Welcome to the module from the course Animal Physiology and biochemistry, From the programme Bachelors of Science, Zoology subject, myself Dr. K.K. Therisa. The name of the module is Lineweaver-Burk plot from the unit Enzyme.

The outline of the presentation is, Introduction to Lineweaver-Burk plot and applications of Lineweaver Burk plot.

The learning outcome expected at the end of this module, the student will be able to understand the concept of Lineweaver Burke plot and the applications of Lineweaver Burk plot.

To introduce about this concept, let me revise that we have learnt in the previous module on enzyme kinetics. Instead, kinetics is the study of rate of enzyme catalysed chemical reaction, and this was studied using and Michaelis Menten equation. Who derived it in a simplified manner so that in an experimental condition the VMAX that is the maximum velocity and the Michaelis constant can be determined.

And this however, have been difficult because the graph that shows in the expression of Michaelis Menten equation was showing a hyperbolic graph and using such graph in experimental condition, it is difficult to determine the KM value and the VMAX value. And that was solved by Lineweaver through their concept of Lineweaverk Burk plot wherein they use the inverse of the Michaelis Menten equation and plotted a graph wherein there was a straight line which was easy to determine, became value, and the VMAX value so to begin with, Lineweaver's Burk plot.

Let us introduce the concept, Lineweavers Burk plot commonly used plot in examining the enzyme kinetics. It is the inverse of the reaction rate that is plotted against the inverse of the substrate concentration and that is what is Lineweaver Burk plot.

This plot is also being known as the double Reciprocal plot and described by Hans Lineweaver and Dean Burk in 1934. A method of graphical representation of Lineweaver Burk, equation of enzyme kinetics is the main concept of this plot, and the plot provides a method of analysis of the Michaelis Menten equation in a simplified manner.

Why Lineweaver Burke plot was required? It is usually difficult to determine the VMAX of enzyme catalyzed reaction accurately. The interpretation of the data to calculate the values of Michaelis constant. That is the KM and the maximum velocity. That is the VMax. By plotting a graph of maximum velocity versus the substrate concentration.

And as this was difficult because as exact values cannot be obtained from a hyperbola obtained when a graph was plotted in the Michaelis menten equation. So, because of this difficulty, the Michaelis menten equation was transformed into an equation for a straight line by line Weaver burk plot, and this led to more accurate determination of the Michaelis constant, that is, the KM& VMAX values.

So, this is our double reciprocal plot. This plot is basically the derivation of the Michaelis Menten equation, which is represented as one by V is equal to KM up on VMAX into substrate concentration plus one by VMAX and this is the inverse of the Michaelis Menten equation. And the inverse of Michaelis Menten equation now called as Lineweavers Burk equation.

Now in this plot, which was otherwise seen as a hyperbola. In enzyme kinetics study what we have learned in the previous module. But the double reciprocal plot which is plotted using the equation of inverse of Michaelis menten equation gives a straight line with a slope that gives the KM versus VMAX value. On the other side, the graph that intersects the straight line that intersects the X axis gives the K value. That is, the KM, Michaelis constant. The inverse of the Michaelis constant, and the straight line that intersects on the Y axis gives the 1/ VMAX. So the Lineweavers Burk plot was widely used to determine the Km and Vmax values.

As I said, the Y intercept of the graph is equivalent to the inverse of VMAX. That is 1/VMAX and the X intercept of graph represents the -1/ KM, and here is the slope that provides the Km, VMAX in this graph.

The application of Lineweaver Burke plot. Is that it provides more information of enzyme inhibition. Quickly by visual impression of the plot by looking at the graph itself, it becomes very easy to understand whether the enzyme inhibition is a competitive inhibition, is a noncompetitive inhibition or the uncompetitive inhibition, and that is the most important application as far as Lineweaver Burk plot is concerned. So when used for determining the type of enzyme inhibition, the Lineweaver Burke plot can distinguish all these three.

Competitive inhibition wherein the inhibitor will compete with the enzyme to bind to the active site of the substrate a noncompetitive inhibition because the inhibitor will alter the enzyme. Confirmation in such a way that again it will affect the normal enzyme catalyzed reaction. Similarly, the uncompetitive inhibition as well have the impact on the enzyme catalyzed reaction, and all these three types of inhibitors can be determined by observing the Lineweaver Burke plot. It is also been used to determine important terms in enzyme kinetics such as the KM and VMAX. Before the wide availability of the powerful computers and nonlinear regression software. Here is the graphical representation depicting the competitive inhibition using the Lineweaver Burke plot.

In this plot, the slope. Shows are change. The Y intercept is the same. The KM Is increased and the VMAX remains unaffected. While the VMAX remains, unaffected whereas the KM Value increases in case of competitive inhibition.

Whereas in uncompetitive inhibition? The slope remains the same, but the Y intercept shows a change.The KM value shows the Reduction as well as the VMAX value.

Whereas in noncompetitive inhibition? The slope change, the Y intercept shows again the change and the KM remains unaffected, whether it is inhibitors present or not, so this is how the Lineweaver Burk plot can

be used to determine whether there is a competitive, uncompetitive or noncompetitive inhibition in our enzyme catalyzed reaction.

To sum up, with the enzyme kinetics and the Lineweaver Burk plot, the Michaelis constant, that is the KM and VMAX are characteristics for a given enzyme, substrate interaction. KM is often defined as the concentration of the substrate required to attain half maximal velocity.

That means half of the active sites present in the solution are saturated with the enzymes with the substrate. The studies and determination of KM and VMAX enable us to understand the effect of modulators on the enzyme catalyzed reactions. They are especially useful in the diagnosis of the type of inhibitor and inhibition, and that is what is achieved easily using the lineweavers Burk plot.

These are the references used to prepare this module.

Thank you.