Fundamentals of Protein Chemistry
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Module - 07
Enzyme Kinetics and Enzyme Inhibition
Lecture - 31
Enzyme Kinetics - I

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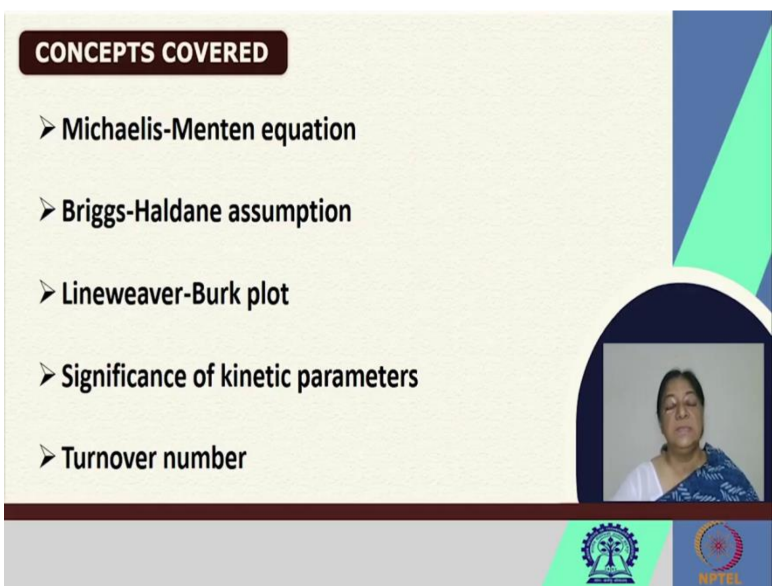

In this module we begin our discussion on enzyme kinetics, enzymes and enzyme mechanisms. In this particular model, we will be looking at the Michaelis-Menten equation, Brigg-Haldane assumption, the Lineweaver-Burk plot and what the significance of these kinetic parameters are and what we mean by the turnover number of an enzyme.

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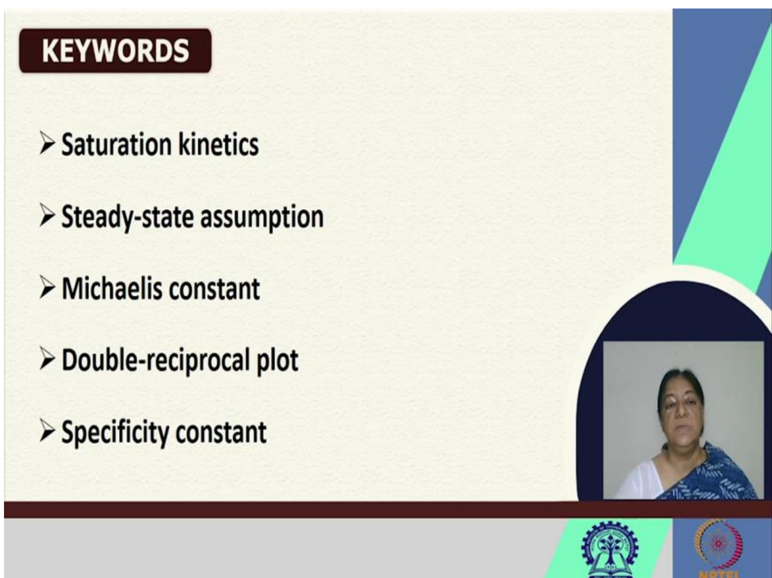

These concepts are important in understanding the saturation kinetics and the specificity of specific substrates, specific enzymes, how we can design specific inhibitors and how we can compare over inhibitors.

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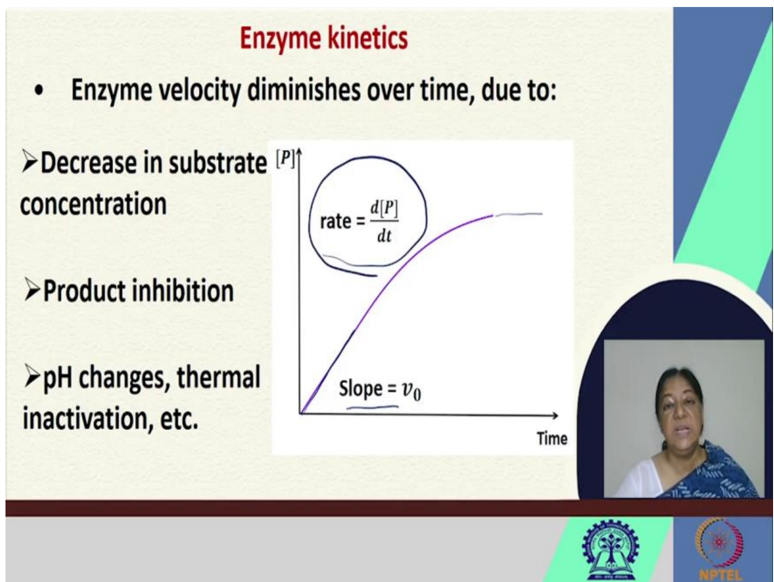

If we look at the concept of enzyme kinetics, we are looking at a velocity as the reaction. The enzyme-substrate reaction proceeds, where we have the formation of the enzyme-substrate complex. In the event it will form the product as we have seen in the previous module.

The velocity of this reaction decreases if there is a decrease in substrate concentration. Product inhibition occurs, there are pH changes and thermal inactivation of the enzyme itself, as we saw that the enzymes work at an optimal temperature and at an optimal pH. If we look at the formation of the product with time or we follow the rate of the product with time, then what we

can look at is the formation of the product and this [refer to slide] slope gives us our initial velocity.

Over time, we see that we reach a saturation as the product is formed indicating that their substrate has been converted into product.

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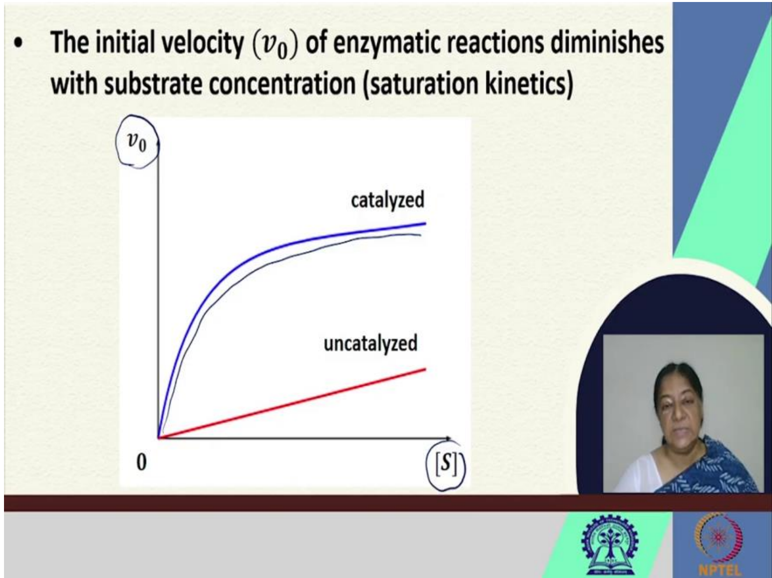

As we look at a reaction of the initial velocity versus the substrate concentration, what we see that in a catalyzed reaction we can reach this saturation much earlier than before. So the initial velocity of this enzymatic reaction, diminishes with substrate concentration, giving us saturation kinetics. Indicating that with time the enzyme is saturated with the substrate and further reaction is not possible.

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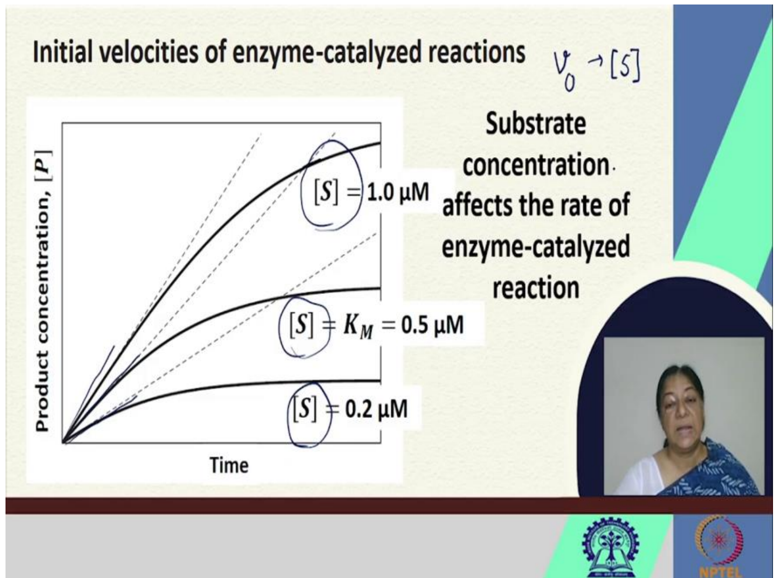

Initial velocities of these enzyme-catalyzed reactions can vary with the substrate concentration, in the sense that the more of the substrate we have, the product concentration; as would be expected, would increase. But for each of these initial values we will get a velocity associated with the substrate.

We will have an initial velocity as corresponding to each substrate concentration. This is important in understanding how we can define our system. The substrate concentration will affect the rate of the enzyme-catalyzed reaction.

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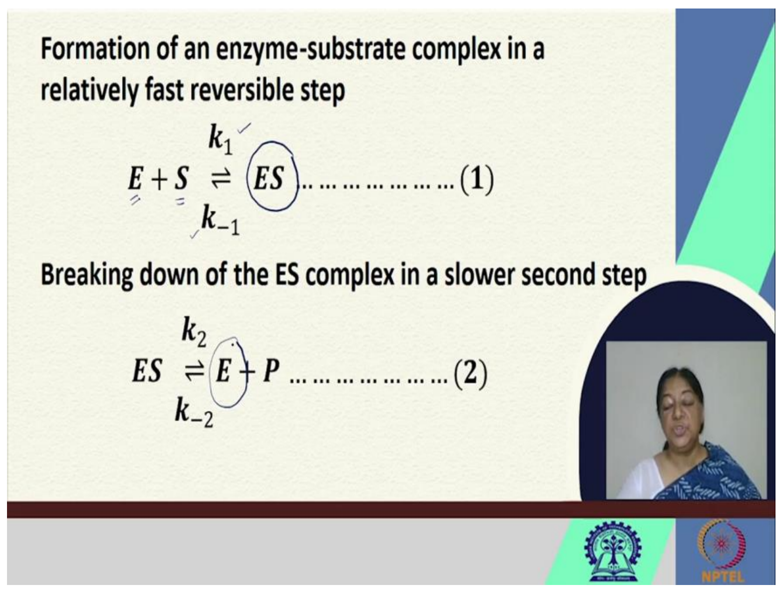

The formation of this enzyme-substrate complex occurs in a relatively fast reversible step. So this [refer to slide] is our first reaction that occurs, where we have our substrate forming a complex with the enzyme, in this enzyme-substrate complex.

The enzyme-substrate complex has a forward rate constant of k_1 and an inverse rate constant of k_{-1}. This is similar to the protein ligand binding studies that we considered in a previous module, where we looked at the ligand binding to the protein in a forward rate constant and a reverse rate constant, which would amount to the dissociation in this case of the enzyme-substrate complex, to form the enzyme and the substrate again.

This [refer to slide] breakdown of the ES complex will result in a product formation in a simple representation, where we look at the enzyme-substrate complex breakdown to form the enzyme and the product. So, this enzyme now is ready to form or to accept another substrate.

In this specific set we are looking at another equilibrium, but considering that the product formation occurs at a rate where we follow the formation of the product, it at times is considered unlikely for this to revert back to the enzyme-substrate complex in a reversible reaction.

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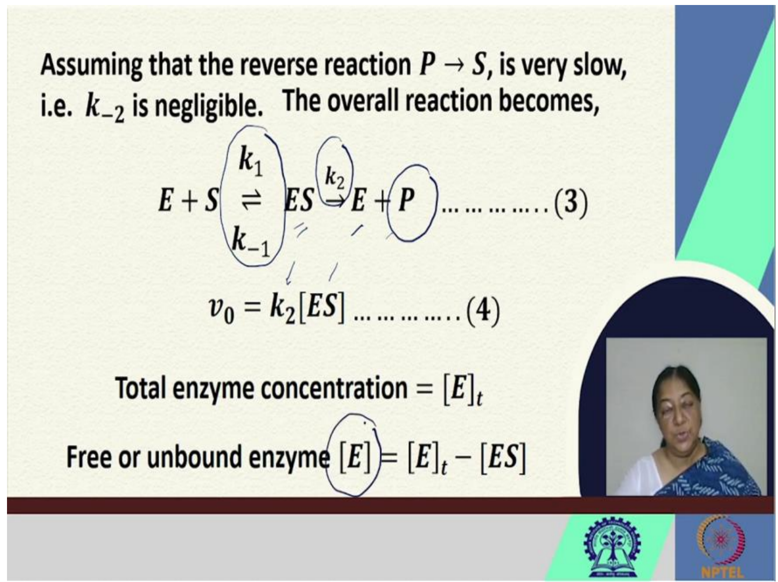

Assuming that this reverse reaction P to S is very slow, we can say that the rate constant associated with k_{-2}; that is the reverse of the second reaction or the second step, is very slow. As a result, the overall reaction can now be written in a fashion, where we have what is called a pre-equilibrium step associated with the enzyme-substrate complex and its possible dissociation. The second step that involves a rate constant k_2, in the dissociation of the enzyme-substrate complex, forms the enzyme and the product.

The rate constant of the formation of the product therefore is our k_2 rate constant and the concentration of the enzyme-substrate complex. The total enzyme concentration as we know is $[E]_t$. Like our ligand concentration, we would know the amount of the free enzyme in solution, as in protein ligand binding kinetics.

Here the enzyme is either in the free form or in the bound form. This bound form is in the complex of the enzyme-substrate. So, we have the total enzyme concentration minus the

enzyme-substrate concentration, would give us the free or the unbound enzyme and from this we can study different kinetics of the enzyme.

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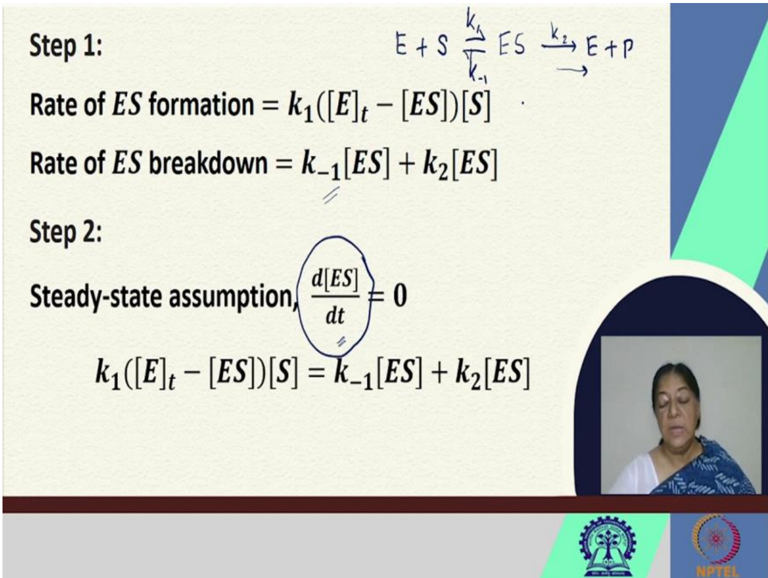

The step 1 therefore, looks at the rate of the enzyme-substrate formation. The enzyme-substrate formation is followed by k_1, that is the first reaction that we have. Where we have E + S in our first step, in the specific rate constants k_{-1} forming our ES complex. This, then dissociating into E + P in our second step. This is the rate of ES formation and we have the rate of ES breakdown, given by the dissociation in the reverse direction. The k_{-1} that we have here [refer to slide] in the forward direction, forms the product.

In step 2, we use what is called a steady-state assumption. In the steady-state assumption, we assume that the concentration of ES with time, does not change. d[ES]/dt = 0.

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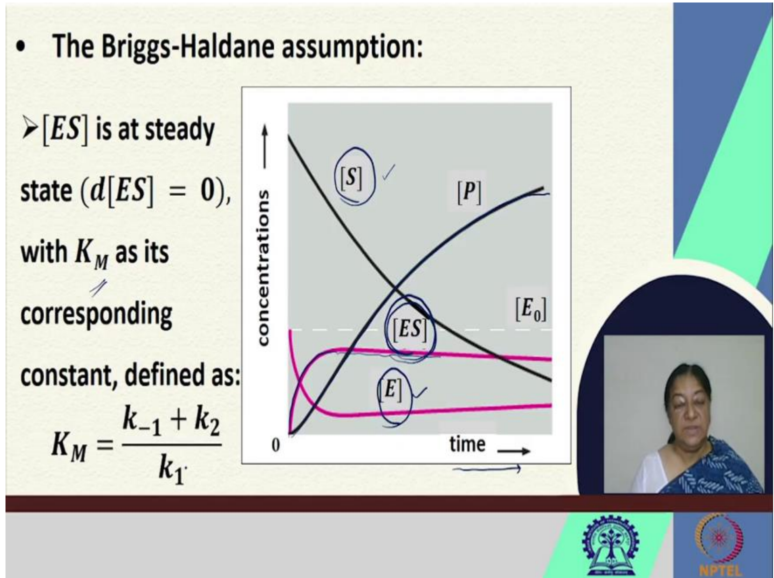

We can equate the rate of ES formation to the rate of ES breakdown and this leads us to a formation that we can consider in a graphical representation like this [refer to slide]; where we have our enzyme concentration and our substrate concentration, both of which decrease with time. Our time is on the x axis and the concentrations of the different species in solution is given as these different lines.

We have our enzyme concentration and our substrate concentration, both decreasing with time and our enzyme-substrate concentration initially rising and then being constant. While our product rises and then reaches saturation with the depletion of the substrate.

This is the Briggs-Haldane assumption, where ES that is our enzyme-substrate complex, is at a steady-state saying that there is no change in the concentration with time, as the corresponding constant is K_M, that is the Michaelis constant defined by $k_{-1} + k_2$ divided by k_1.

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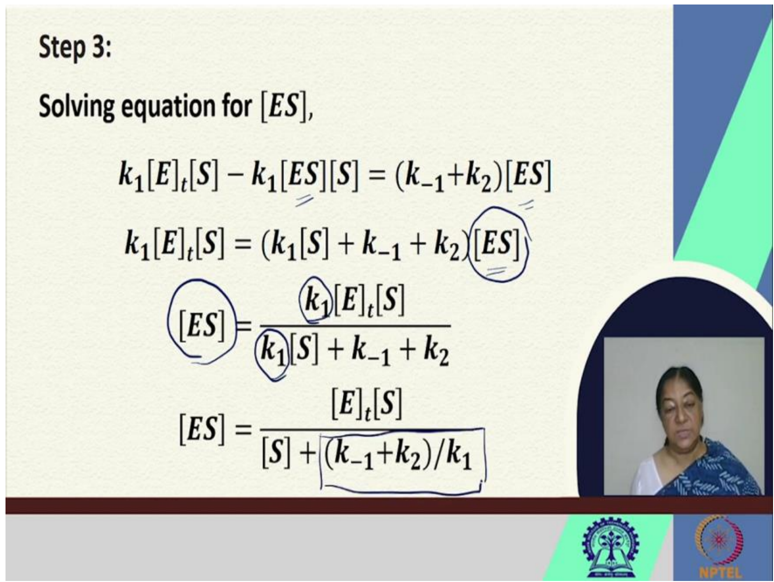

Step 3, involves solving this equation for ES. So we have the equality related to the formation of ES and the disintegration of ES, which we have equated. From that with a bit of algebra, where we take the concentration of the substrates and the enzyme-substrate complexes to one side.

And we have the enzyme-substrate complex concentration that we have excluded from the brackets here [refer to slide]. What we have is, we have an expression for the enzyme-substrate concentration divided by k_1 throughout this equation, giving us the specific constant.

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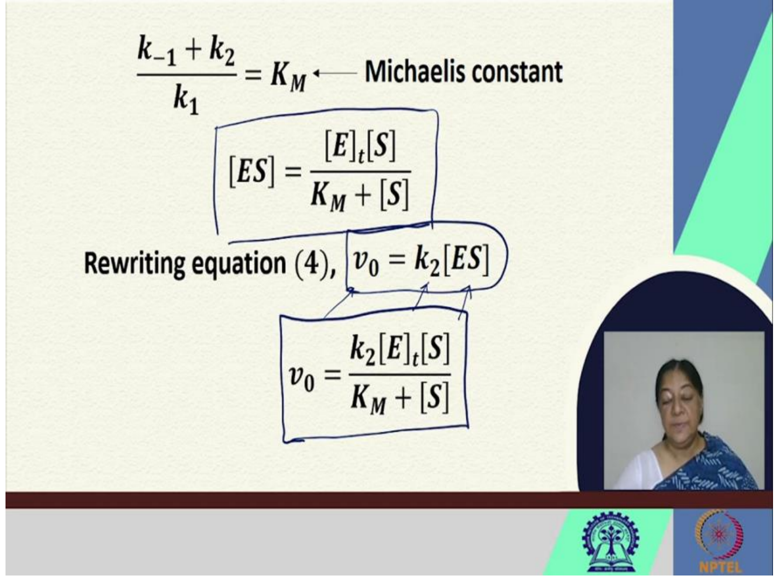

This we work out to be the Michaelis-Menten constant. This Michaelis constant is given by: $K_M = (k_{-1} + k_2) / k_1$, which we will look at in more detail to understand about the expressions. If we rewrite equation 4, where we have our v_0, that is the initial velocity given as the k_2, that is the

second step and the product of the ES complex, we get an expression that is equal to this. This helps us in understanding the enzyme-substrate formation.

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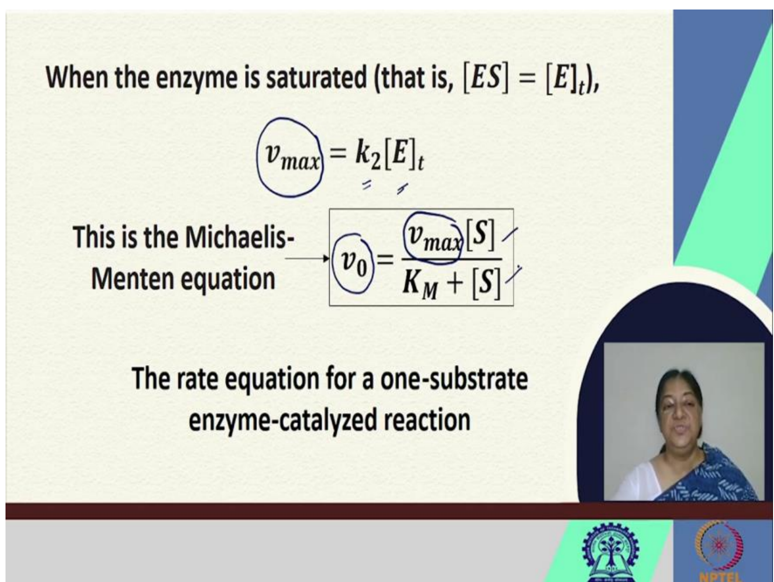

We can work out specific options or specific possibilities. If we say that the enzyme-substrate complex concentration is equal to the total enzyme concentration, this means that the enzyme is saturated, which means that we have reached the maximum velocity possible. That is $k_2[E]_t$ considering that the reaction is to completion.

So, we can write out $k_2[E]_t$ as $v_{max}[S]$ from our previous expression. We have an initial velocity, we have the maximum velocity, and we have this in terms of the substrate concentration. The Michaelis-Menten equation is:

$$v_0 = \frac{v_{max}[S]}{K_M + [S]}$$

The rate equation is for a one-substrate enzyme-catalyzed reaction.

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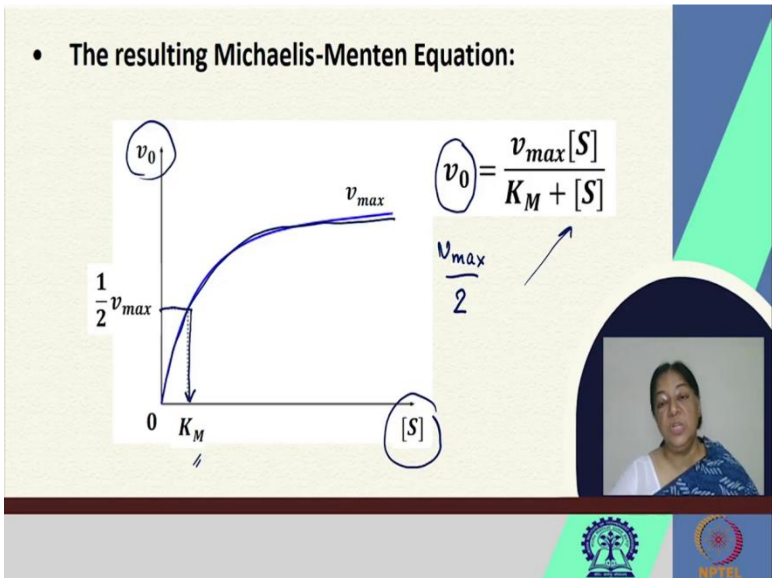

The resulting Michaelis-Menten equation that is shown here [refer to slide], we have the v_0 versus the substrate concentration, we will reach the maximum velocity and at half the v_{max}, the substrate concentration corresponding to half v_{max}, we will see if we can plug these values in here, where we make v_0 equal to v_{max} by 2, that is half v_{max}. We will get K_M corresponding to the substrate concentration.

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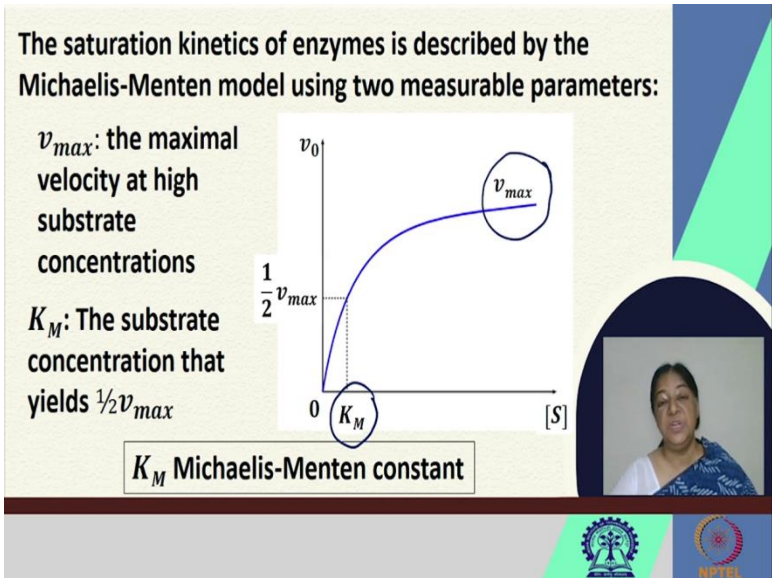

The Michaelis-Menten constant gives us a constant that is useful in comparing substrates and the saturation kinetics of enzymes described by these two measurable parameters, that is the v_{max} of the reaction and the K_M of the reaction. This is what describes our enzyme kinetics. So it is the substrate concentration that yields half of v_{max} and v_{max} is the maximal velocity at high substrate concentrations.

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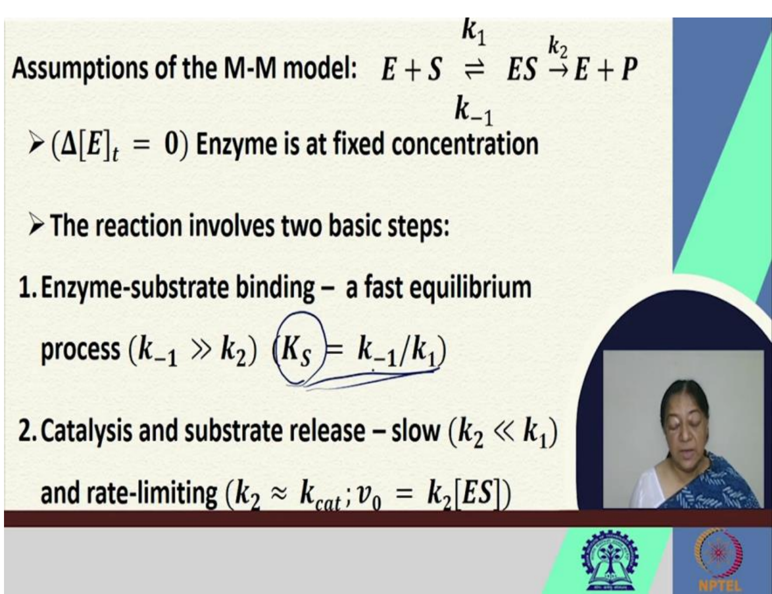

Let us look at various possibilities. [Refer to slide] here is our equation, our specific expression for the enzyme-substrate complex formation and its subsequent formation of the products.

The assumptions of this Michaelis-Menten model say that the enzyme is at a fixed concentration and the reaction involves two basic steps. One is a fast equilibrium process where we can have the fast formation, where we have now an equilibrium which we define as the backward reaction rate, divided by the forward reaction rate.

Where we have our k_{-1} process much larger than the k_2. We can have the catalysis and substrate release that is slow, where we have a k_2 less than a k_1 and rate limiting where we have what is called a k_{cat} that is associated with the k_2, where we will have our initial velocity correspond to the second step of our reaction, multiplied by the product of the enzyme-substrate concentration.

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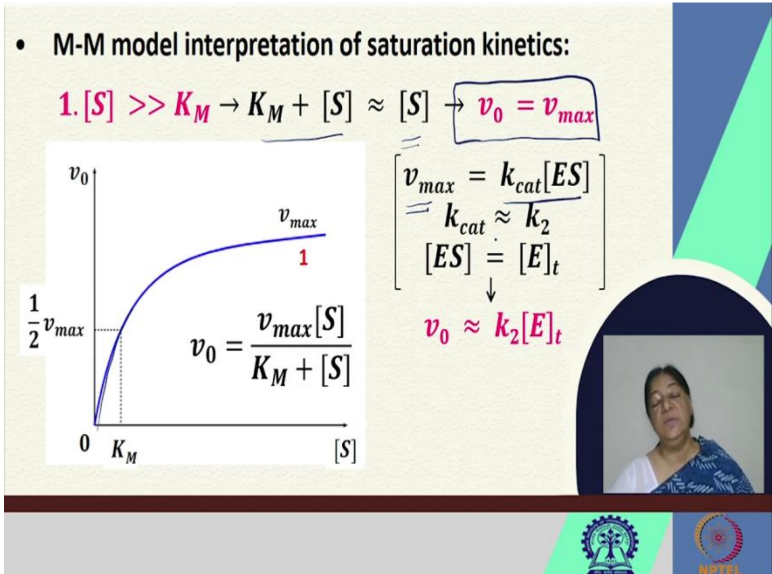

If we now look at the interpretation of the saturation kinetics, in terms of the curve where we plot the initial velocity versus the substrate concentration. Initially we considered a first order reaction followed by a second order reaction. So, this [refer to slide] is our expression.

If we look at the substrate concentration that is much greater than the Michaelis constant, then we can form our equation in such a way that if we just plug this possibility into this equation, the K_M plus the substrate concentration, is very close to the total substrate concentration. As a result of which we can get the v_0 equal to the v_{max} when the substrate concentration is very high.

When we reach this v_{max} possibility, we have k_{cat}[ES] and k_{cat} is approximately equal to k_2, when the enzyme-substrate complex corresponds to the total enzyme concentration available. So, we have our v_0 equal to $k_2[E]_t$ instead of [ES].

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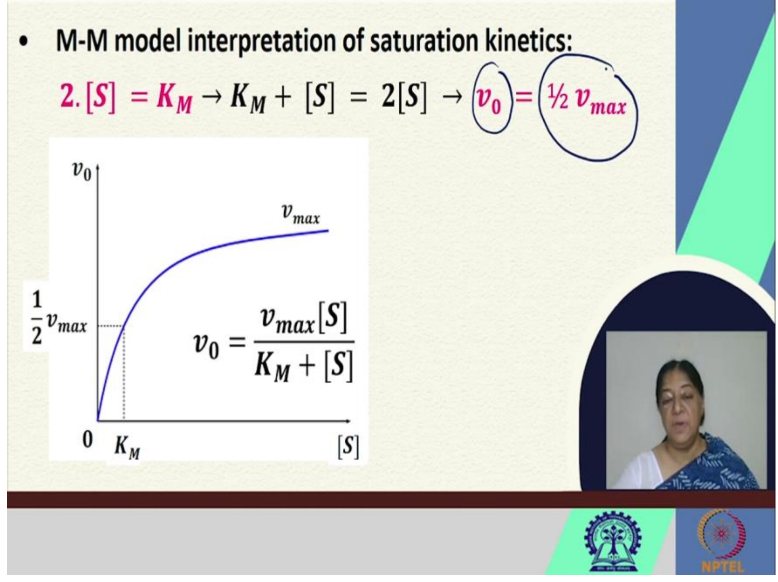

Another possibility that we can look at is when the substrate concentration is equal to the K_M, the way in which we defined our K_M value; that is the v_0 would be equal to v_{max} by 2 at K_M. So, the K_M definition is the substrate concentration, when the velocity has reached half maximum velocity.

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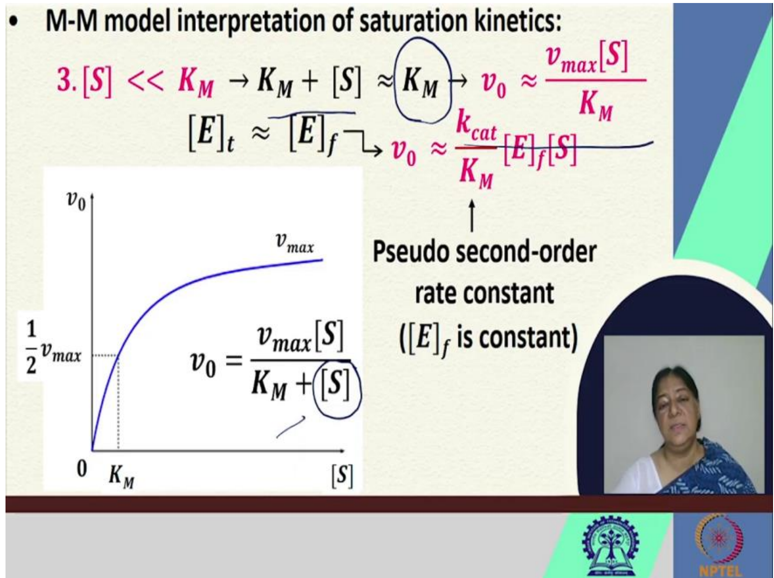

Another possibility would be where we have the substrate concentration much less than the K_M value. In this case, our K_M + [S] is approximately equal to the K_M and we rearrange this equation where we neglect the [S] in the denominator and we get an expression for v_0.

This means that the [E] is equal to the final concentration and we can say that k_{cat} by K_M in an expression for the [S], giving us a pseudo second-order rate constant, where $[E]_f$ is a constant; considering that the [S] is less than the K_M value.

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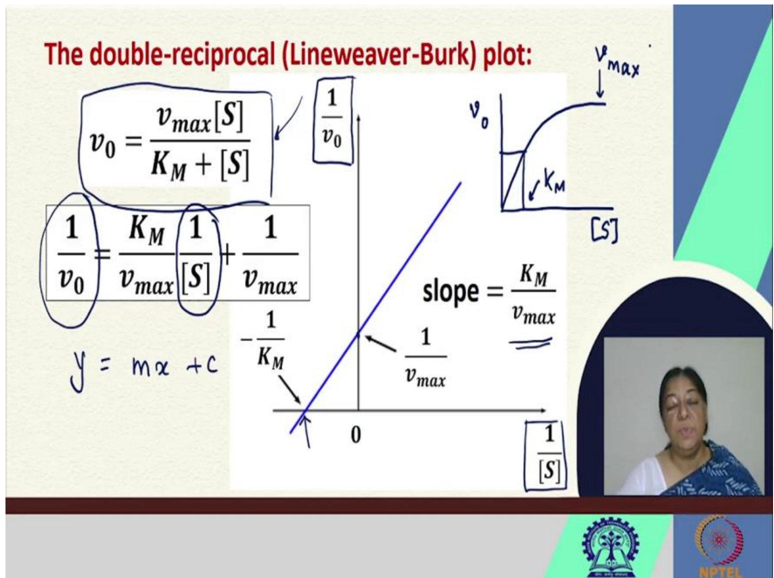

Our next expression looks at a linearization of our Michaelis-Menten. This is by far the most useful plot in enzyme kinetics, where we can actually determine the value of v_{max} and K_M from a double reciprocal product as it is called, where we get the form of y = mx + c, where we realize that if we plot 1 by v_0 versus 1 by [S], our slope is going to correspond to K_M by v_{max}. So this [refer to slide] is what we plot in our double reciprocal plot, 1 by v_0 versus 1 by [S].

This linearization gives us a value for 1 by v_{max} and our the negative intercept on the x axis as -1 by K_M. This intersection, the intercept on the y axis gives us 1 by v_{max}.

We realize that this is a much easier representation in the sense, when we plot the v_0 versus the substrate concentration we get a plot that is in this [refer to slide] fashion. Identifying or trying to determine the v_{max} or the half of the v_{max} that is going to correspond to the K_M value, is not an easy task. However, this double reciprocal plot makes the job much easier.

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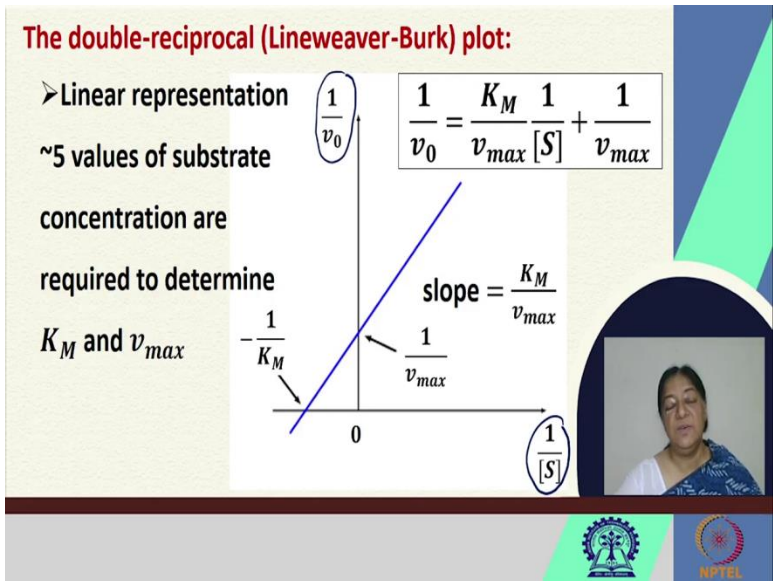

In our double reciprocal plot, we found out that if we plot 1 by v_0 versus 1 by [S], we can get the slope and the intercepts that will give us the values of K_M and v_{max}. So, this [refer to slide] is our expression. This linear representation can work with even 5 values of the substrate concentration and we can determine K_M and v_{max}, which is what we can use to compare over different substrates; comparing the K_M constants and comparing the maximum velocities.

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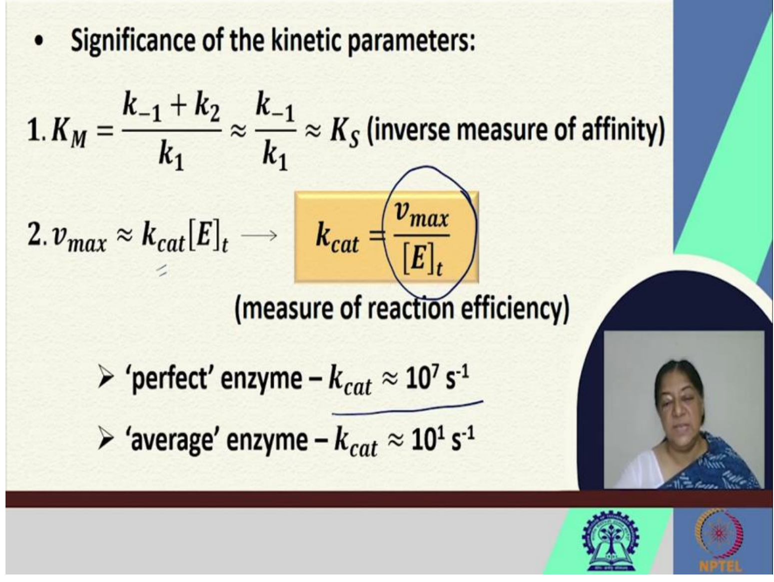

Now if we look at the significance of these kinetic parameters. We have the $K_M = (k_{-1} + k_2) / k_1$, neglecting the value of k_2 considering that it is really small, we can get an inverse measure of the affinity of the enzyme-substrate complex. So, this is like the association and dissociation constants that we considered for protein ligand binding.

Now, for the enzyme-substrate concentration or the enzyme-substrate complex formation, we do not want this to be too strong. Because if the enzyme-substrate complex is a very tight complex then the substrate or the reaction would not proceed and we would not have product formation.

Another possibility is the v_{max} that corresponds to the maximum velocity, where we have the k_{cat} value that gives us the v_{max} divided by the [E]. This gives us a measure of the reaction efficiency. So we have the affinity, we have the efficiency. And the perfect enzyme would have a k_{cat} value of the order 10^7 s^{-1} and an average enzyme, a rule of thumb would be 10^1 s^{-1}.

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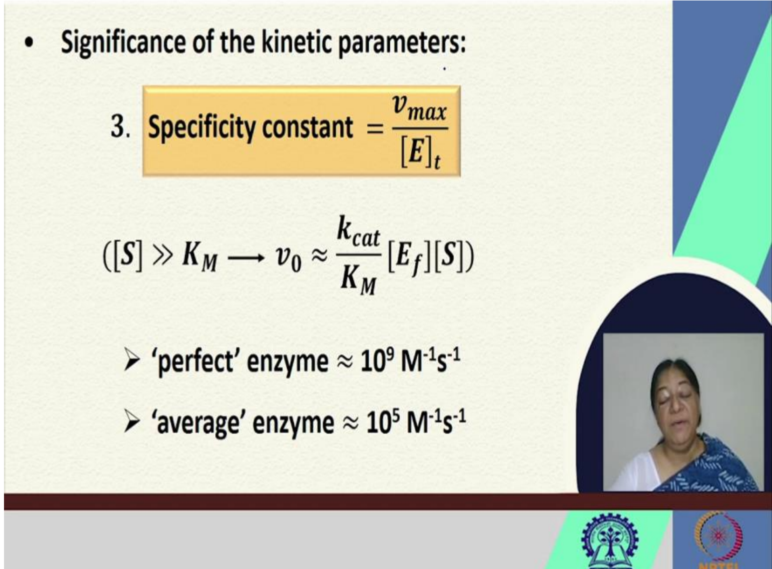

So, we have a specificity constant that would give us v_{max} by $[E]_t$. And we have the specific variations that we looked at, that tell us what a perfect enzyme would be in terms of its specificity. So we would want our catalytic protein, that is an enzyme to be specific in nature, to be efficient and to have a perfect kinetics in the sense of product formation.

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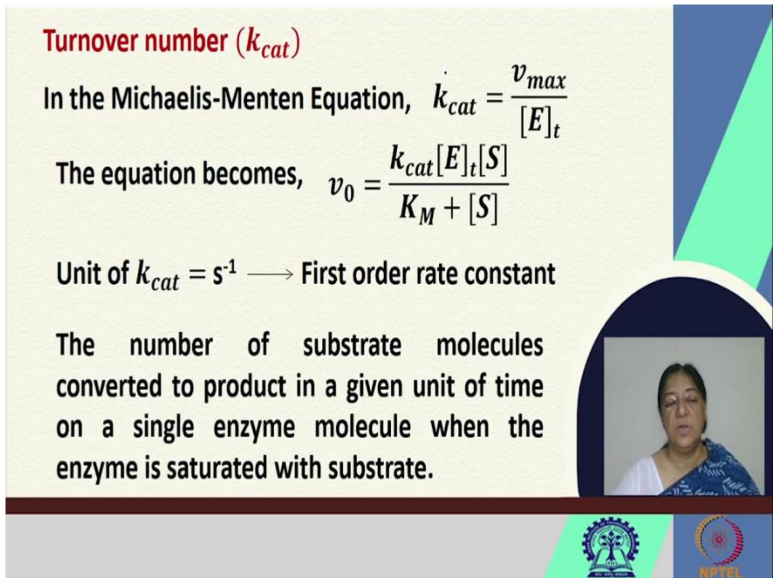

The turnover number is the number of substrate molecules that are converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with the substrate.

Now in the Michaelis-Menten equation, we look at that as k_{cat} by v_{max} by $[E]_t$ and we can work this out in terms of our expression and the unit of k_{cat} is s^{-1}. So, it tells us how the turnover of the substrate molecule to the product is; it is a first order rate constant.

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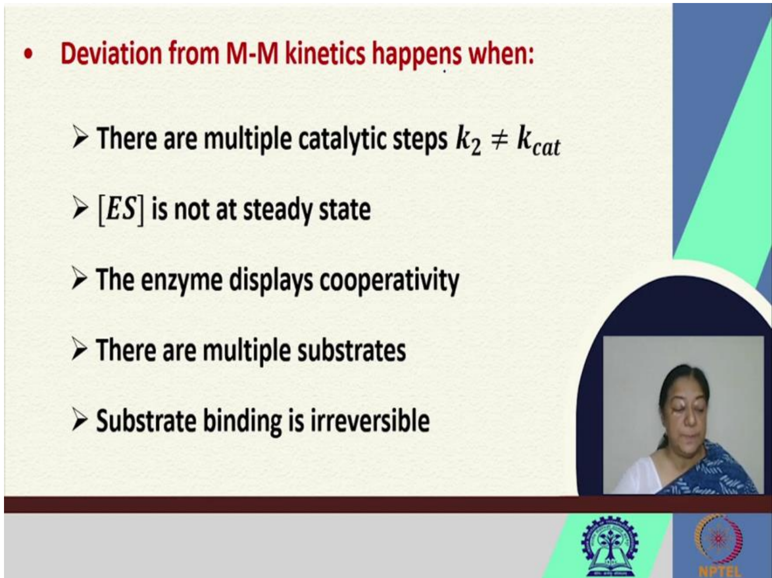

Similarly, we can have deviations from the kinetics as well and these deviations occur when there are multiple catalytic steps, where we cannot equate the k_2 with the k_{cat}. ES, the enzyme-substrate complex is not exactly at steady state.

And the enzyme displays cooperativity, where we can have distinctions, which we looked at in the protein ligand situation, where the binding of the substrate could have an effect on the binding of another substrate molecule, where we will be looking at double bisubstrate kinetics; where we have multiple substrates. This substrate binding is irreversible, in the sense that there is a covalent linkage that cannot be broken.

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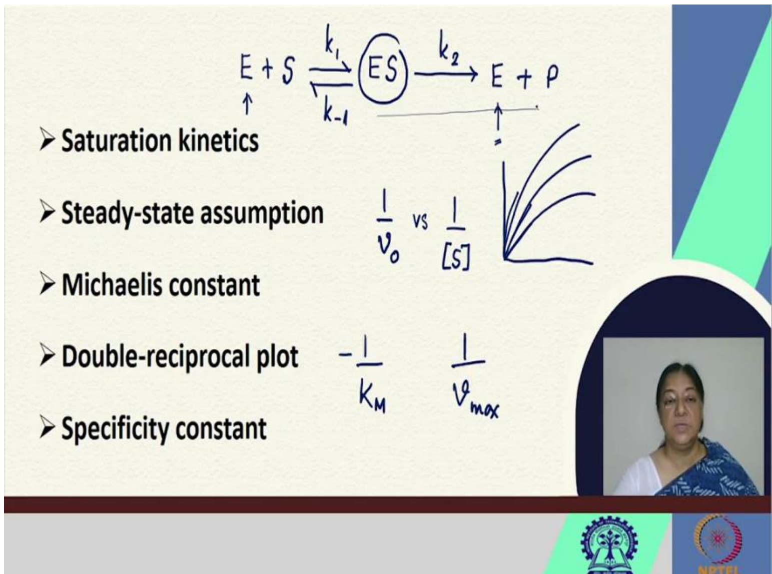

In the Michaelis-Menten kinetics we look at a saturation. A saturation indicating that we have our enzyme of interest that is now bound to a substrate. A pre-equilibrium step, that involves a forward reaction rate of k_1 an inverse, reverse reaction rate of k_{-1}, forming our ES complex.

This in a simplistic way, using the rate constant k_2, is going to give our E + P. In the enzyme mechanisms that we looked at, we found out that our enzyme that we started off with, is now available for further substrate bound to it, to have a further reaction occur to form the product.

Our expression for the Michaelis constant and Michaelis-Menten kinetics indicated that we could consider a steady-state assumption for the ES complex, where we equated its rate of formation with its rate of disintegration and we got expressions for the Michaelis constant. We then found out that if we look at the double reciprocal plot where each of these plots at various substrate concentration, give us specific initial velocities that were the initial slopes of the graphs.

We now plot 1 by v_0 versus 1 by [S] in our double reciprocal plot, the Lineweaver-Burk plot, we are able to get on the x axis the value equal to - 1 by K_M; the intercept on the x axis. And our intercept on the y axis corresponded to the 1 by v_{max} information about our proteins, about our enzyme. We also could understand what a specificity constant is because we would like our enzyme to have a strong affinity for the substrate. But the binding should not be too tight because we would want this further reaction to go forward to form the product.

This is the basics of enzyme kinetics. We will look at multi-substrate reactions and see how we can describe their kinetics in the next lecture.

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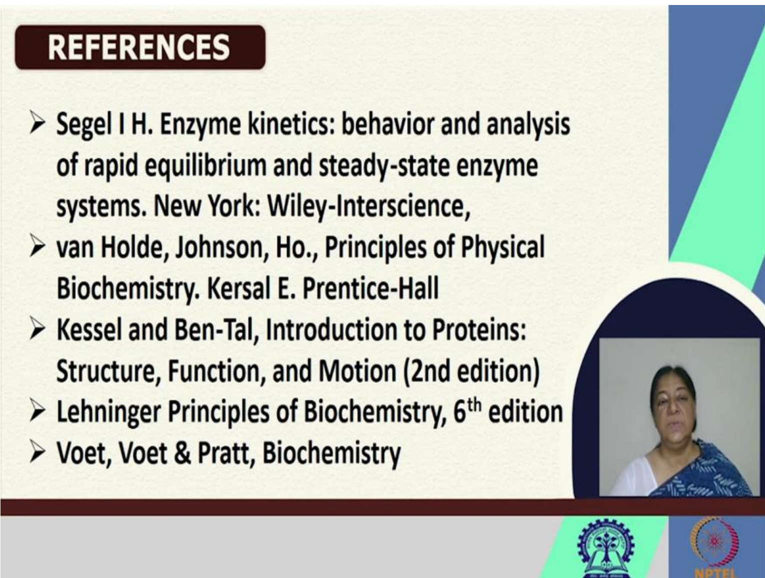

These [refer to slide] are the references.

Thank you.