate$ of line $Y_3$ for attaining the BQ is more than half $[1 ÷ 2]$; this value is easy to plot on the graph; The Baramati Quotient (BQ) is easy to calculate. The method deserve wide applicability. It may have a demerits, like: the reading of maximum velocity ($V_{max}$.) obtained in the Bioassay attempt may exert influence on the calculation of Km through Baramati Quotient (BQ); The slope of the Line $Y_1$ is extremely low and that of $Y_2$ is extremely high.

Keywords: Baramati Quotient (BQ) , Enzyme Kinetics ; Km

INTRODUCTION
Enzymes accelerate, or catalyze, biochemical reactions. The substrates are the compounds that initiates the process of biochemical catalytic reactions. Almost all metabolic processes in a cell need enzymes in order to occur at rates fast enough to sustain life (Stryer, et al., 2002). The set of enzymes made in a cell determines which metabolic pathways occur in that cell. Enzymes are known to catalyze more than 5,000 biochemical reaction types (Schomburg, 2013). All the enzymes are proteins, although a few are catalytic RNA molecules. Enzymes’ specificity comes from their unique three-dimensional structures. Like all the catalysts, enzymes increase the rate of a reaction by lowering its activation energy. Some of the enzymes
can make their conversion of substrate to product occur many millions of times faster. For example, the enzyme “orotidine 5'-phosphate decarboxylase”, allows a reaction that would otherwise take millions of years to occur in milliseconds (Radzicka and Wolfenden, 1995; Callahan and Miller, 2007). The enzymes are like any catalyst and are not consumed in chemical reactions, nor do they alter the equilibrium of a reaction. The enzymes differ from most other catalysts by being much more specific. Enzyme activity can be affected by other molecules: inhibitors are molecules that decrease enzyme activity, and activators are molecules that increase activity. Many of the drugs and poisons are enzyme inhibitors. An enzyme’s activity decreases markedly outside its optimal temperature and pH. Some enzymes are used commercially, for example, in the synthesis of antibiotics. Some household products use enzymes to speed up chemical reactions: enzymes in biological washing powders break down protein, starch or fat stains on clothes, and enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.

The enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. The data in the form of “rate of reaction (v)” used in kinetic analyses is commonly obtained from enzyme assays. In 1913, Leonor Michaelis and Maud Leonora Menten proposed a quantitative theory of enzyme kinetics, which is referred to as Michaelis–Menten kinetics (Michaelis and Menten, 1913). According to Michaelis and Menten, enzyme reactions in two stages. The substrate binds reversibly to the enzyme, forming the enzyme-substrate (ES) complex in the first stage. The enzyme-substrate complex is sometimes called the Michaelis-Menten complex in their honor. In the second step, enzyme then catalyzes the reaction and releases the product. This work of Leonor Michaelis and Maud Leonora Menten was further developed by G. E. Briggs and J. B. S. Haldane, who derived kinetic equations that are still widely used today (Briggs and Haldane, 1925).

The enzyme rates depend on solution conditions and substrate concentration. To find the maximum rate of an enzymatic reaction (v), the substrate concentration [S] is increased until a constant rate of product formation is seen. The saturation happens because, as substrate concentration increases, more and more of the free enzyme is converted into the substrate-bound ES complex. At the maximum reaction rate (V_max) of the enzyme, all the enzyme active sites are bound to substrate, and the amount of ES complex is the same as the total amount of enzyme (Stryer, et al, 2002).

Source for the Image: https://www.studyblue.com/notes/note/n/enzyme-kinetics/deck/4656513

The maximum reaction rate (velocity) (V_max) is only one of several important kinetic parameters. The amount of substrate required to achieve a given rate of reaction is also important. This is represented by
the Michaelis-Menten constant \( (K_m) \), which is the substrate concentration required for an enzyme to reach one-half its maximum reaction rate \( (V_{\text{max}} / 2) \); generally, each enzyme has a characteristic \( K_m \) for a given substrate. Another useful constant is \( k_{\text{cat}} \), also called the turnover number, which is the number of substrate molecules handled by one active site per second (Stryer, et al., 2002). The Michaelis–Menten kinetics relies on the law of mass action, which is derived from the assumptions of free diffusion and thermodynamically driven random collision. Many biochemical or cellular processes deviate significantly from these conditions, because of macromolecular crowding and constrained molecular movement (Ellis, 2001). More recent, complex extensions of the model attempt to correct for these effects (Kopelman, 1988).

**Determination of constants**

The typical method for determining the constants like Maximum velocity \( (V_{\text{max}}) \) and Michaelis-Menten constant \( (K_m) \) involves carrying out a series of enzyme assays at varying substrate concentrations \( [S] \) and measuring the initial reaction rate \( (v) \). 'Initial velocity' here is taken to mean that the reaction rate/velocity is measured after a relatively short time period, during which it is assumed that the enzyme-substrate complex has formed, but that the substrate concentration held approximately constant, and so the equilibrium or quasi-steady-state approximation remain valid (Segel and Slemrod, 1989). By plotting reaction rate against concentration, and using nonlinear regression of the Michaelis–Menten equation, the parameters may be obtained (Segel and Slemrod, 1989). Before computing facilities to perform nonlinear regression became available, graphical methods involving linearisation of the equation were used. A number of these were proposed, including the Eadie–Hofstee diagram, Hanes–Woolf plot and Lineweaver–Burk plot; of these, the Hanes–Woolf plot is the most accurate (Segel and Slemrod, 1989). However, while useful for visualization, all three methods distort the error structure of the data and are inferior to nonlinear regression (Leskovac, 2003). Nonetheless, their use can still be found in modern literature (Hayakawa, et al., 2006). This is because they deserve fortified concept of enzyme kinetics. In 1997 Santiago Schnell and Claudio Mendoza derived a closed form solution for the time course kinetics analysis of the Michaelis–Menten kinetics (Schnell and Mendoza, 1997). The plot of \( (v) \) versus \( [S] \) is not linear; although initially linear at low \( [S] \), it bends over to saturate at high \( [S] \). Before the modern era of nonlinear curve-fitting on computers, this nonlinearity could make it difficult to estimate \( K_M \) and \( V_{\text{max}} \) accurately. Therefore, several researchers developed linearisations of the Michaelis–Menten equation, such as the Lineweaver–Burk plot, the Eadie–Hofstee diagram and the Hanes–Woolf plot. All of these linear representations can be useful for visualising data, but none should be used to determine kinetic parameters, as computer software is readily available that allows for more accurate determination by nonlinear regression methods. The Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934. The inverse values of initial velocities \( (1 / v) \) of enzyme catalyzed biochemical reaction are plotted against the inverse of substrate concentrations \( (1 / S) \). Therefore, the plot is said to be double reciprocal.
Practical Problems with the Lineweaver–Burk plot:

The Lineweaver–Burk plot is classically used in older texts, but is prone to error, as the y-axis takes the reciprocal of the rate of reaction – in turn increasing any small errors in measurement. Also, most points on the plot are found far to the right of the y-axis (due to limiting solubility not allowing for large values of [S] and hence no small values for 1/[S]), calling for a large extrapolation back to obtain x- and y-intercepts. According to Lineweaver – Burk, only the initial velocities (v) (possibly, the velocities less than the half of it’s maximal / Vmax.) of enzyme catalyzed biochemical reactions are obey to occupy significant position in the double reciprocal plot. In theoretical sense, there is no substrate concentration for maximal velocity (Vmax.) and it is for half of it’s maximal (Vmax / 2), which is nothing but the Km value. There is a possibility of human error in the process of considering the values of reciprocal of velocities (1/v) and... and to plot the line passing through the reciprocal value of maximal velocity (Vmax.). There is no any perfect method for calculation of Km value through the Lineweaver – Burk plot. This may be reason, for restriction of Lineweaver – Burk plot for the practical use. For the purpose to improve and fortify the method of calculation of Km values for enzyme catalyzed biochemical reactions, the present attempt on Baramati Quotient has been planned.

MATERIAL AND METHODS

The method to calculate the Km value for enzyme catalyzed biochemical reactions involve steps, which include: Theoretical Considerations; Calculation of Baramati Quotient; Calculation of slope and it’s utilization for knowing the Km value of enzyme catalyzed biochemical reaction.

(I). Theoretical Considerations:
Let us consider three lines, Y₁, Y₂ and Y₃, equations of which correspond to:

\[ Y_1 = \frac{2K_mV_{max}}{K_mV_{max}^2-1}X + \frac{1}{V_{max}} \]

\[ Y_2 = \frac{K_mV_{max}^2-1}{2K_mV_{max}}X + \frac{K_mV_{max}^2-1}{2K_mV_{max}^2} \]
Y₃ = - X + 1 respectively.

The lines Y₁ and Y₂ intersect with each other at a point (b).

Let us find the x – co-ordinate and y – co-ordinate of the point (b).

\[
\frac{2 \cdot Km \cdot V_{\text{max}}}{Km \cdot V_{\text{max}}^2 - 1} \cdot X + \frac{1}{V_{\text{max}}} = \frac{Km \cdot V_{\text{max}}^2 - 1}{2 \cdot Km \cdot V_{\text{max}}} \cdot X + \frac{Km \cdot V_{\text{max}}^2 - 1}{2 \cdot Km \cdot V_{\text{max}}^3}
\]

\[
\frac{2 \cdot Km \cdot V_{\text{max}}}{Km \cdot V_{\text{max}}^2 - 1} - \frac{Km \cdot V_{\text{max}}^2 - 1}{2 \cdot Km \cdot V_{\text{max}}} \cdot X = \frac{1}{V_{\text{max}}} - \frac{Km \cdot V_{\text{max}}^2 - 1}{2 \cdot Km \cdot V_{\text{max}}^3}
\]

\[
\frac{(Km \cdot V_{\text{max}}^2 - 1)(Km \cdot V_{\text{max}}^2 - 1) - (2 \cdot Km \cdot V_{\text{max}})(2 \cdot Km \cdot V_{\text{max}})}{(2 \cdot Km \cdot V_{\text{max}})(Km \cdot V_{\text{max}}^2 - 1)} \cdot X = \frac{2 \cdot Km \cdot V_{\text{max}} - Km \cdot V_{\text{max}}^2 + 1}{V_{\text{max}}(2 \cdot Km \cdot V_{\text{max}}^2)}
\]

\[
\frac{(Km \cdot V_{\text{max}}^2 - 1 - 2 \cdot Km \cdot V_{\text{max}})(Km \cdot V_{\text{max}}^2 - 1 + 2 \cdot Km \cdot V_{\text{max}})}{(2 \cdot Km \cdot V_{\text{max}})(Km \cdot V_{\text{max}}^2 - 1)} \cdot X = \frac{-(Km \cdot V_{\text{max}}^2 - 1 - 2 \cdot Km \cdot V_{\text{max}})}{V_{\text{max}}(2 \cdot Km \cdot V_{\text{max}}^2)}
\]

\[
\frac{(Km \cdot V_{\text{max}}(V_{\text{max}} - 2) - 1)(Km \cdot V_{\text{max}}(V_{\text{max}} + 2) - 1)}{(2 \cdot Km \cdot V_{\text{max}})(Km \cdot V_{\text{max}}^2 - 1)} \cdot X = \frac{-(Km \cdot V_{\text{max}}(V_{\text{max}} - 2) - 1)}{V_{\text{max}}(2 \cdot Km \cdot V_{\text{max}}^2)}
\]

\[
X = \frac{-1}{V_{\text{max}} Km \cdot V_{\text{max}}(V_{\text{max}} + 2) - 1} = (b)
\]

This point of intersection of Y₁ and Y₂ is X = \( \frac{-1}{V_{\text{max}} Km \cdot V_{\text{max}}(V_{\text{max}} + 2) - 1} \); let us find the value of X – co-ordinate; when Y₃ attains (b).
Let us label this \( X \) value as Baramati Quotient (BQ)

\[
\frac{1}{\frac{K_m V_{\text{max}}^2 - 1}{V_{\text{max}} K_m V_{\text{max}}(V_{\text{max}} + 2) - 1}} = -X + 1
\]

\[
X = \frac{1}{1 - \frac{K_m V_{\text{max}}^2 - 1}{V_{\text{max}} K_m V_{\text{max}}(V_{\text{max}} + 2) - 1}}
\]

\[
X = \frac{K_m V_{\text{max}}^2 (V_{\text{max}} + 2) - V_{\text{max}} - K_m V_{\text{max}}^2 + 1}{K_m V_{\text{max}}^2 (V_{\text{max}} + 2) - V_{\text{max}}}
\]

\[
X = \frac{K_m V_{\text{max}}^2 (V_{\text{max}} + 2 - 1) - (V_{\text{max}} - 1)}{K_m V_{\text{max}}^2 (V_{\text{max}} + 2) - V_{\text{max}}}
\]

\[
X = \frac{K_m V_{\text{max}}^2 (V_{\text{max}} + 1) - (V_{\text{max}} - 1)}{K_m V_{\text{max}}^2 (V_{\text{max}} + 2) - V_{\text{max}}}
\]

\[
X = \frac{1}{V_{\text{max}}} \frac{K_m V_{\text{max}}^2 (V_{\text{max}} + 1) - (V_{\text{max}} - 1)}{K_m V_{\text{max}} (V_{\text{max}} + 2) - V_{\text{max}}}
\]

This will help to calculate the Baramati Quotient (BQ) through the use of preliminary data \([ S]\) and velocity of enzyme catalyzed biochemical reaction \((v)\).
This Baramati Quotient (BQ) is going to serve the efficient tool for the calculation of $K_m$, the substrate concentration $[S]$ at which the velocity $(v)$ of enzyme catalyzed biochemical reaction attains half of it’s maximum, that is \( \frac{V_{\text{max}}}{2} \).

Mathematical formula for calculation of $K_m$ through the use of Baramati Quotient (BQ):

\[
\frac{(B.Q. \cdot V_{\text{max}}) + (1) - (V_{\text{max}})}{B.Q. \cdot V_{\text{max}} \cdot (V_{\text{max}} + 2) - V_{\text{max}} \cdot (V_{\text{max}} + 1) \cdot V_{\text{max}}}
\]

Steps for the calculation of Baramati Quotient (BQ) from the data pertaining the substrate concentration $[S]$ and the velocity $(v)$ of enzyme catalyzed biochemical reaction:

(II). Calculation of $[S \cdot (V_{\text{max}} - v)]$:

The very first step towards the calculation of Baramati Quotient, to arrange the substrate concentrations $(S)$ and respective values of velocities $(v)$, obtained in practical course, in a tabular form. Through the simple subtraction method, it is possible to calculate the value : $(V_{\text{max}} - v)$ for each of the $(S)$ and $(V)$. This $(V_{\text{max}} - v)$ is multiplied by respective $(S)$ values, which results into the parameter : $[S \cdot (V_{\text{max}} - v)]$. This parameter $[S \cdot (V_{\text{max}} - v)]$ appears to exhibit increasing tendency along the values of $(S)$ and $(V)$. Practically, it seems that, this increasing tendency of $[S \cdot (V_{\text{max}} - v)]$ get blocked at certain point of $(S)$. It is proposed that, the values of $[S \cdot (V_{\text{max}} - v)]$ with increasing tendency along respective $(S)$ and $(V)$ should be considered for further use in knowing the Baramati Quotient.

(III). Calculation of $D$:

Multiply $[S \cdot (V_{\text{max}} - v)]$ with $[V_{\text{max}} \cdot (V_{\text{max}} + 2)]$.

And now subtract $[v]$ from $[S \cdot (V_{\text{max}} - v)] \cdot [V_{\text{max}} \cdot (V_{\text{max}} + 2)]$. That is to say, calculate $[S \cdot (V_{\text{max}} - v)] \cdot [V_{\text{max}} \cdot (V_{\text{max}} + 2)] - [v]$.

Therefore, $D = [S \cdot (V_{\text{max}} - v)] \cdot [V_{\text{max}} \cdot (V_{\text{max}} + 2)] - [v]$.

(IV). Calculation of $N$:

Calculate the $[v(V_{\text{max}} - 1)]$ and $[S(V_{\text{max}} - v)] \cdot [V_{\text{max}}^2 \cdot (V_{\text{max}} + 1)]$.

Subtract $[v(V_{\text{max}} - 1)]$ from $[S(V_{\text{max}} - v)] \cdot [V_{\text{max}}^2 \cdot (V_{\text{max}} + 1)]$.

This will give the value of $N$ (Numerator):

$N = [S(V_{\text{max}} - v)] \cdot [V_{\text{max}}^2 \cdot (V_{\text{max}} + 1)] - [v(V_{\text{max}} - 1)]$. 

(V). Calculation of Km:

Divide \([N] \) by \([D] \).
The quotient thus obtained is further divided by the value of \(V_{\text{max}}\).

Therefore, \(Km = \left(\frac{[N]}{[D]} \right) \times \frac{1}{V_{\text{max}}} \)

RESULTS AND DISCUSSION

The results are summarized through the points, which include: Intersection of the two equations \((Y_1 \) and \(Y_2 \)); Baramati Quotient through the Equation \(Y_3 \); Simplification of Baramati Quotient (BQ); and demonstration with suitable.

(I). Intersection of the two equations \((Y_1 \) and \(Y_2 \)):
The intersection of the two lines, \(Y_1 \) and \(Y_2\)

That is to say;

\[
Y_1 = \frac{2Km \, V_{\text{max}}}{Km \, V_{\text{max}}^2 - 1} X + \frac{1}{V_{\text{max}}}
\]

\[
Y_2 = \frac{Km \, V_{\text{max}}^2 - 1}{2Km \, V_{\text{max}}} X + \frac{Km \, V_{\text{max}}^2 - 1}{2Km \, V_{\text{max}}^2}
\]

results into the point “(b)”

The lines \(Y_1 \) and \(Y_2\) intersect with each other at a point (b).

The \(x\) – co-ordinate and \(y\) – co-ordinate of the \(b\) correspond to:

\[
X = \frac{-1}{V_{\text{max}}} \quad \text{and} \quad \frac{1}{V_{\text{max}} \, (V_{\text{max}} + 2) - 1}
\]

(II). Baramati Quotient (BQ) through the Line / Equation \(Y_3 \):

The line equation for \(Y_3 = -X + 1\)

With the help of this line \((Y_3 = -X + 1)\), it became possible to find out the Baramati Quotient (BQ). Mathematically, this Baramati Quotient (BQ) correspond to the \(x\) – co-ordinate of the point, when \(Y_3\) attains the \(y\) – co-ordinate of the point \(b\); which is equal to:
(III). Simplification of Baramati Quotient (BQ):

In terms of Substrate concentration [S] and velocity of enzyme catalyzed biochemical reaction (v); the Baramati Quotient (BQ) can be written as:

\[
x = \frac{1}{V_{\text{max}}} \frac{K_m V_{\text{max}}^2 (V_{\text{max}} + 1) - (V_{\text{max}} - 1)}{K_m V_{\text{max}} (V_{\text{max}} + 2) - 1}
\]

This will help to calculate the Baramati Quotient (BQ) through the use of preliminary data [S] and velocity of enzyme catalyzed biochemical reaction (v).

This Baramati Quotient (BQ) is going to serve the efficient tool for the calculation of Km, the substrate concentration [S] at which the velocity (v) of enzyme catalyzed biochemical reaction attains half of its maximum, that is \( \frac{V_{\text{max}}}{2} \).

In terms of the Substrate concentration [S] and the velocity of enzyme catalyzed biochemical reaction (v); the Baramati Quotient (BQ) can be written as:

\[
x = \frac{1}{V_{\text{max}}} \frac{V_{\text{max}}^2 (V_{\text{max}} + 1)[S (V_{\text{max}} - v) - v(V_{\text{max}} - 1)]}{V_{\text{max}} (V_{\text{max}} + 2)[S (V_{\text{max}} - v) - v]}
\]

The above mathematical formula for Baramati Quotient (BQ) is self explaining. The data in terms of Substrate concentration [S] and the velocity of enzyme catalyzed biochemical reaction (v) obtained practically through the bioassay of any enzyme is going to serve to the purpose calculate the Numerator (N) and Denominator (D) in the mathematical formula of Baramati Quotient (BQ). It is better to calculate the Denominator (D) firstly. It should be followed by the calculation of Numerator (N). The Numerator (N) is divided by the Denominator (D). The quotient thus obtained is further divided by maximum velocity (Vmax) of enzyme catalyzed biochemical reaction.

The product / parameter \([S (V_{\text{max}} - v)]\) seems to appear in both Numerator (N) and the Denominator (D) of the mathematical formula for Baramati Quotient (BQ). It indicates that, this parameter \([S (V_{\text{max}} - v)]\) should be preferred to consider to calculate at the first. It appears that, the parameter \([S (V_{\text{max}} - v)]\) is increasing. This tendency of increase seems to up to some certain
extent of the [S]. The present attempt is considering the [S] and (v) that are exhibiting the increasing tendency in their [S(Vmax – v)].

Mathematical formula for calculation of Km through the use of Baramati Quotient (BQ):

\[
\frac{(B.Q. V_{\text{max}}) + (1) - (V_{\text{max}})}{[B.Q.V_{\text{max}} (V_{\text{max}} + 2)] - [V_{\text{max}} (V_{\text{max}} + 1)]} \times \frac{1}{V_{\text{max}}}
\]

Steps for the calculation of Baramati Quotient (BQ) from the data pertaining the substrate concentration [S] and the velocity (v) of enzyme catalyzed biochemical reaction:

(A). Calculation of [S (Vmax. – v)]:

The very first step towards the calculation of Baramati Quotient, to arrange the substrate concentrations (S) and respective values of velocities (v), obtained in practical course, in a tabular form. Through the simple subtraction method, it is possible to calculate the value: (Vmax. – v) for each of the (S) and (V). This (Vmax. – v) is multiplied by respective (S) values, which results into the parameter: [S (Vmax. – v)].

This parameter [S (Vmax. – v)] appears to exhibit increasing tendency along the values of (S) and (v). Practically, it seems that, this increasing tendency of [S(Vmax. – v)] get blocked at certain point of (S). It is proposed that, the values of [S(Vmax. – v)] with increasing tendency along respective (S) and (v) should be considered for further use in knowing the Baramati Quotient.

(B). Calculation of D:

Multiply [ S(Vmax. – v) ] with [Vmax (Vmax. + 2) ].

And now subtract [v] from [ S(Vmax. – v) ][Vmax (Vmax. + 2) ]. That is to say, calculate [ S(Vmax. – v) ][Vmax (Vmax. + 2) ] - [v].

Therefore, \( D = [ S(V_{\text{max}}. - v) ][V_{\text{max}} (V_{\text{max}}. + 2) ] - [v] \)

(C). Calculation of N:

Calculate the [v(Vmax. – 1)] and [S(Vmax. – v)][Vmax\(^2\) (Vmax.+1)].

Subtract [v(Vmax. – 1)] from [S(Vmax. – v)][Vmax\(^2\) (Vmax.+1)].

This will give the value of N (Numerator):

\( N = [S(V_{\text{max}}. - v)][V_{\text{max}}^2 (V_{\text{max}}. +1)] - [v(V_{\text{max}}. - 1)]. \)
(E). Calculation of Km:

Divide [N] by [D].

The quotient thus obtained is further divided by the value of Vmax.

Therefore, $\text{Km} = \frac{[N]}{[D]} \times \frac{1}{V_{\text{max}}}$

(IV). Demonstration with Example:

It is most relevant to entertain the matter through suitable example. The data here taken belongs to the biochemical reaction catalyzed by the mid gut protease in the fifth instar larva of silkworm, *Bombyx mori* (L) (Vitthalrao B. Khyade, 2004).

**Table – 1**: Data on the substrate Concentration [S] and Velocity (v) of enzyme catalyzed biochemical reaction.

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<thead>
<tr>
<th>[S]</th>
<th>(v)</th>
</tr>
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<td>5</td>
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</tr>
<tr>
<td>10</td>
<td>7.6</td>
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<tr>
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<td>10.8</td>
</tr>
<tr>
<td>30</td>
<td>13.6</td>
</tr>
<tr>
<td>40</td>
<td>15.8</td>
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<tr>
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<tr>
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<td>17.2</td>
</tr>
<tr>
<td>100</td>
<td>17.2</td>
</tr>
</tbody>
</table>

Source of Data: The mid gut protease activity (v) in the mid-gut homogenate at 120 hours after the fourth moult in silkworm, *Bombyx mori* (L) (Race: PM x CSR$_2$) (Vitthalrao B. Khyade, 2004).

**Table – 2**: Data pertaining various parameters on the calculation of Baramati Quotient (B.Q.).

| [S] | (v) | [S(V_{\text{max}} – v)] | D = V_{\text{max}}(V_{\text{max}}+2) \times [S(V_{\text{max}} – v)] – v | N = V_{\text{max}}^2(V_{\text{max}}+1) \times [S(V_{\text{max}} – v)] – [v(V_{\text{max}}-1)] | B.Q. = \frac{[N]}{[D]} \times \frac{1}{V_{\text{max}}}$
<table>
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<td>20470.08</td>
<td>333748.096</td>
<td>0.94791808677**</td>
</tr>
<tr>
<td>10</td>
<td>7.6</td>
<td>96*</td>
<td>31695.44</td>
<td>516768.528</td>
<td>0.9479181183**</td>
</tr>
<tr>
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<td>10.8</td>
<td>128*</td>
<td>42259.92</td>
<td>689013.904</td>
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<tr>
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<td>0</td>
<td>-17.2</td>
<td>-278.64</td>
<td>0.94186046511</td>
</tr>
<tr>
<td>70</td>
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<td>0</td>
<td>-17.2</td>
<td>-278.64</td>
<td>0.94186046511</td>
</tr>
<tr>
<td>80</td>
<td>17.2</td>
<td>0</td>
<td>-17.2</td>
<td>-278.64</td>
<td>0.94186046511</td>
</tr>
</tbody>
</table>
* : The [S(Vmax. – v)] readings eligible for further calculations.

** : The readings to be considered to be significant.

(IV). Merits of the system of Calculation of Km through Baramati Quotient (BQ) :

1. Easy to understand to the non-mathematical students. It is not essential to go through plotting the graph. Therefore, it deserve easiness for the non-mathematical faculties / candidates.
2. The parameter [S (Vmax – v)] allows to screen the data on enzyme bioassay in the form of substrate concentration [S] and velocity (v) of enzyme catalyzed biochemical reaction. This is because, mostly, all the [S] and corresponding (v) in the Bioassay attempt are not obeying the modulation of Lineweaver–Burk plot.
3. The method is keeping the concept of response of (v) to the [S].
4. The x – co-ordinate of line Y3 for attaining the BQ is more than half [1 ÷ 2]. This value is easy to plot on the graph.
5. The Baramati Quotient (BQ) is easy to calculate.
6. It deserve wide applicability.
7. 

(V). Demerits of the system of Calculation of Km through Baramati Quotient (BQ) :

1. The reading of maximum velocity (Vmax.) obtained in the Bioassay attempt may exert influence on the calculation of Km through Baramati Quotient (BQ).
2. The slope of the Line Y1 is extremely low and that of Y2 is extremely high.

CONCLUSION:

The substrate Concentration [S] at which the velocity of enzyme catalyzed reaction attains half of it’s maximum [Vmax ÷ 2] is termed as Michaelis-Menten constant (Km). Mathematically, the Baramati Quotient (BQ) correspond to the X – co-ordinate of the point, for the line Y3 ( - X + 1) when it attains the point which is equal to:

\[ x = \frac{1}{Vmax} \frac{KmVmax^2(Vmax + 1) - (Vmax - 1)}{Km Vmax(Vmax + 2) - 1} \]

The method of Calculation of Km through Baramati Quotient (BQ) is easy to understand to the non-mathematical students; the parameter [S (Vmax – v)] allows to screen the data on enzyme bioassay in the form of substrate concentration [S] and velocity (v) of enzyme catalyzed biochemical reaction. This is because, mostly, all the [S] and corresponding (v) in the Bioassay attempt are not obeying the modulation of Lineweaver–Burk plot. The method is keeping the concept of response of (v) to the [S]. The x – co-ordinate of line Y3 for attaining the BQ is more than half [1 ÷ 2]; this value is easy to plot on the graph;
The Baramati Quotient (BQ) is easy to calculate. The method deserve wide applicability. The demerit of the present method lies in exerting influence of the reading of maximum velocity (Vmax.) obtained in the Bioassay attempt. It may exert influence on the calculation of Km through Baramati Quotient (BQ). And one more demerit of the method is in the slope of the Line Y1 is extremely low and that of Y2 is extremely high. Otherwise present attempt deserve efficiency as a tool in enzyme klinetics.

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