Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 07
Enzyme Kinetics and Enzyme Inhibition
Lecture - 35
Discussion Class

In the final class of enzymes, we will be looking at some specific examples and some specific problems related to the topic.

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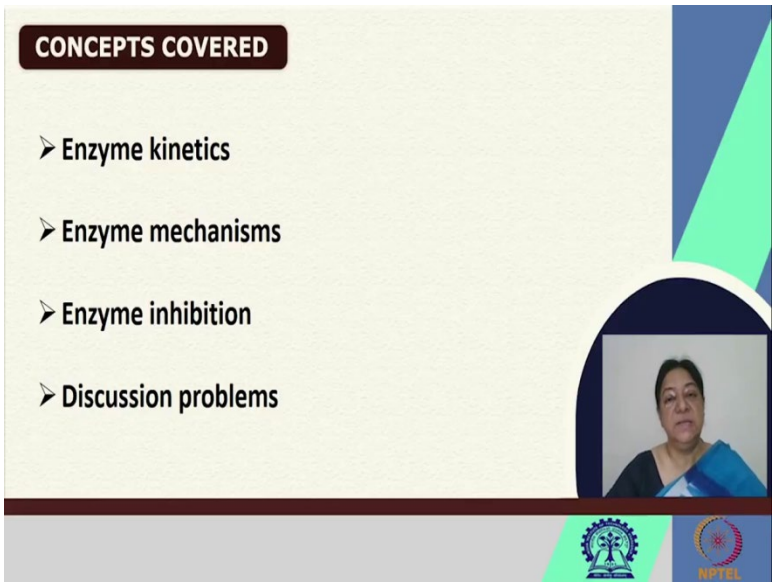

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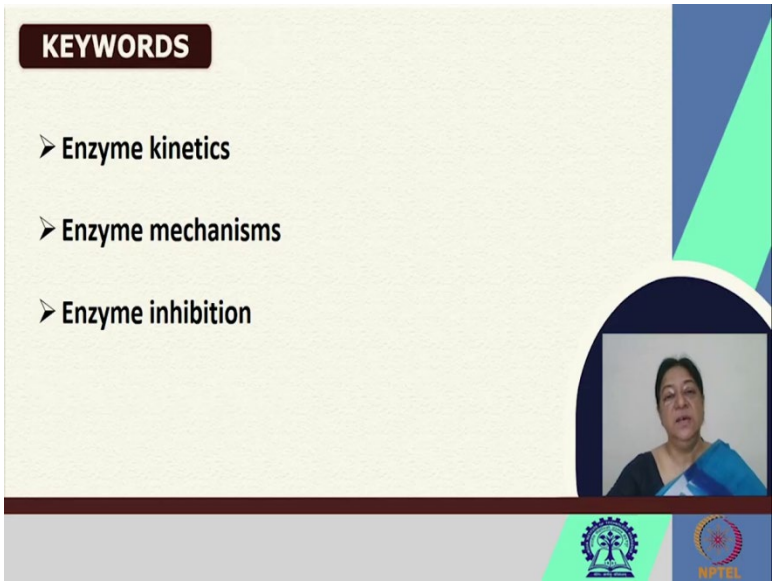

We have been studying enzyme kinetics, enzyme mechanisms, different enzyme classes and then enzyme inhibition. Today, we will be looking at these topics again, just to reiterate the specific ideas related to enzymes.

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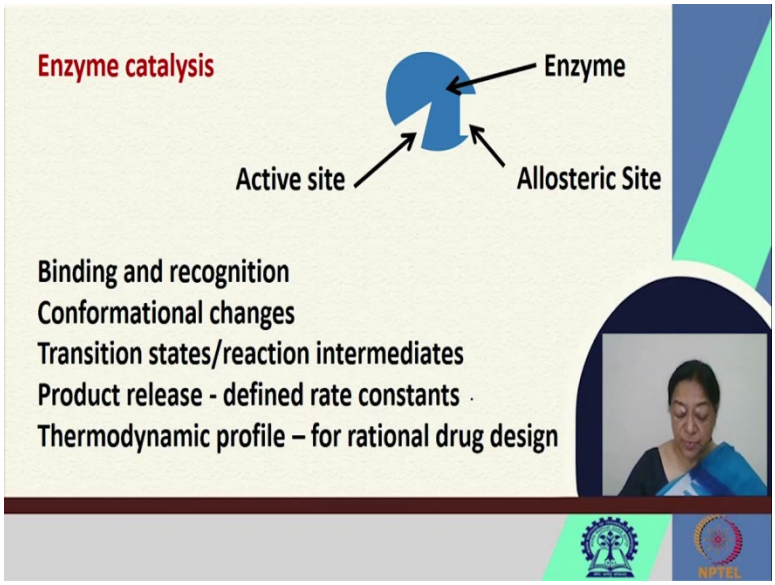

Their extreme importance in considering that we have our enzyme substrate complex, we have our active site, our allosteric site, the formation of products and the fact that they are used for targeted drug design.

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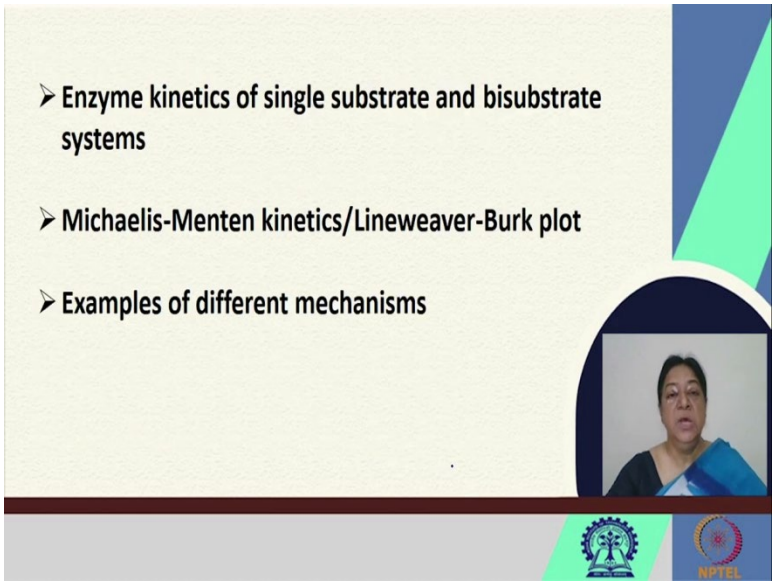

We also discussed enzyme kinetics related to single substrate and bisubstrate systems; Michaelis-Menten kinetics, the Lineweaver-Burk plot and several mechanisms of specific types of enzymes.

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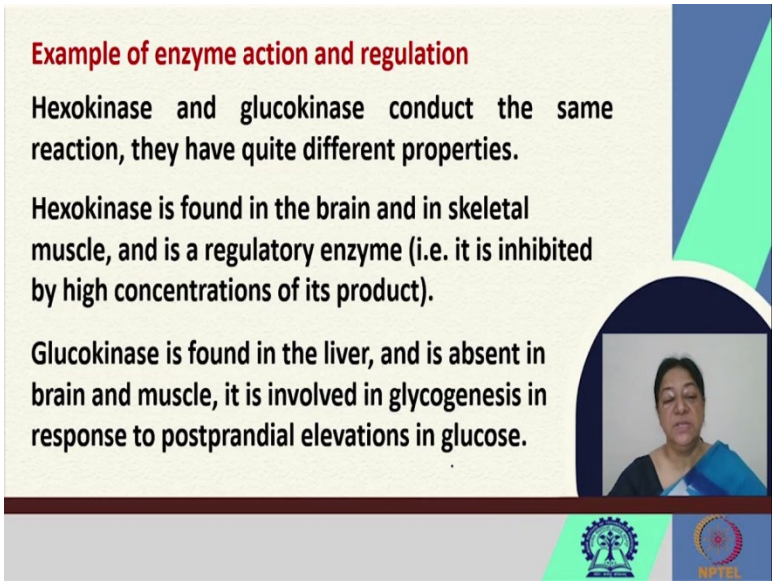

If we look at an example of enzyme action and regulation, a very beautiful example is that of hexokinase and glucokinase. They conduct the same reaction, but their properties are different. Hexokinase for example, is found in the brain and in skeletal muscle and is a regulatory enzyme. This enzyme is inhibited by high concentrations of its product, like we saw in feedback inhibition. Glucokinase on the other hand, is found in the liver and is absent in the brain and muscle and it is involved in glycogenesis, when there are elevated levels in glucose.

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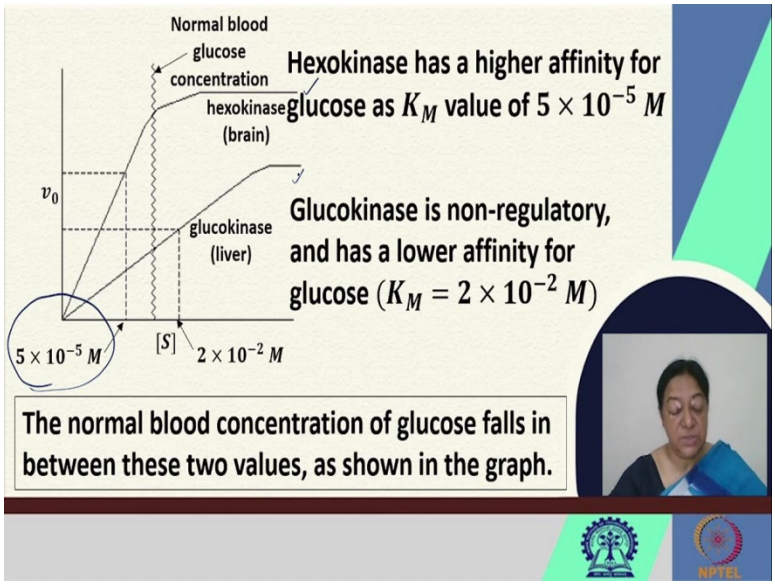

Let us look at the way of their operation to understand how we can look at a specific v_0/S curve, a Michaelis-Menten kinetics curve and see how we can understand the regulation related to levels of glucose. This [refer to slide] is the hexokinase plot and this is the glucokinase plot. As mentioned before, the hexokinase is found in the brain and glucokinase in the liver and the normal blood level concentration is as indicated.

We have the 2 K_M values associated with this protein and hexokinase is found to have a higher affinity for glucose because of its K_M value, considering that of glucokinase being non-regulatory, this has a lower affinity for glucose which is also apparent from the enzyme kinetics plot that we can see. Now the normal blood level concentration falls in between these two levels of K_M.

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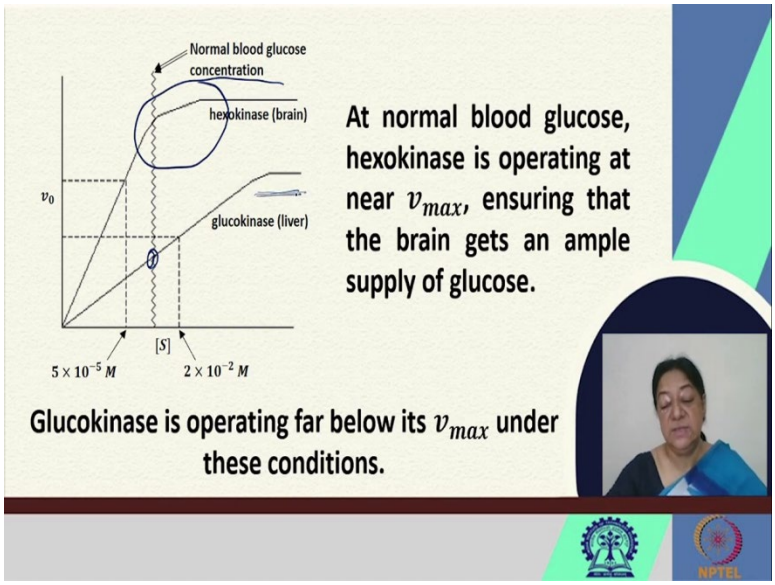

When we have normal blood glucose, hexokinase is operating at near v_{max} because this is the value and this ensures that the brain gets an ample supply of glucose. However, glucokinase is operating far below its v_{max}. Under these conditions as is apparent from these values here [refer to slide], here is the value of the normal glucose level and that is the v_{max} of glucose.

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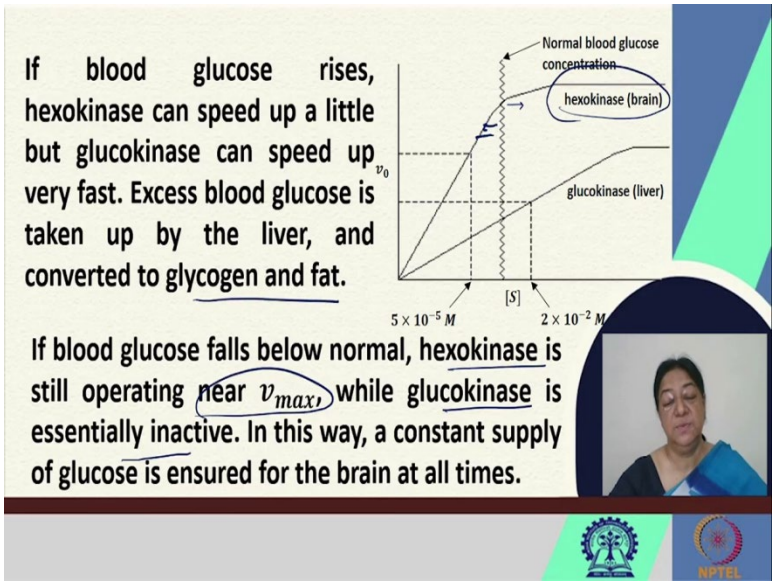

However when the blood glucose level rises, the concentration of blood glucose level will be this [refer to slide]. But hexokinase is already near its v_{max}, so it cannot speed up as much. However, glucokinase can speed up dramatically. As a result of which the excess blood glucose level will be taken up by the liver and converted to glycogen and fat.

If the blood glucose falls below normal, hexokinase is still operating near its v_{max} because glucokinase is essentially inactive. In this way, a steady supply of glucose is ensured at the brain in all times. So this is a fascinating way in which levels are controlled by the action of these two very similar enzymes that actually work on the same reaction.

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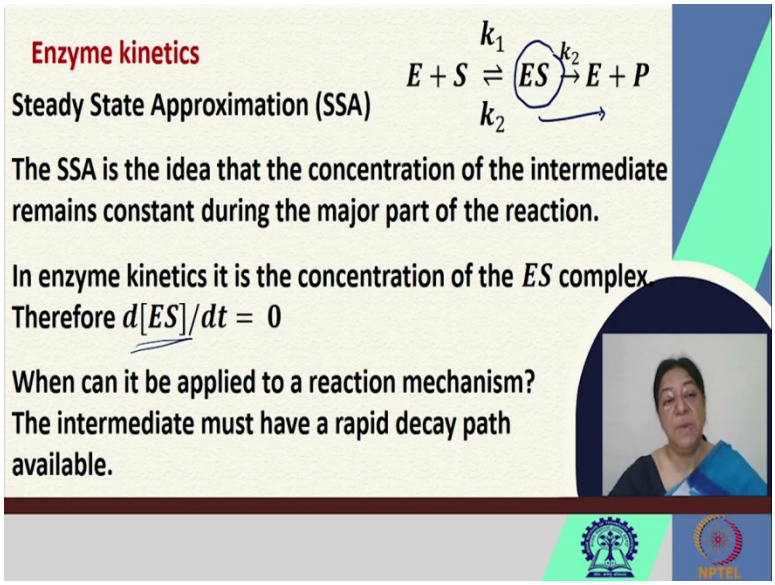

If we revisit our topics on enzyme kinetics, we have the steady state approximation idea that the concentration of the intermediate, that is our enzyme substrate complex, remains constant during the major part of the reaction. And we have the expression d[ES]/dt; that means the concentration of ES does not change in time. The intermediate has a rapid decay path to form the enzyme and the product.

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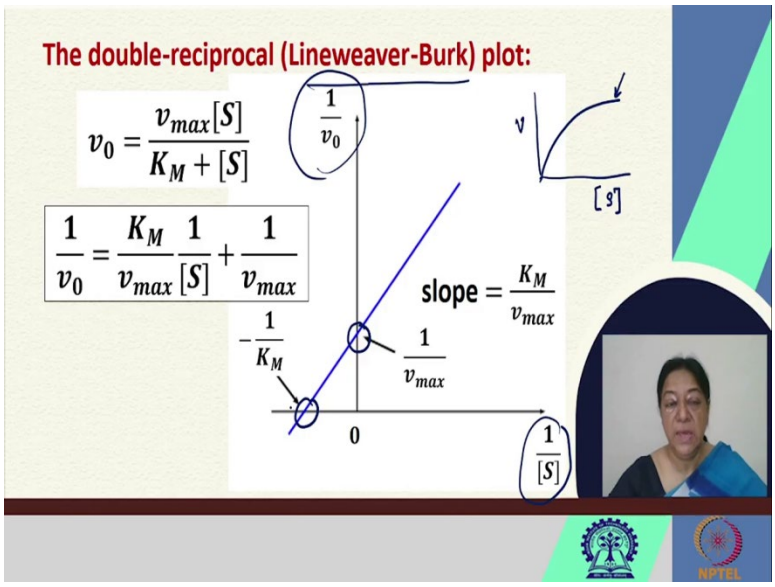

When we now look at the double reciprocal plot or the Lineweaver-Burk plot, we have the $1/v_0$ versus the 1/S, that is our reciprocal of the concentration of the substrate. We realize from the enzyme Michaelis-Menten normal curve, it was difficult to determine the v_{max} value, where the v was plotted against the S.

It was also difficult to determine the K_M value for that matter. So the double reciprocal plot; the Lineweaver-Burk plot is plotted in this case, to give us our y intercept and our x intercept that gives us the value for the v_{max}, the reciprocal of the v_{max} and the - $1/K_M$ value.

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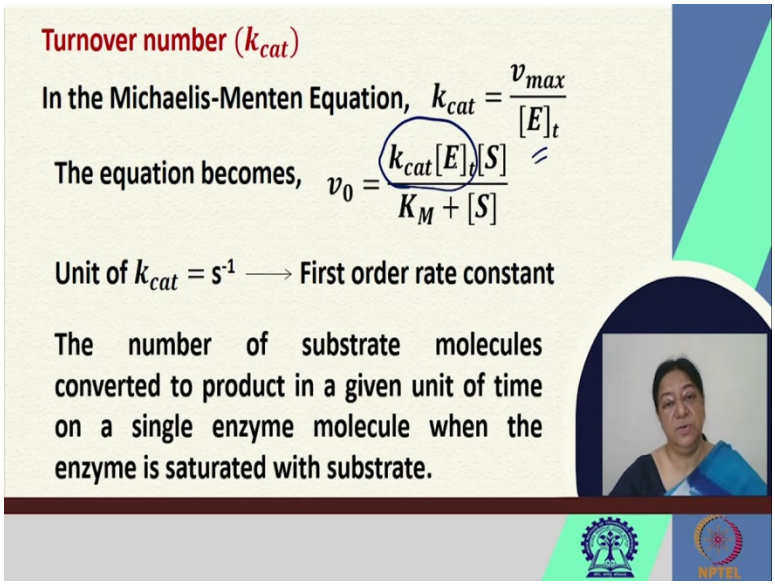

We also discussed the turnover number as to how efficient the enzyme is and this is the number of substrate molecules that are converted to product in a given unit of time on a single enzyme molecule, when the enzyme is saturated with the substrate. In the Michaelis-Menten equation, we have k_{cat} which is $v_{max}/[E]_t$, considering that the substrate has occupied all active sites on the enzyme available to it.

So we have an equation, where we have the modification of the Michaelis-Menten equation, where our v_{max} is now replaced by $k_{cat}[E]_t$ and we can determine the value of k_{cat}. This is a first order rate constant, determining where we have our enzyme kinetics or the number of the conversion, rather of the substrate to the product.

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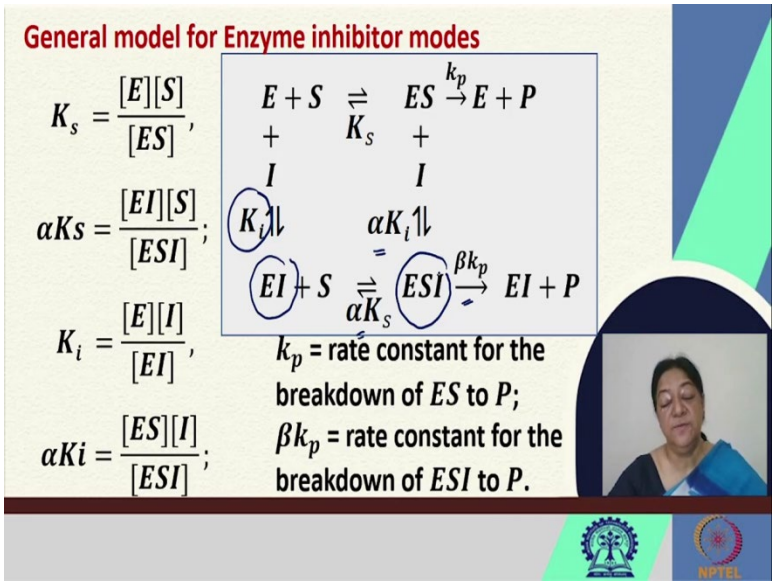

When we looked at several inhibitor models now, we had specific kinetics and specific equilibria associated with the presence of the inhibitors, where we looked at constants related to an inhibition constant and then the αK_i, the αK_s values and the β values were related to the types of enzyme inhibition models that we had.

Whether it was the enzyme forming, the enzyme inhibitor complex or the formation of the ternary complex, it was important to realize how the enzyme could act on the substrate to be converted to its product.

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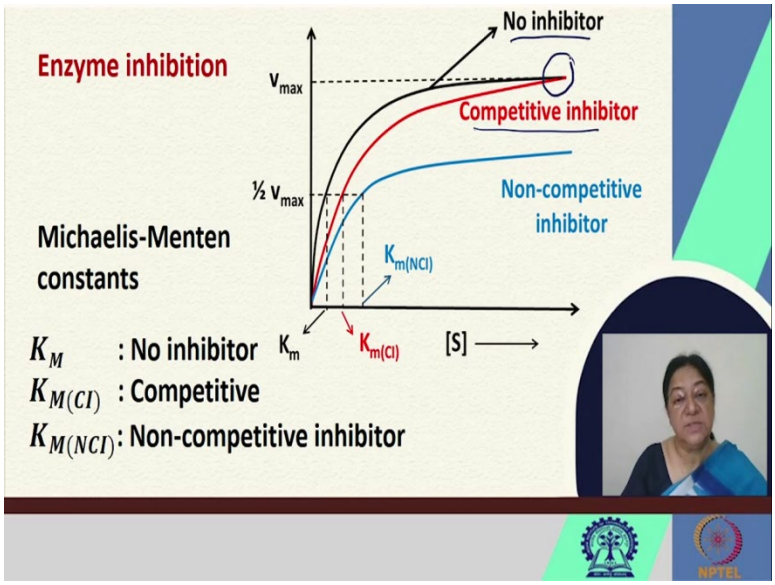

This was the specific kinetics that we looked at in terms of an understanding, where we had no inhibitor, a competitive inhibitor that would reach the v_{max} that would be expected and the non-competitive inhibitor that could not reach the v_{max} because some of the sites of the enzyme were

occupied or the allosteric sites were occupied, rendering the enzyme inactive or unable to bind to the substrate.

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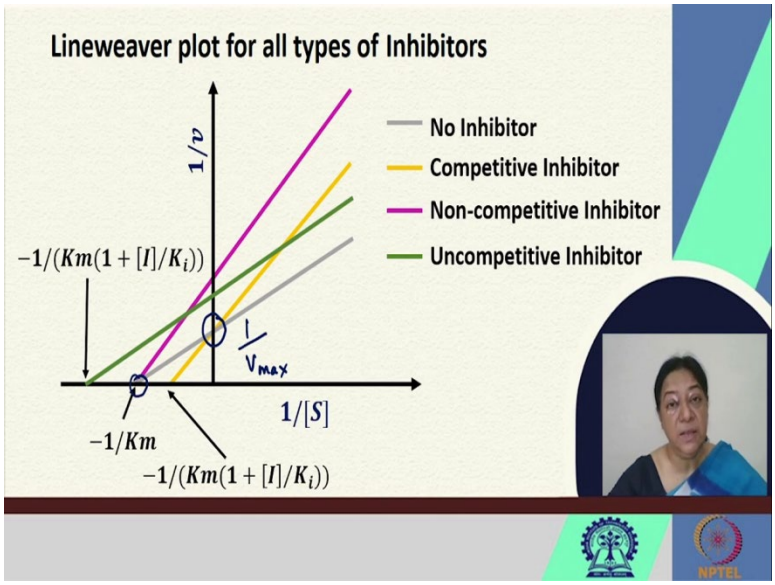

Then if we look at a Lineweaver-Burk plot for all the types of inhibitors, this [refer to slide] is one where we have no inhibitor. The one with the competitive inhibitor would have the same v_{max} value. We know that this corresponds to $1/v_{max}$ and the non-competitive inhibitor would have the same K_M value and a uncompetitive inhibitor would be parallel to that of the one with no inhibitor. So this summarizes all the plots that are available for the inhibitors and the uninhibited enzyme.

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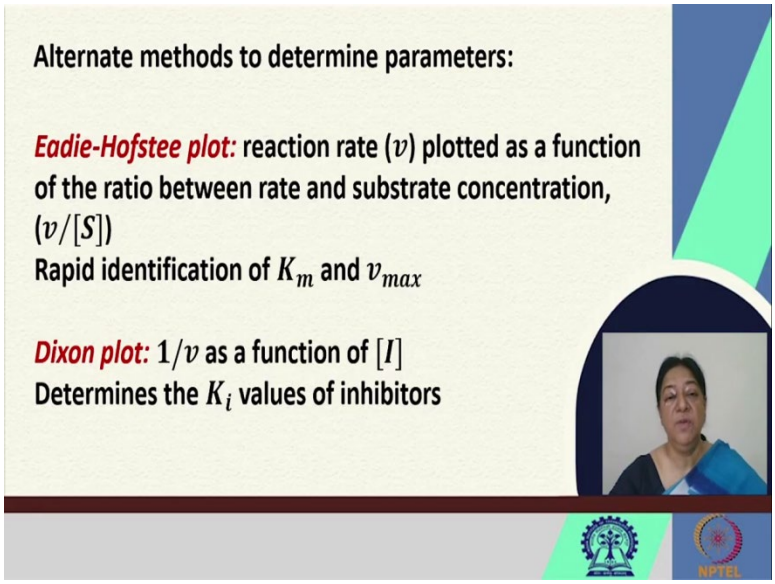

Now if we look at alternate methods to determine these parameters, there are other plots that are available. One is the Eadie-Hofstee plot, where the reaction rate is plotted as a function of the ratio between the rate and the substrate concentration, where we can get an identification of the K_M and the v_{max} values, which is what our intention is when we look at an enzyme kinetics reaction.

And the Dixon plot, where 1/v is plotted as a function of the inhibitor concentration and this is generally used to find out the inhibition constant of inhibitors.

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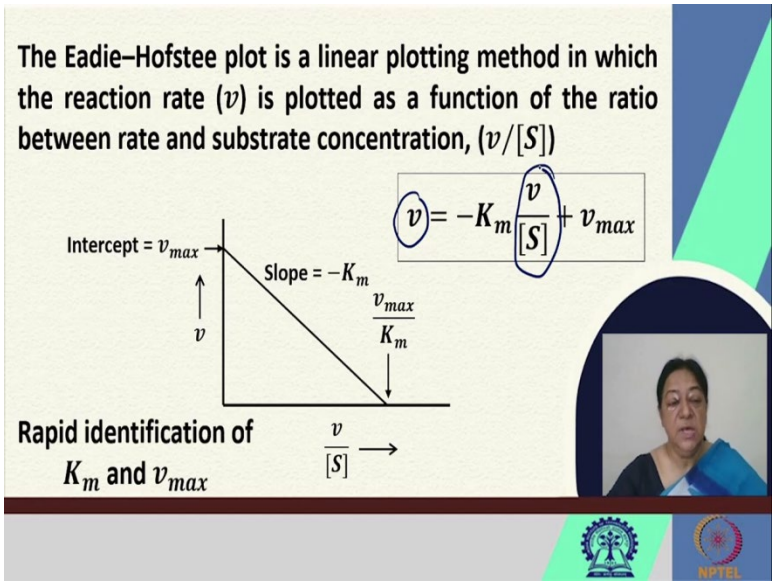

So, the Eadie-Hofstee plot is a linear plotting method, where we have the specific reaction [refer to slide], kinetics expression given by this and we have a plot and from this [refer to slide] plot, we can find out the slope that corresponds to -K. We can see if we plot v versus v/S, we have the $-K_M$ value for the slope and we have the intercept as v_{max}. So, this gives us an idea about the Eadie-Hofstee plot and a rapid identification of K_M and v_{max} is possible.

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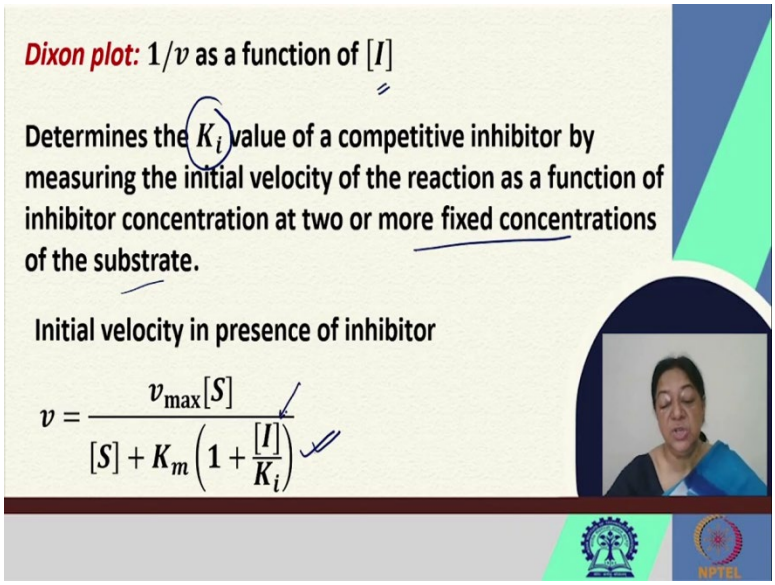

In the Dixon plot, where we plot the 1/v versus the function of the inhibitor concentration, we can determine the K_i value by measuring the initial velocity of the reaction as a function of the inhibitor concentration, but we have to have two or more fixed concentrations of the substrate. In this case, we have the initial velocity in the presence of the inhibitor. This is where we can use the inhibitor concentration and the K_i value that needs to be determined.

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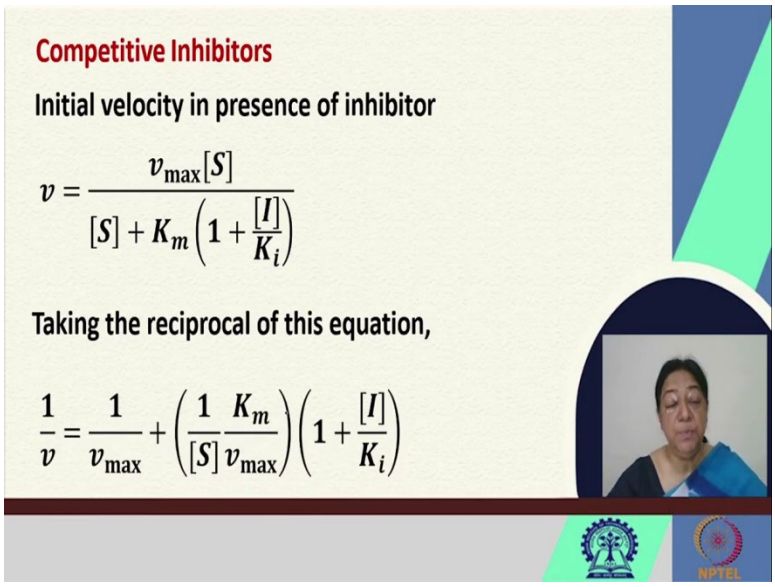

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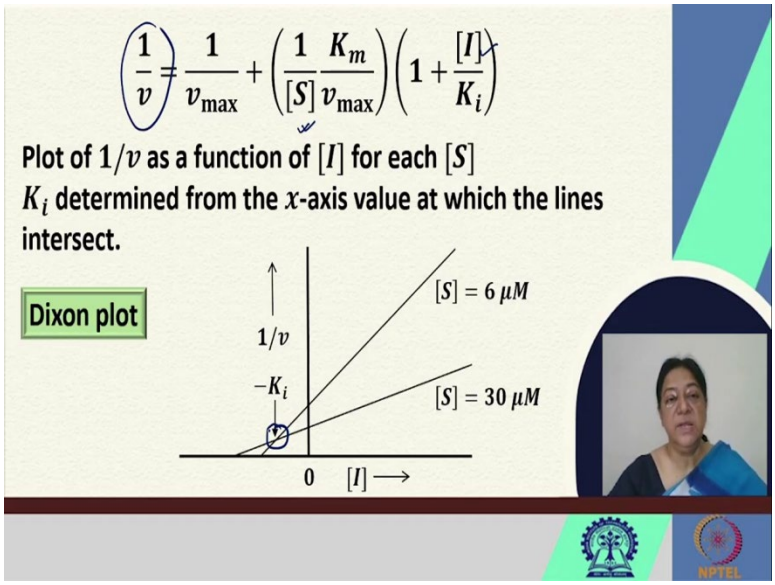

For competitive inhibitors, when we have the initial velocity in the presence of the inhibitor, we have the specific expressions associated and the specific plots associated with the Dixon plot that where we have the plot of 1/v as a function of I for the different substrate concentrations and the plot is such that the point of intersection actually gives us our K_i value.

So we have the two substrate concentrations and we have the K_i value, the $-K_i$ value given by the point of intersection to determine the K_i value of the competitive inhibitor in this case. This is our Dixon plot.

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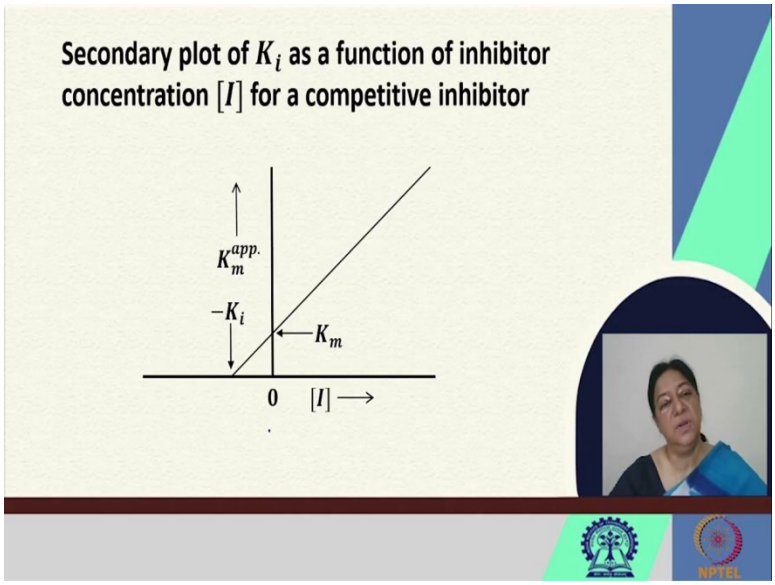

Similarly, we can get the secondary plot of the K_i as a function of the inhibitor concentration and determine an apparent K_M value.

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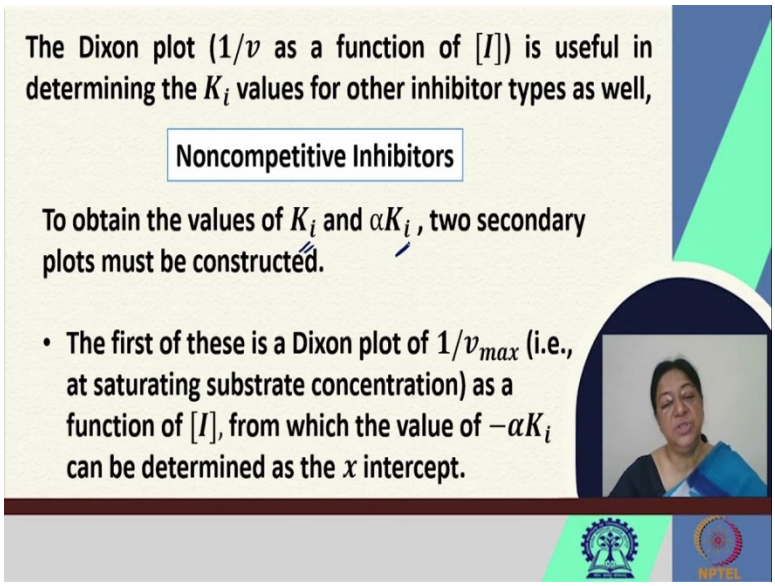

For the other inhibitor types, for the non-competitive inhibitor types, similar plots can also be made and we have the K_i and we have the αK_i value. For this case there has to be several plots, two secondary plots rather, that must be constructed to determine these values.

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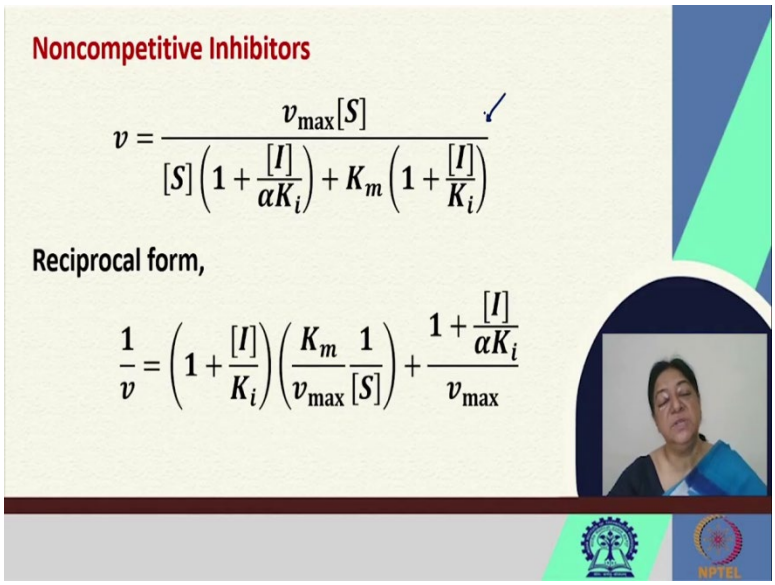

The idea is similar, the same expressions modified because of the presence of the inhibitors. Now we have the specific types of inhibitors and the specific types of kinetic expressions, the kinetic parameters and the equilibria that are going to change because of the presence of these inhibitors.

While we monitor the inhibitor, the substrate concentration and the inhibitor concentration, we can work around these to find out what the inhibition constants are, depending upon the type of inhibitor that we have.

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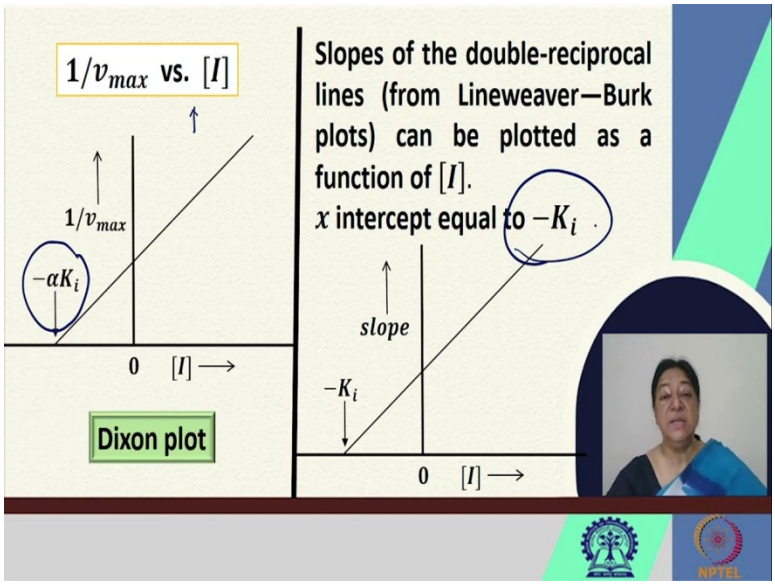

So we have the different types of plots, where we have $1/v_{max}$ versus I in this particular case, where we can get the value of α. Then the slopes of the double reciprocal lines, that is a Lineweaver-Burk plot, can also be plotted and from the x intercept we can get $-K_i$.

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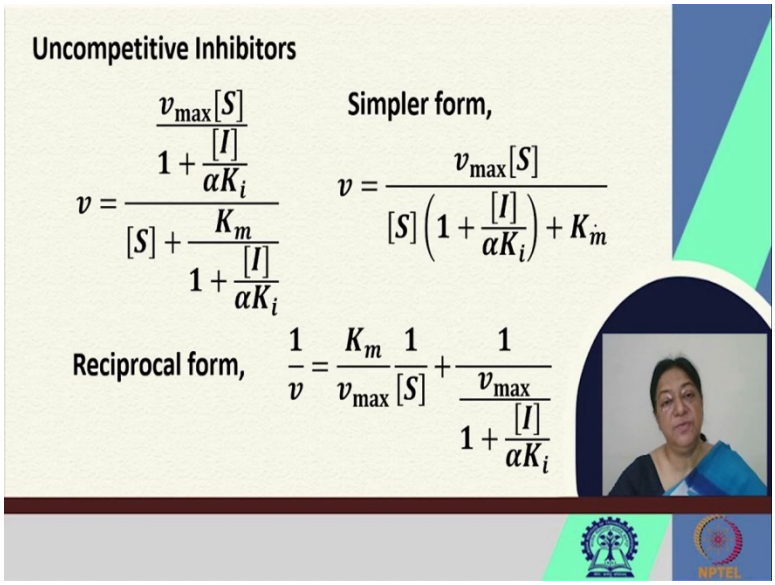

An understanding of the enzyme kinetic parameters in terms of the Dixon plot gives us a similar idea, modifications related to the algebraic expressions for the Michaelis-Menten kinetics using

the reciprocal forms like we do for the Lineweaver-Burk plot. Based on that, looking at the specific plots and determining what the x intercept and the y intercept can give.

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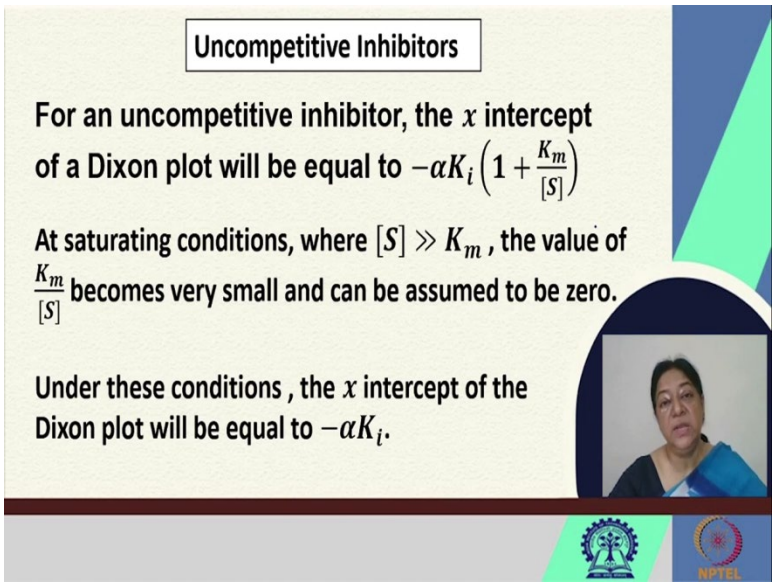

So these kinetic examples give us a specific way to determine, to compare different inhibitors from the specific K_i values, the K_M values and the v_{max} values.

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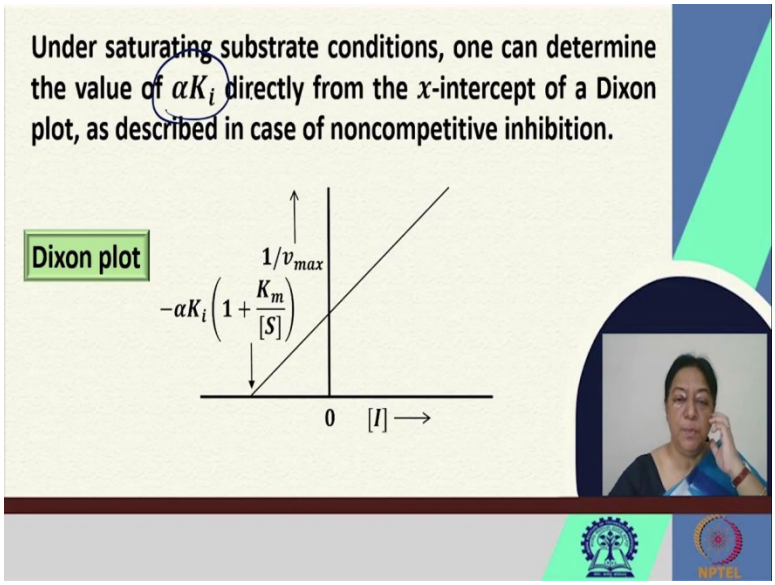

So this [refer to slide] is the saturated condition, where we can find again the αK_i directly from the x-intercept of a Dixon plot, as it is in the case of non-competitive inhibition.

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If we now look at some sample problems related to the topics that have been covered so far, we will be looking at examples where we have the substrate concentration given and the velocities given for different types of examples.

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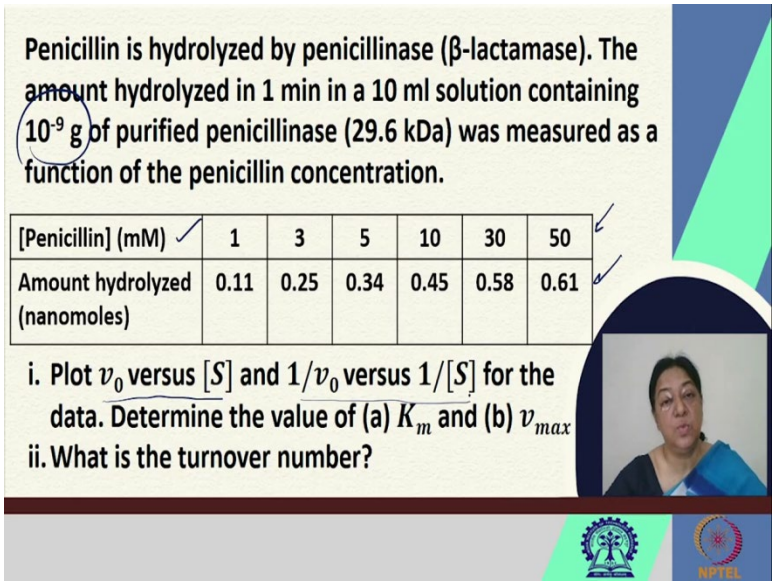

If we have an example, where we have the penicillin hydrolysis by penicillinase, the protein that is the β-lactamase, the amount hydrolyzed in 1 minute in a 10 ml solution that contains 10^{-9}g amount of the protein, it was measured as a function of the penicillin concentration; the penicillin being the substrate. So, we have the substrate concentration and we have the amount hydrolyzed in 1 minute, that gives us an idea of the extent of the reaction.

So, the requirements here are to plot v_0 versus S and again, $1/v_0$ versus 1/S in a Lineweaver-Burk plot for the data and from that to determine the K_M and the v_{max} values; and also from the information given, to find out the turnover number.

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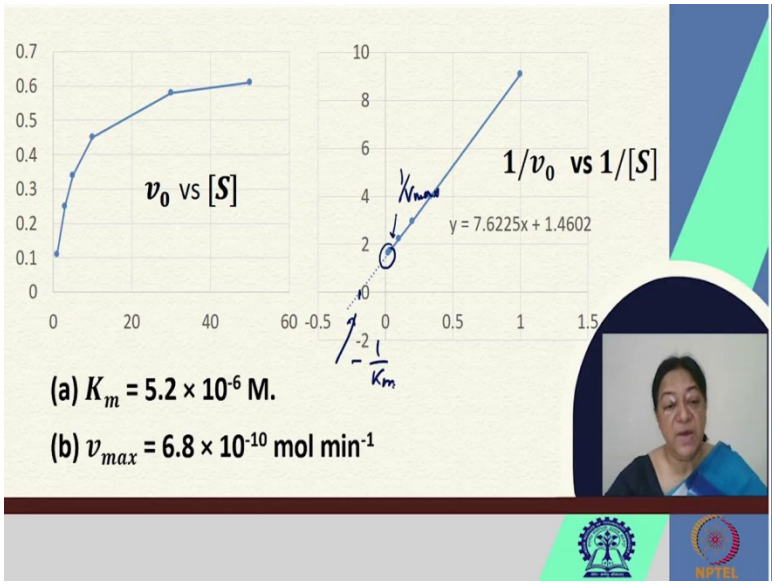

So, this [refer to slide] is our v_0 versus S plot from the data that has been provided and the $1/v_0$ versus 1/S, that gives us an indication of the Lineweaver-Burk plot and we know that the point of intersection here is going to give us our $1/v_{max}$ value and this is going to give us our -1 by K_M value. Based on this, we can get the information related to the K_M value and the v_{max} value.

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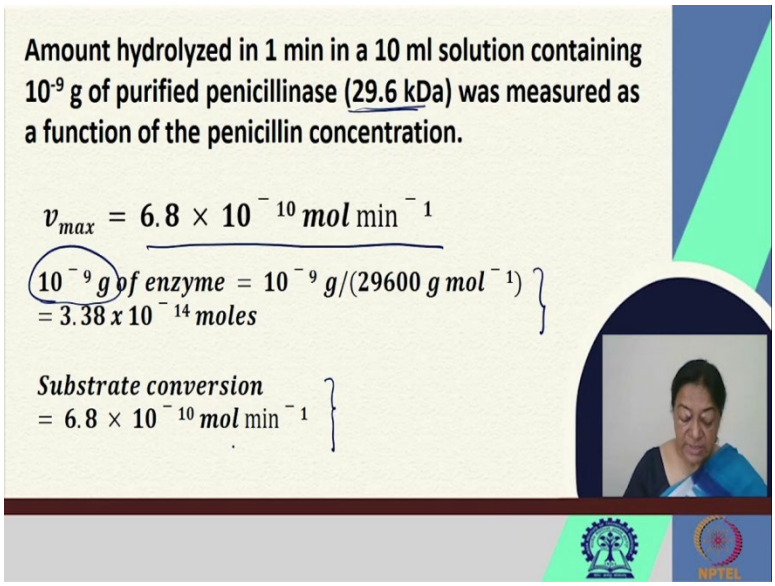

Now, from the v_{max} value that we got from the previous plots, we know that we started off with 10^{-9}g of enzyme. The molecular weight has been given to us, so it is possible to determine the number of moles in the reaction for the specific enzyme. We have a substrate conversion rate that is given by 6.8 x 10^{-10}g.

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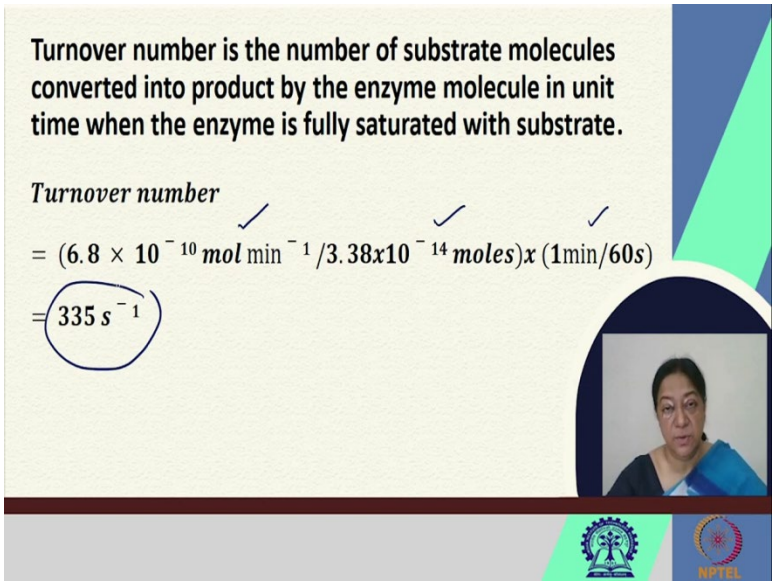

If 6.8 x 10^{-10} mol min^{-1} is the substrate conversion rate, 3.38 x 10^{-14} moles is the number of moles available and the problem said that this was the conversion for 1 minute. We have our turnover number 335 s^{-1} because we know that the definition of the turnover number is the number of substrate molecules that are converted into product by the enzyme molecule in unit time, when the enzyme is fully saturated with the substrate.

This gives us an idea of how to determine the K_M and the v_{max} values and the turnover number from a series of values for the substrate concentration and the specific amount hydrolyzed in this case.

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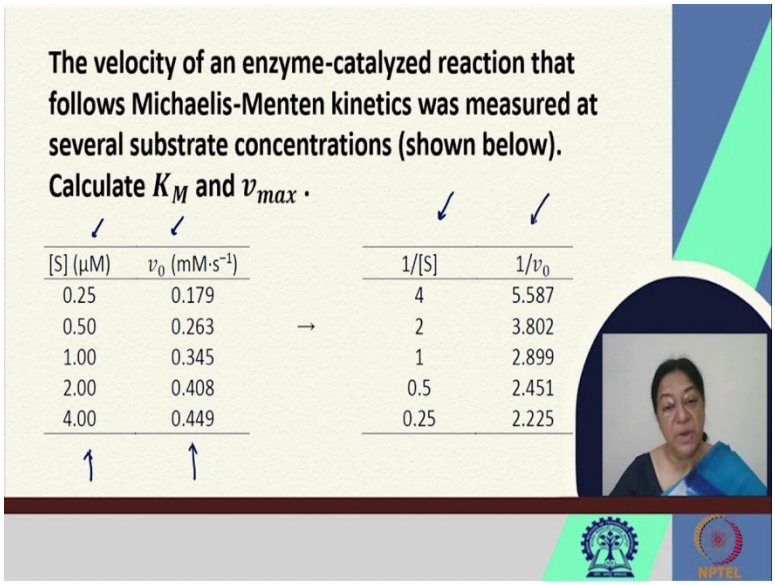

If we look at a velocity reaction or a substrate concentration, where we have the v_0 values and the substrate concentration and we need to calculate the K_M and the v_{max} in a similar manner.

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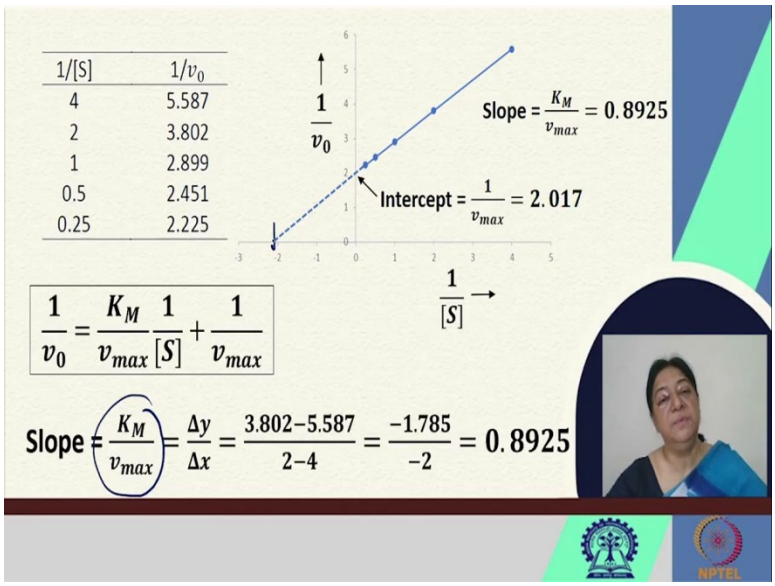

We know we have to determine the 1/S and the $1/v_0$ and basically plot this in the Lineweaver-Burk plot, the reciprocal plot and from that we can get the intercept and the slope that is K_M/v_{max} and we can also determine the K_M value from this. So this gives us an idea of how to approach these problems, where we have a series of Michaelis-Menten kinetics experiments conducted.

We find out the v_0 for each of these experiments for the different substrate concentrations. Look at the double reciprocal drop to determine the v_{max} and the K_M values.

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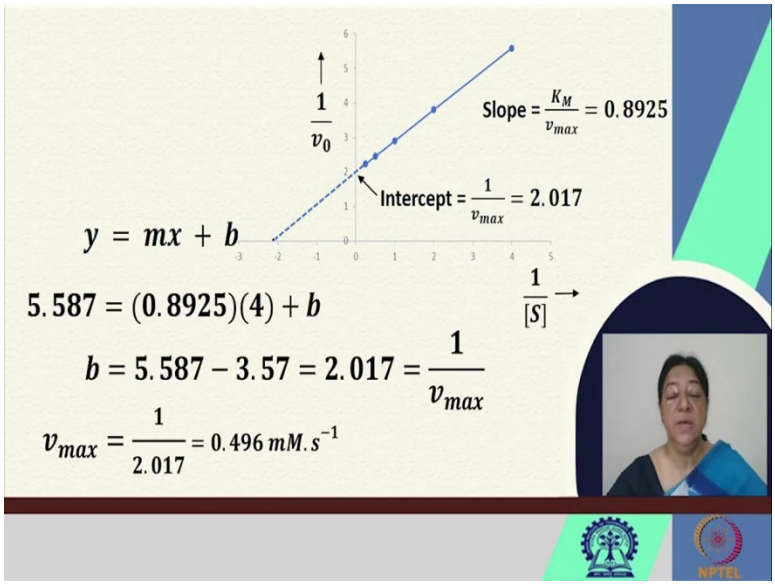

From y = mx + b, we can also get the equation of the line and from that we can also determine the v_{max} value and also the K_M value.

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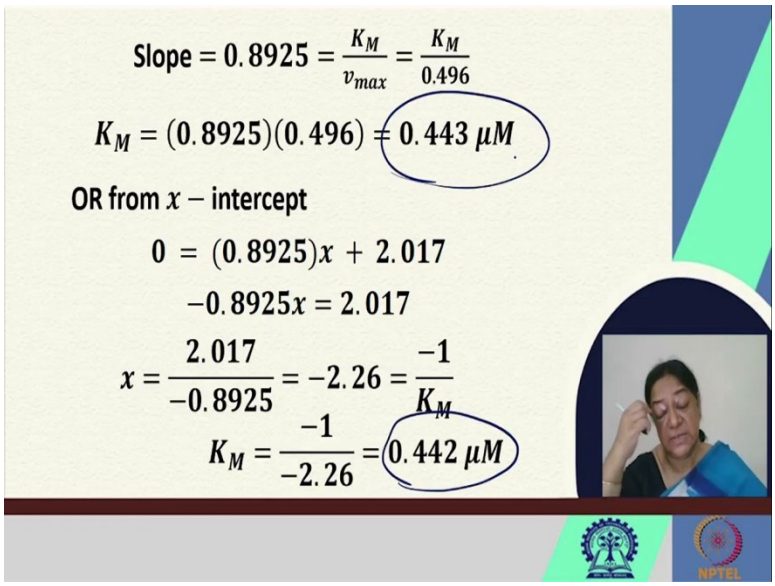

This is how we can determine the K_M value or from the x intercept we can also get this value from the equation that we have and we see that the values are very close to each other. So from the x intercept that is the $-1/K_M$ from the plot or from the slope that we can get the similar values of the K_M that is required for this particular problem.

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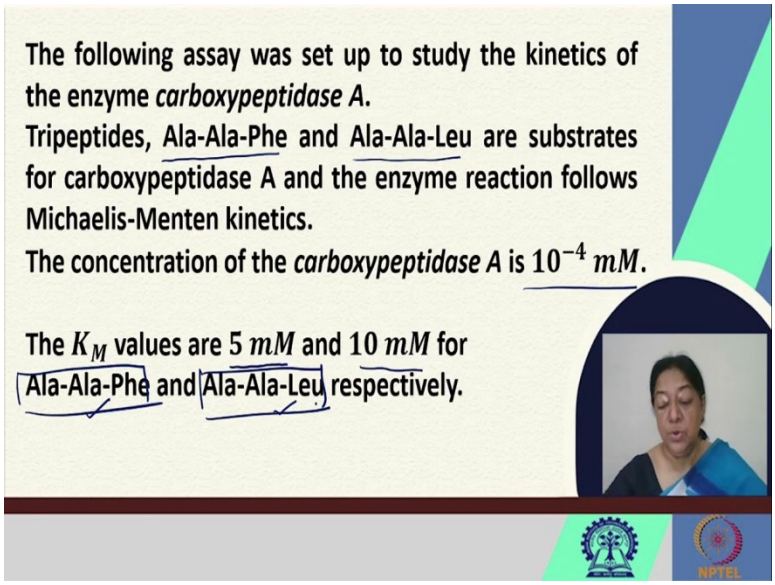

In another problem, if we look at the following assay that was set up to study the kinetics of an enzyme carboxypeptidase A. So there were two tripeptides as has been given here Ala-Ala-Phenylalanine and Ala-Ala-Leu. These are the substrates for the enzyme and the enzyme reaction is known to follow Michaelis-Menten kinetics.

The concentration of the enzyme is given as 10^{-4} mM. The K_M values are already provided one is 5 mM and one is 10 mM, for the two tripeptides provided. So we have our enzyme carboxypeptidase A. This is working on the two tripeptides, an Ala-Ala-Phenylalanine and an Ala-Ala-Leucine.

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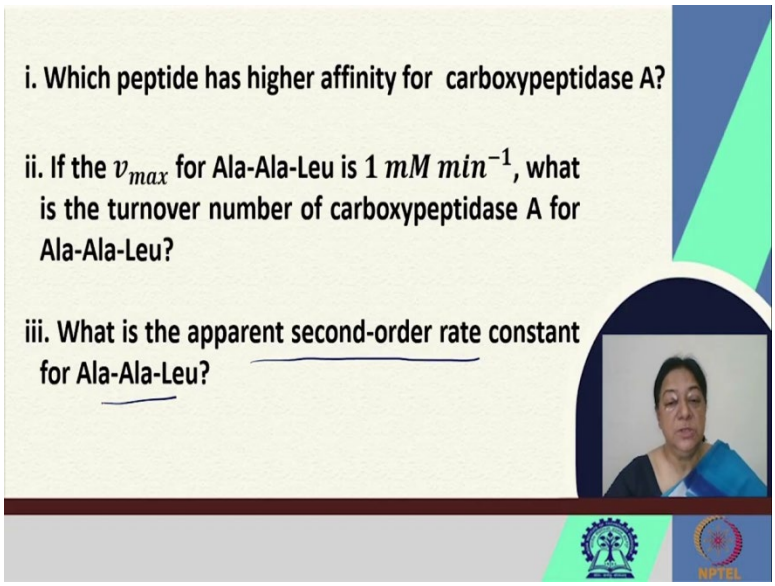

We want to know is we have to find out which peptide has a higher affinity for carboxypeptidase A. So what we have is we have the two values of K_M. The next question is if the v_{max} is given as

1 mM min^{-1}, what is the turnover number and the apparent second order rate constant of this particular reaction, of this particular enzyme experiment.

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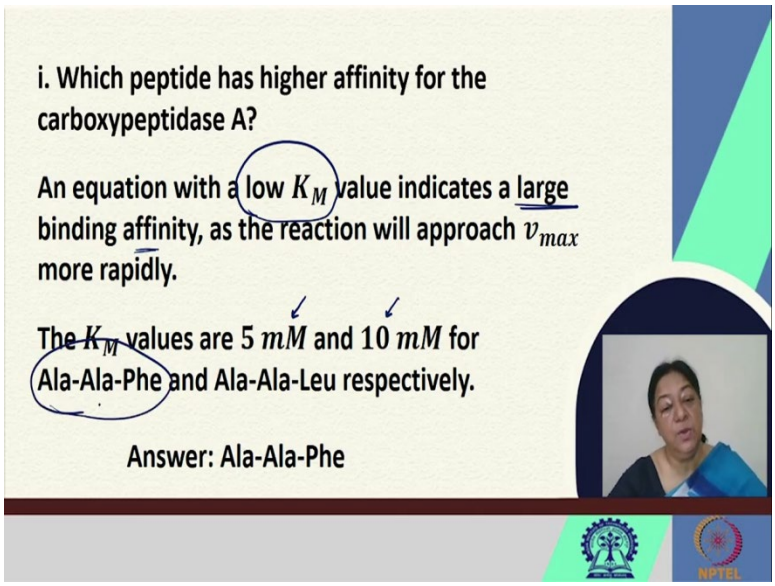

So, the first question is which peptide has a higher affinity. An equation where we have a low K_M value indicates a higher binding affinity, as the reaction is going to approach our v_{max} faster. In that case, we have the K_M values that were given as 5 mM and 10 mM for the two tripeptide substrates. We can definitely say that the answer is Ala-Ala-Phe because in this case, we have a lower K_M value compared to the Ala-Ala-Leu tripeptide.

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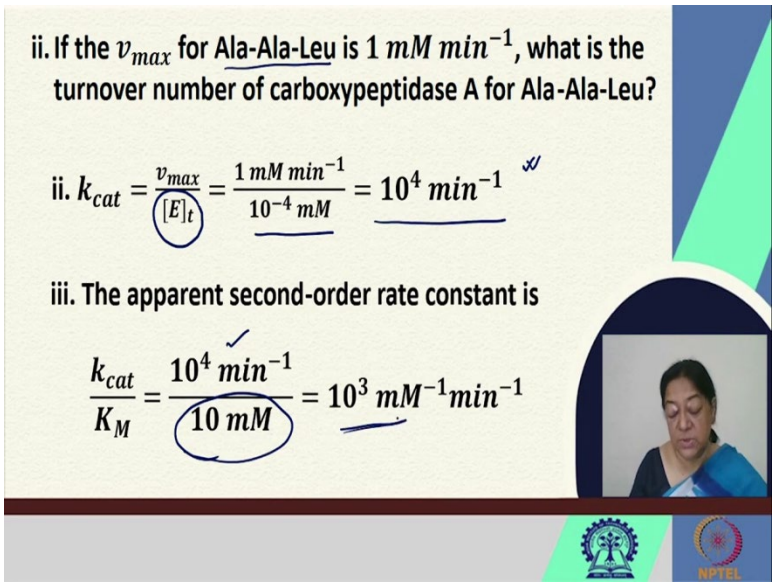

In the second question, if the v_{max} for the particular tripeptide is 1 mM min^{-1}, we want to know what the turnover number is. What do we need for that is, we need the k_{cat} value. We actually need the total concentration of the enzyme, which was given as 10^{-4} mM. The total concentration of the enzyme, the v_{max} value provided to us and we need to keep our units correct.

10^4 per min^{-1} is the turnover number and the apparent second order rate constant that is given by k_{cat}/K_M. So, this [refer to slide] is the K_M value of the second tripeptide that we were looking at, that is Ala-Ala-Leucine and the k_{cat} is something we calculated from a knowledge of the v_{max} and the knowledge of the total enzyme available to us and from that we can determine what the second order rate constant is.

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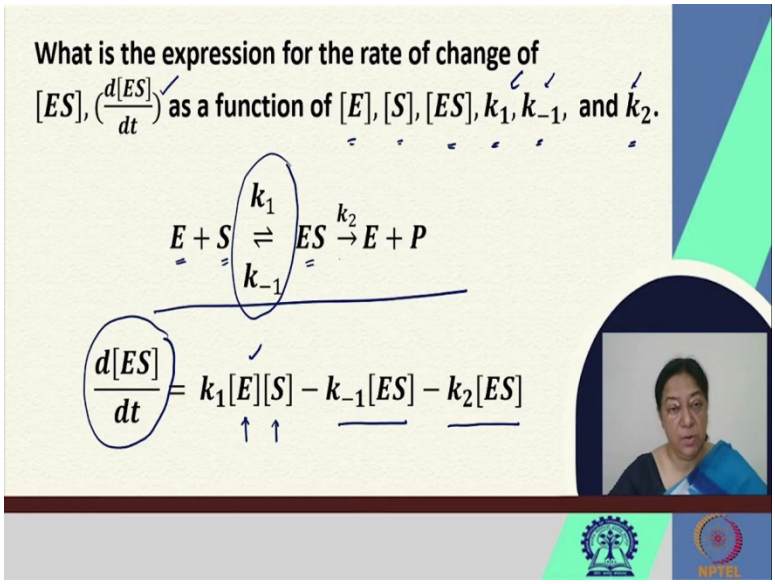

If we want to know the expressions for the rate of change of [ES] that is the d[ES]/dt as a function of [E], [S], [ES], k_1, $k_{-1}$and k_2. The small ks indicate rate constants of the specific reactions that are involved. So we have our overall kinetic mechanism given in this case, where we have a pre-equilibrium step and we have a final product formation step. If we want to apply the steady state approximation as we looked at, we have our expression related to the change of the enzyme substrate concentration with time.

The formation of the enzyme substrate complex is from k_1, from the product of the concentrations of E and S, it is disintegrated by the reverse of this reaction in its concentration and also in the formation of the products.

So, this is going to give us the expression for the rate of change of the enzyme substrate concentration with time and given if we know we can monitor this, then we can find out the specific rate constants or as we looked at the overall expressions for the enzyme kinetics of the Michaelis-Menten kinetics, we can find out the specific values for the K_M also.

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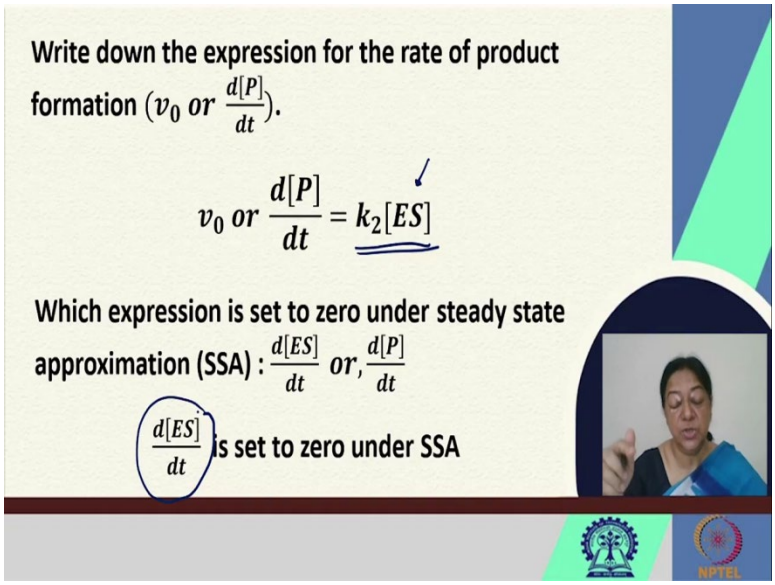

In another expression now, if we want to find out the expression for the rate of product formation that is d[P]/dt. We know from our previous expression that is it is going to be just k_2[ES] because that is from where the product is going to appear. So, we want to know which expression is set to 0 under steady state approximation. Is it d[ES]/dt or d[P]/dt?

Now, we know that the steady state approximation tells us that any intermediate concentration will remain as 0 because we want the intermediate formation and the intermediate disintegration has a constant rate. The rate of formation is going to be the rate of disintegration. This is set until 0 under the steady state approximation.

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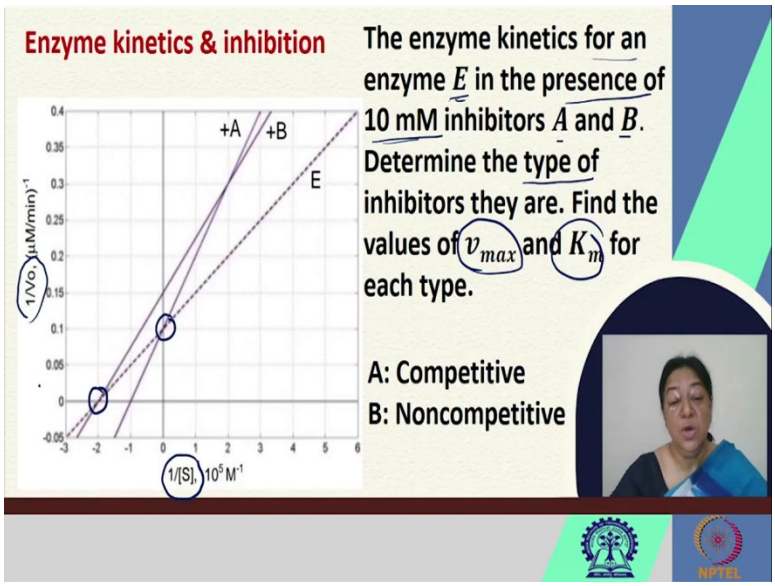

If we now look at a some enzyme kinetics and inhibition problems, we have the enzyme kinetics for an enzyme E in the presence of 10 mM inhibitors A and B and from a plot, we have to

determine the type of inhibitors that they are and from the plot, we have to see whether we can determine the v_{max} and the K_M value for each type.

So, given that this [refer to slide] is our plot, we have on the y-axis the $1/v_0$ and the 1/S on the x-axis and we realize that this is our Lineweaver-Burk plot and from the points of intersection here and here, we can determine that A is a competitive type, B is a non-competitive type and from the values given in the y and the x-axis, we can determine the value of the v_{max} and the K_M.

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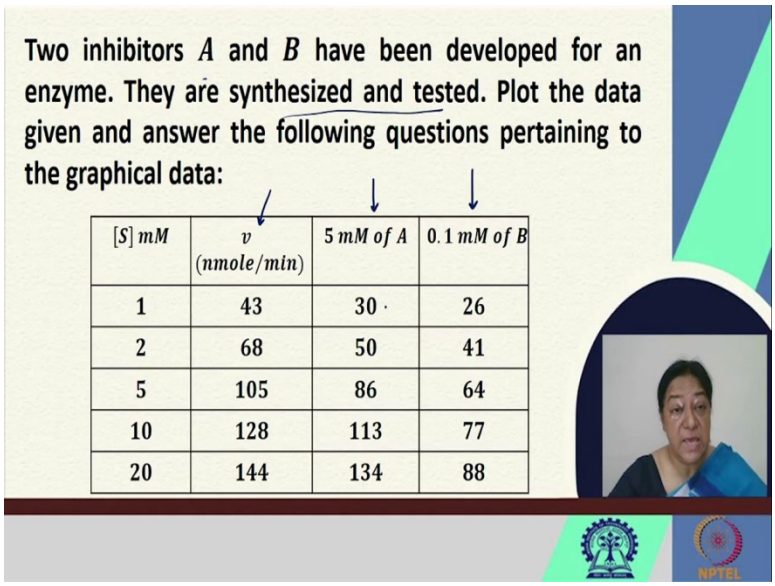

We look now at a specific example again, where we have two inhibitors A and B that have been developed for a specific enzyme. They have been synthesized and tested and we have the data [refer to slide] associated with 5 mM of A and 0.1 mM of B and these are the various velocities associated, where we do not have any inhibitor.

This is the uninhibited enzyme at the several substrate concentrations that are given in the first column and the specific kinetics associated with that is the nmole/min for the v for the different inhibitors A and B.

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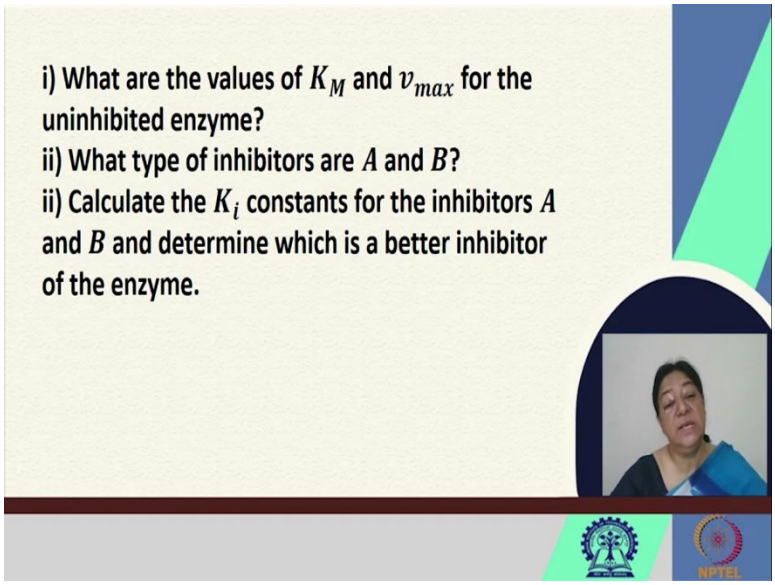

So we want to know the values of the K_M and the v_{max} for the uninhibited enzyme types of inhibitors of A and B and the K_i constants in this case as well and to determine which one is the better inhibitor of the enzyme.

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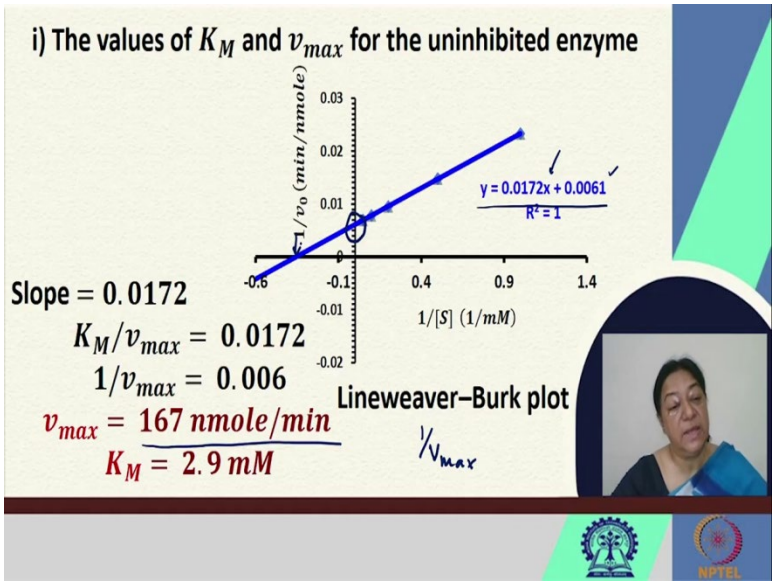

So if we plot [refer to slide] for the uninhibited enzyme, the Lineweaver-Burk plot that is the double reciprocal plot, we can get the slope that gives us the K_M/v_{max} because we see that this form is y = mx + c. We get the c here and we get the v_{max} value from the expression the $1/v_{max}$, that is our y intercept here.

So we can put x = 0 to get the y intercept, we can put our value here; we can put y = 0 to get our x intercept and from that we can get the K_M and the v_{max} values.

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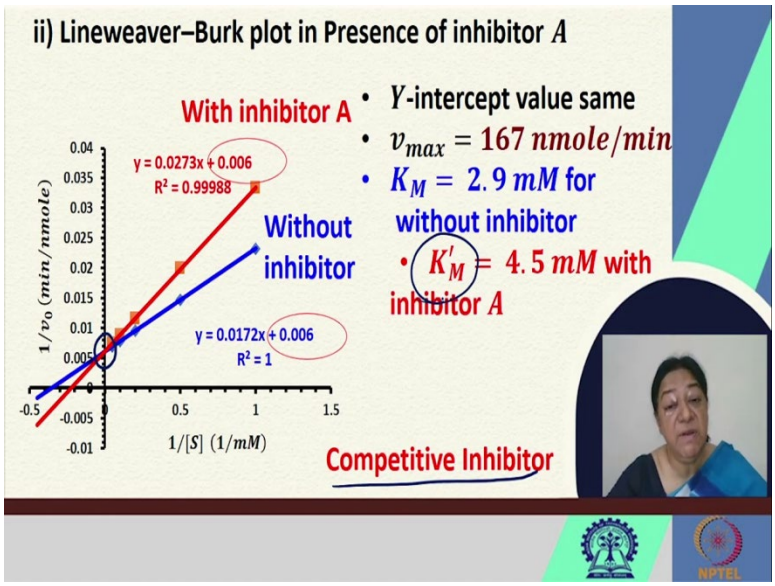

If we want to plot it in the presence of the inhibitor, then we see [refer to slide] that with inhibitor A, we have a point of intersection that is given here. So, we know that it is a competitive inhibitor. Now given the values that we have here, we see that the y intercept value is the same, which means that the v_{max} is the same and we have the K_M for the uninhibited enzyme that is given in blue.

In this case the point of intersection on the x-axis, that is without inhibitor and we can find out the value with inhibitor, where we see a modified K_M value with the specific inhibitor A in this case.

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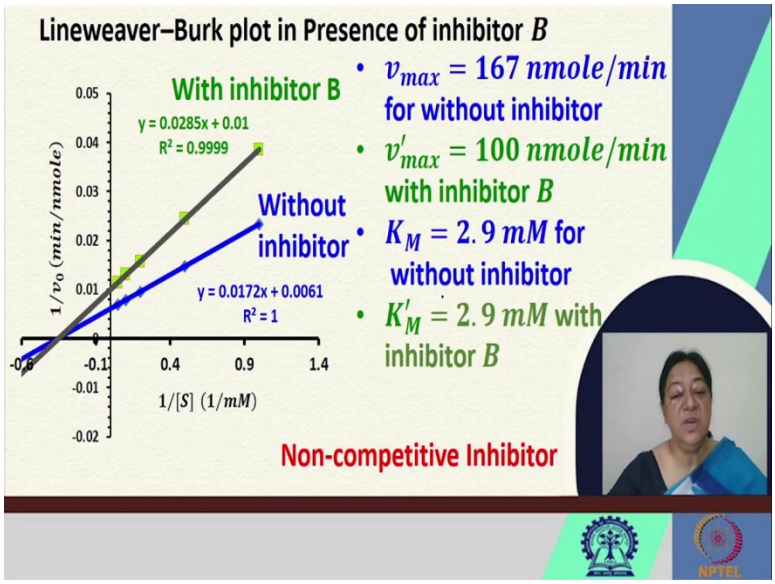

Similarly if we do it for the inhibitor B, we find out that we have a varying v_{max} but the K_M is the same; the point of intersection now is on the x-axis. K_M without inhibitor is 2.9 mM and K'_M with the inhibitor B is 2.9 mM. We know that we have a point of intersection on the x-axis indicating that B is a non-competitive inhibitor.

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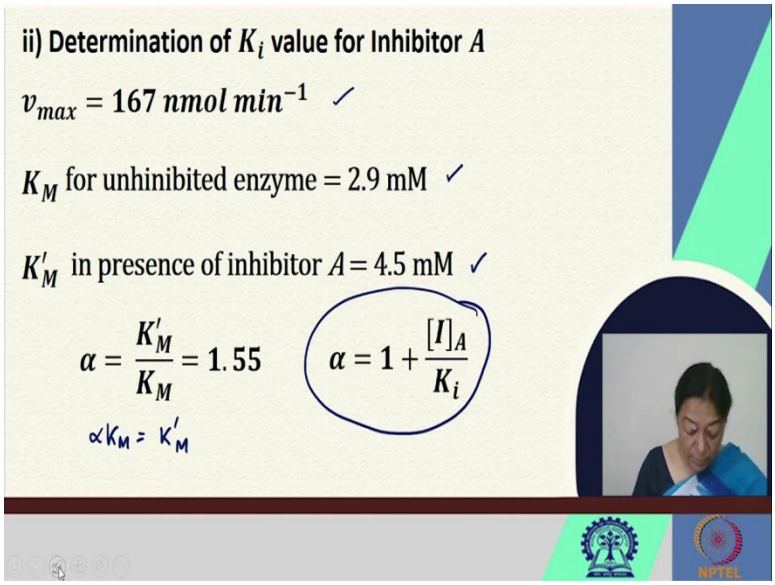

Now, if we want to find out the specific values of the K_i, the way to determine that is the information that we need is the v_{max} value, the K_M for the uninhibited enzyme and the K'_M in the presence of the inhibitor. The ratio of this is going to give us our α value and the αK_M that we see here is the K'_M. So, from an idea of the concentration of the inhibitor, we can actually determine the value of our K_i.

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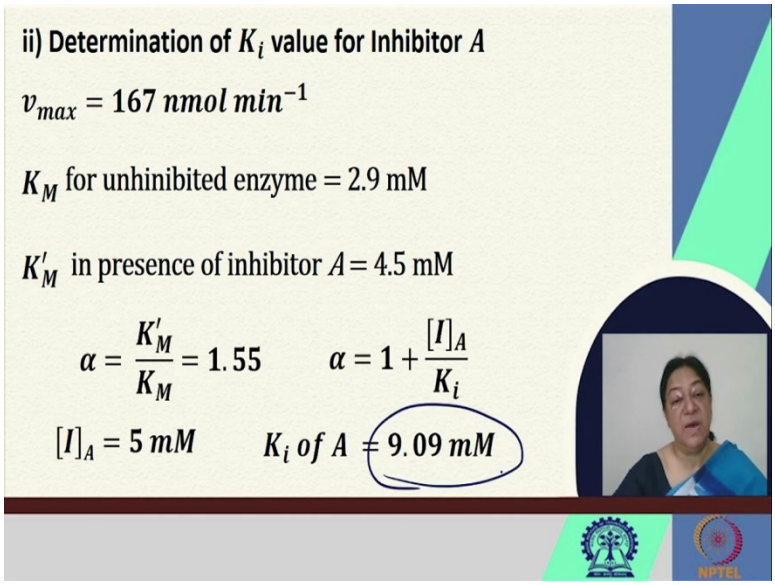

We have the inhibitor concentration at 5 mM and the K_i value at 9.09 mM from the values that we have determined.

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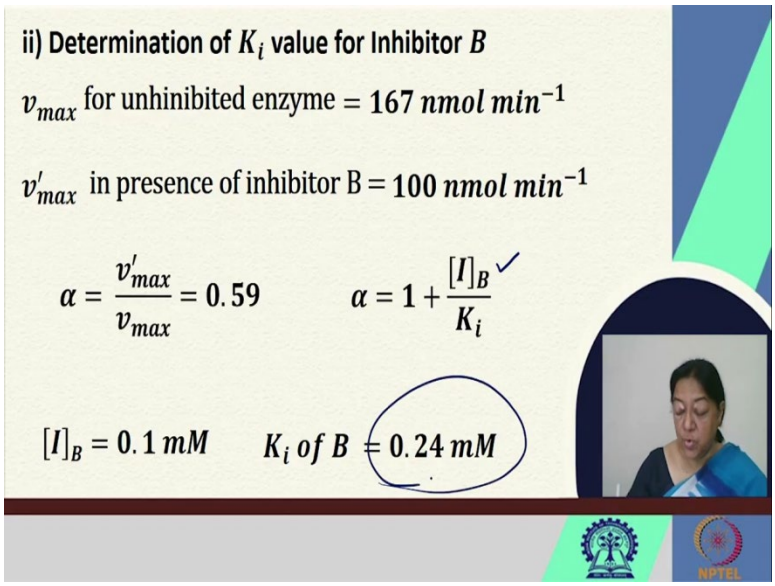

Similarly, if we want to find the K_i value of inhibitor B, again we have the v_{max} value and the v'_{max} value. In this case the K_M values are the same because we know that B is a non-competitive inhibitor. We get a variation in the α values that is going to also give us the $1 + [I]/K_i$. So, the variation is going to be the α because we have a v'_{max} in this case. So the K_i of B is 0.24 mM.

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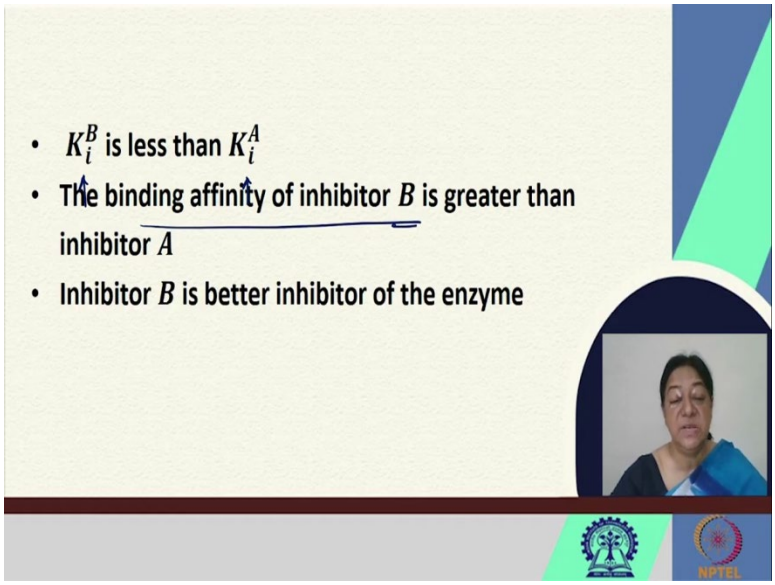

So, now if we look at both the values, we have the K_i B value that is the inhibitor inhibition constant for B that was less than that the one was 0.24 and the previous one was 9.09. We see

that we have in this case a better inhibitor in case of B because we have a lower inhibition constant, a tighter binding complex. So inhibitor B is the better inhibitor of the enzyme.

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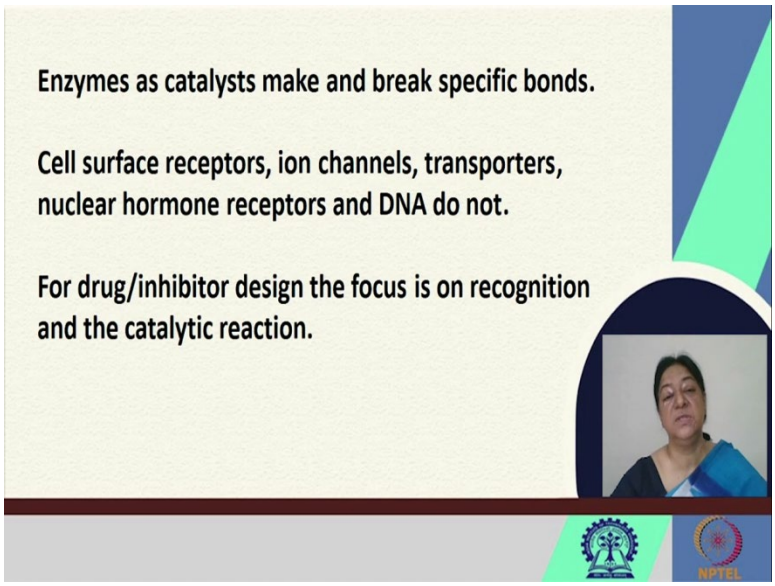

So, in our discussions of enzymes, we have looked at them as catalysts to break specific bonds. They act as we have seen in various manners as cell surface receptors, channel, ion channels, transporters, and for the drug inhibitor design, we can focus on their catalytic mechanism or their structural aspects.

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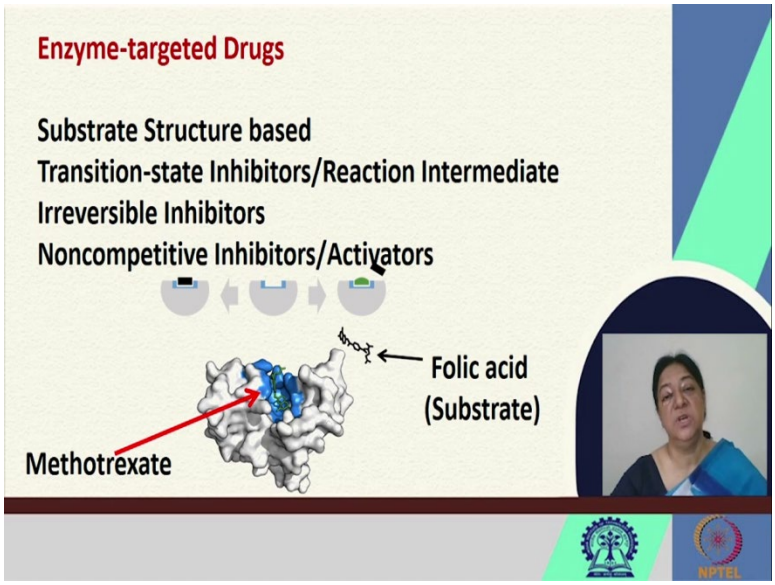

And determine substrate or structure based inhibitors, transition state inhibitors as we look at enzyme targeted drugs for the development of specific inhibitors for enzymes.

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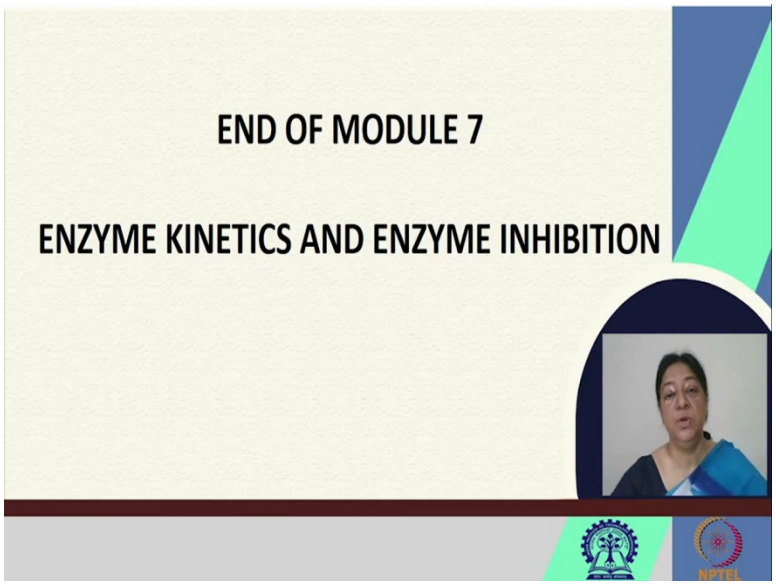

This is the end of module 7 that dealt with enzymes, enzyme mechanisms, enzyme kinetics and enzyme inhibition.

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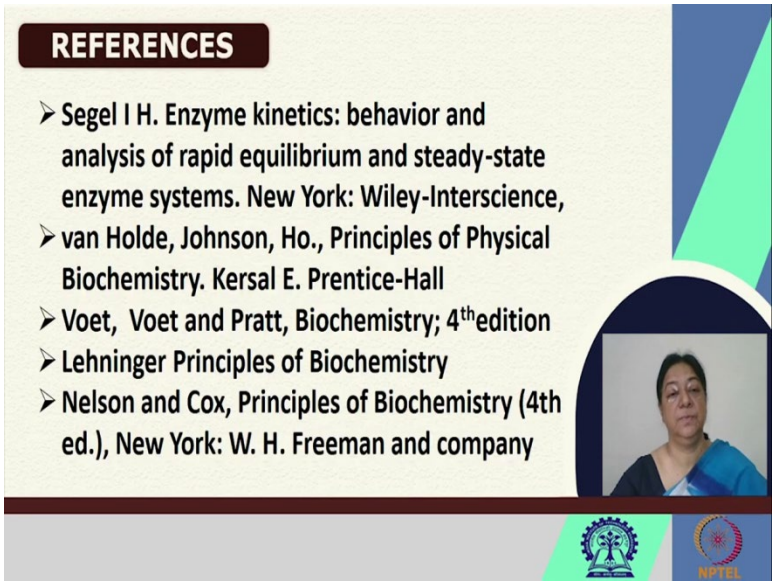

These [refer to slide] are the references that we have followed.