

Rate Processes
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Module No. # 01

Lecture No. # 15

Kinetics of Some Specific Reactions (contd...)

Hello, Good morning everybody.

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- Reaction Rates and Rate Laws
- Effect of Temperature on Reaction Rate
- Complex Reactions
- Theories of Reaction Rate
- Kinetics of Some specific Reactions
- Kinetics of Catalyzed Reactions
- Fast Reactions
- Reactions in Solutions
- Ultrafast processes
- Reaction Dynamics

So, today we will continue with rate processes and we were discussing kinetics of some specific reactions and under that heading, we will talk about enzyme catalyzed reactions.

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Simplified Model for Enzyme Catalysis

- E ≡ enzyme
- S ≡ substrate
- P ≡ product



$$\text{rate} = k [ES]$$

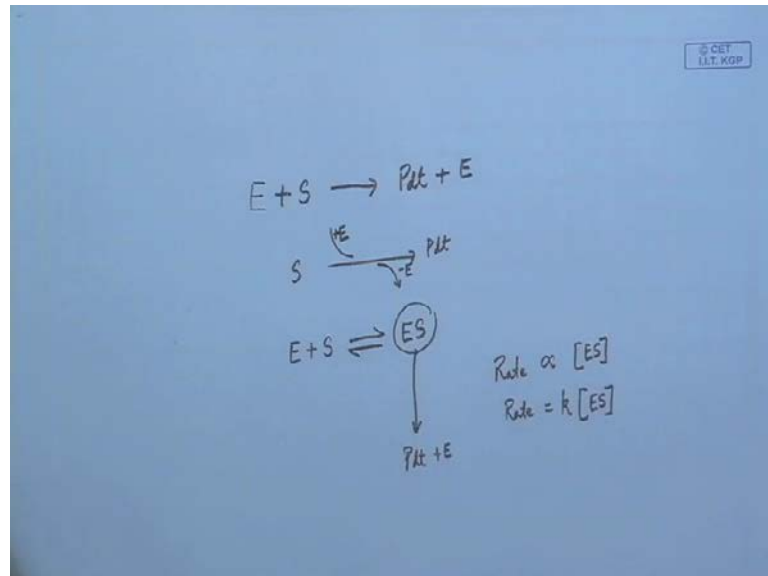
- The reaction rate depends directly on the concentration of the substrate

So, catalysis, we already have talked about. It is a substance that generally, what it does is, it increases the rate I mean, efficiency of the process. So, in absence of catalyst, maybe some reactions are found to be very slow or the yield of reaction that is, amount of product that is formed is very small. But, if we apply catalyst then, it is found that the yield is increased and **it takes** sometimes, it takes less time for the reaction to occur. So, like catalysis, **so** enzyme catalysis is typically a biological catalysis.

Basically enzymes are large molecules. I mean, enzymes are generally proteins. So, proteins means they are composed of amino-acid residues joined together by peptide bonds and these basically are large peptides. I mean proteins. So, having many number of amino acid residues. **They are** you know, when they are bonded together to form the protein. So, these protein molecules have a typical three dimensional structure and that three dimensional structure is very much very specific. And these protein molecules **those were** those which are called enzymes **are acting** are you know, acting as biological catalysis for you know, many reactions which **which** are found to occur in maybe **in** human systems or maybe plants maybe animals. So, **and** now a days, these enzymes are used in **in** synthesis, in synthesizing many chemicals or doing many chemical processes in the **in the** industries. So, industrial applications of enzymes are **are** huge now a days. **Now**, if we **if we** start to talk with about these enzyme catalyst reactions, **so** there are three things, one is enzyme that is a biomolecule, large biomolecule, then substrate on which this enzyme will act. So, substrate will **will** be converted to a product. So,

basically what is happening that you have got the enzyme E and then this enzyme **will enzyme will** with your substrate it will give you product plus enzyme back.

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So, it is you know, used again. So, it is recycled in this way **so** basically, substrate you know, to product. So, this enzyme is entering and that enzyme is coming, so plus E this way minus E. So, enzyme is eliminated, enzyme is included. So, this is known to occur again via a **a** complexation process. That is, enzyme first reacts with your substrate and producing it is producing enzyme substrate complex. So, enzyme substrate complex is in equilibrium with enzyme plus substrate. So, it is your reactant side, this is your intermediate side and this enzyme substrate will further undergo reaction to give you product plus enzyme back.

So, basically, this is the intermediate and we have to may be, apply **we have to apply** this steady-state approximation onto this. And the reaction rate is found to depend directly on the concentration of substrate **directly on the concentration of substrate**. And therefore, your overall rate is proportional to ES. And so, rate will, because this is your **this is your** you know, product formation steps therefore, it is equal to some constant rate constant into E S. So, **this is** these are the two elementary, I mean, basically three elementary steps, one, two and three elementary steps.

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Enzyme Catalysis

- Enzymes - proteins ($M > 10000$ g/mol)
- High degree of specificity (i.e., they will react with one substance and one substance mainly)
- Living cell > 3000 different enzymes

Now, what are enzymes? Enzymes are proteins having molecular weight more than 10,000 gram per mole that is, 10 kilodalton. And, it has got high degree of specificity. It is very specific. Specific means, one enzyme will recognize a particular substrate or maybe a **a** group of substrates having some specific functionality. Some **some** specific functional groups are there. So, this enzyme will recognize that you know, substrates which are having you know, specific functional groups and high degree of specificity, that is, they will react with one substance and one substance mainly or maybe one group of substances having **having** specific functionality functional groups.

Maybe, now living cells are found to contain you know, more than **you know more than** or maybe around 3,000 different enzymes, about 3,000 different enzymes. So, just imagine imagine a living cell its dimension and in that dimension **in that dimension** there are about 3,000 different enzymes. So, how complicated is the machinery and how complicated is the overall thing. That is, so, many processes are going on within such a **such a** small region. That is, I am talking about a single cell and you see that these reactions are now interconnected. So, if you **if you** chop off any, you know, step then maybe functional things are impaired, cell functionality may be, you know, impaired. So, some some complications may arise. So, **you** just you just imagine that about 3,000 different enzymes, 3,000 different biocatalysts are there. So, it is an amazing situation situation that we can we **may** may be thinking of.

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The Lock and Key Hypothesis

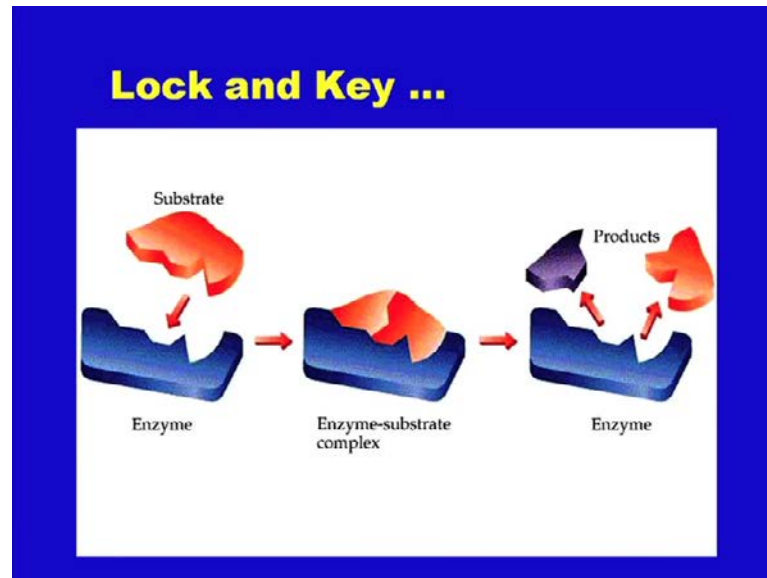
- Enzymes are large, usually floppy molecules. Being proteins, they are folded into fixed configuration.
- According to Fischer, active site is rigid, the structure of substrate exactly fits the “lock” (hence, the “key”).

Now, the lock and key hypothesis you know, previously it was hypothesized that enzymes are you know, according to Fischer, the active site. Active site means, the site where this substrate binds to the enzyme. So, that **that** place is called the active site where, the enzyme does the chemical transformation. That is first this enzyme you know, **you know** substrate **substrate** binds to the active site of the enzyme and **and** active site is rigid and the structure of the substrate is exactly such that, it fits that region. It is just like a lock and key. So, a specific key can recognize a specific lock. So, you know, if you have two keys and two locks. So, you cannot interchange the key. **so** Only one key will **will** be specific for one lock. In the same way, the other key will be specific for the other lock. So, it is just like a lock and key hypothesis.

Now, **now** also enzymes are large molecules. As I told you that, these are usually floppy molecules. Floppy molecules means, they are you know, it is changing with **with** you know, conditions or may be changing with time, changing with **with with** other factors like temperature, maybe pH or maybe other **other** external factors that may, **you know**, **may** lead to alteration of this **this** three dimensional structure. And, being proteins they are folded into a fixed configuration. It has got a definitive folded structure. It is not that suppose, you have got two or three molecules of having same molecular weight and you know same sequence, same you know, amino acid sequence then, three of them will have three different structures, three dimensional three different, three dimensional structure. It is not possible. So, it will have a very specific structure depending on the external

conditions like p h, temperature and maybe ionic strength as well. So, external factors are very important in determining what will be the overall structure of **of** these enzyme or large protein molecules.

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Now, you just look at this diagram you know, picture that you have got. This enzyme, it has got a specific group you know, in a specific manner. And you see that this substrate is having the complementary groups. So, it will just fit into it. It will just come and then, it will bind the substrate, **will bind the substrate** will bind to this enzyme. So you see, that this group is exactly complementary to this part and again this part is complementary to this part. Then, this part is exactly complementary to this part. And so on.

So, that is why you can **you can** say that when substrate binds to the enzyme, producing the enzyme substrate complex at first, so, it is basically you see that these groups are not **not** seen now. So, it is basically, just like a overall thing and a combined a combined stuff. Then with time, this enzyme substrate complex what **will** it will do that, it will do further reaction to give you enzyme back and now products are separated. So, basically you see that, the substrate was like this and your product is that may be from substrate, a portion has been taken out, has been snatched out to give you the other part.

So, basically these are the products. May be **maybe** this basically represents a, you know, say breaking of some big molecule to small molecule, large molecule to small molecule. Large means you know, say moderately large molecule or maybe suppose a substrate is

dissociating into two products via this enzyme. So, enzyme plus substrate producing enzyme substrate complex, then enzyme back and then these are the **these the these** two are the products. So, it **it** is something like lock and key. You know, **it it** this represents some **some** kind of lock and key picture. But, now a days this lock and key picture is no longer that **that** popular.

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The Michaelis-Menten Mechanism

- Enzyme kinetics – use the steady-state approximation to ES complex

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} P + E$$

ES – the enzyme-substrate complex

So, now-a-days, the Michaelis-Menten mechanism, Michaelis-Menten mechanism is thought to be the right mechanism. So, in enzyme kinetics it is **it is** the building block of enzyme, of understanding **of** enzyme kinetics. So, what is that in enzyme kinetics? You know, this enzyme plus substrate producing enzyme substrate complex. So, you just go back to the earlier slide that, this enzyme substrate producing enzyme substrate complex and then it is dissociating to the product. So, you see enzyme plus substrate giving rise to enzyme substrate complex. So, that there is an equilibrium between enzyme substrate and enzyme plus substrate, with k 1 for the forward and k minus 1 for the back reaction.

So, forward rate constant is k 1 for the formation of S and backward, I mean, dissociation of S will be k minus 1 and k 2 is the product formation step. So, it is a quick equilibrium and then maybe slow dissociation to give you product plus enzyme back. So, it is **it is it is** nothing, but, the enzyme substrate complex that dissociates to give you **that dissociates to give you** your desired product. So, you see that, this is the intermediate through which, this enzyme plus enzyme plus substrate or may be substrate with the help of enzyme is

converted to product. **so** That means, we should **we should** apply steady-state approximation to this intermediate complex that is ES. So, we have to apply steady-state condition for ES and then we will find out the **the** concentration of E S **E S**. And then, we will use it further for finding out the overall **overall** rate of reaction. I mean rate constant.

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Applying the Steady State Approximation

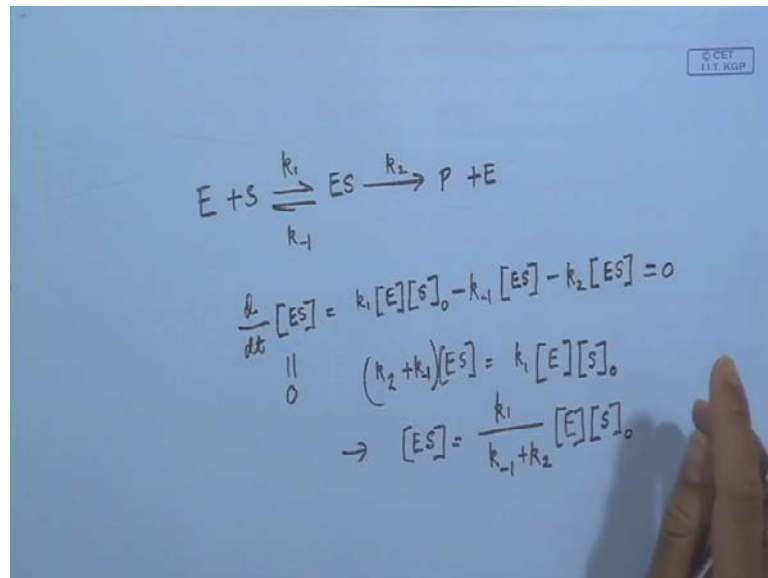
$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} P + E$$

- Note that the formation of the product depends directly on the [ES]
- What is the rate of formation of [ES]?

$$\frac{d[ES]}{dt} = k_1[E][S]_o - k_{-1}[ES] - k_2[ES]$$

So, when applying steady-state approximation, this is the mechanism. So, note that the formation of product depends directly on ES. You see, this step directly depends on this. So, this is one elementary step. **so** Therefore, k_2 into E S concentration **So, give you that** will give you the product that is your **that is your** desired product. Now, what is the **what what is the** rate of formation of ES? Now, rate of formation of ES, is this step, I mean, this is the **this is the** formation step and this is your, you know, dissociation step. The lower the **the** back reaction and this is again, by this route, the amount of ES, that is, ES concentration is depleted. So, that these **these** all three should be taken into consideration **should be taken into consideration** while, finding out the rate of formation of ES. **so** Therefore, **therefore**, the corresponding differential equation will be $\frac{d}{dt}$ of ES, that is, rate of change of ES concentration with time. So, let us write down the, you know, the step E plus S giving rise to ES product plus enzyme back.

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This is k_2 , this is k_1 , this is k_{-1} . So, $\frac{d}{dt}$ of ES is equal to k_1 into ES because, it is the formation step. Then, minus k_{-1} into ES minus k_2 into ES, this is the rate of **rate of** change of ES with time. I mean, time rate of development of ES.

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The slide has a blue background with yellow text. The title is "ES - The Intermediate". Below the title, there is a bullet point: "Apply the steady-state approximation to the equation for $\frac{d[ES]}{dt}$ ". Below this, the equation $\frac{d[ES]}{dt} = 0$ is shown. This is followed by the equation $k_1[E][S]_0 = k_{-1}[ES] + k_2[ES]$. Finally, the equation is solved for [ES]: $[ES] = \frac{k_1[E][S]_0}{k_{-1} + k_2}$.

So now, you apply steady-state approximation. So, you put this to be equal to 0; that means, this is equal to 0. This is equal to 0 means your k_2 plus k_{-1} , k_2 plus **sorry** k_{-1} into ES is equal to k_1 ES or concentration of ES intermediate k_1 by $k_{-1} + k_2$ ES because, you are applying steady-state

approximation. So, **so** and this is your substrate concentration. So, at **at a at** a given substrate concentration, you can **you can** do this. **so** Therefore, let us put this wherever S is there. S_0 initial concentration of the substrate. So, initial concentration of the substrate is given like this. So, you got the **the** concentration of your intermediate. Now, it is **now** **it is** time to find out the rate of formation of product in terms of this E S.

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$$\frac{d[P]}{dt} = k_2 [ES]$$

$$= \frac{k_1 k_2}{k_{-1} + k_2} [E][S]_0$$

$$[E]_0 = [E] + [ES]$$

$$[E] = [E]_0 - [ES]$$

$$[ES] = \frac{k_1}{k_{-1} + k_2} [E][S]_0$$

$$[ES] = \frac{k_1}{k_{-1} + k_2} [S]_0 ([E]_0 - [ES])$$

So, therefore, your $d[P]/dt$, that is, product formation is equal to $k_2 [ES]$. So, that is equal to your $k_1 k_2 / (k_{-1} + k_2) [E][S]_0$ into E enzyme concentration, into S, initial concentration of S, substrate concentration.

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Working Out the Details

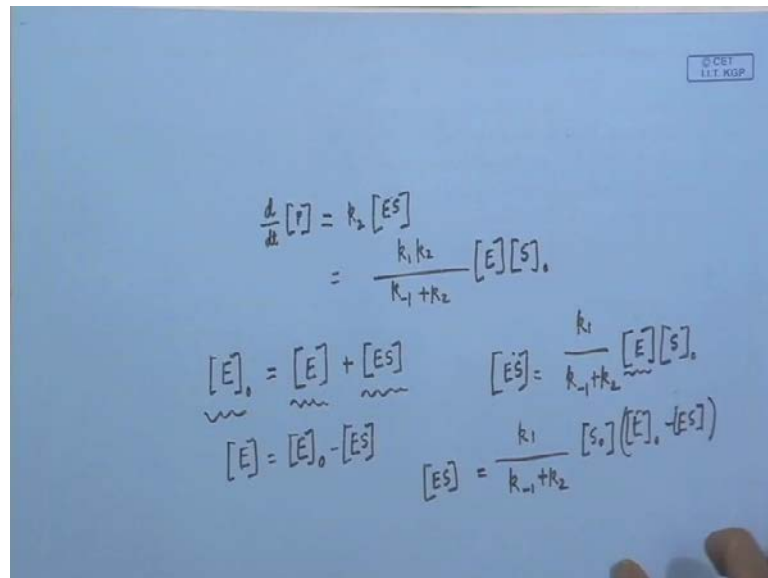
- $[E]_0 = [E] + [ES]$
 - Initial enzyme concentration
 - Free enzyme concentration
 - Concentration of Complex

Also $[E] = [E]_0 - [ES]$

$$[ES] = \frac{k_1 [E]_0 [S]_0}{k_{-1} + k_2 + k_1 [S]_0}$$

Now, **now** let us do one thing that we have to think of the overall concentration of enzyme. So, initial concentration of enzyme is E_0 which is equal to free enzyme concentration plus enzyme substrate concentration. So, this is your initial concentration, this is your free enzyme concentration, this is your enzyme substrate concentration complex concentration. Therefore, your E will be equal to **will be equal to** E_0 minus ES . Therefore, what we have to do is, ES will be your concentration of ES . What we wrote, like ES is equal to k_1 divided by $k_{-1} + k_2$ then, E into S_0 . That, you can replace E with this 1. So, that means, k_1 divided by $k_{-1} + k_2$. Then, **S_0 S_0** **then** S_0 is there. So, E is E_0 minus ES ; that means, left hand side you have ES and right hand side is also with ES . So, that means, if you **if you** transpose then your ES will come out to be equal to your ES **will come out to be equal to $E S$** will be equal to your **you** transpose.

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So, let us do it again. So, let us do transposition. So, ES will be equal to k_1 by $k_{-1} + k_2$ minus $[E]$ plus k_2 . Then, $[S]_0 [E]_0$, this is one term, another term will be minus k_1 by $k_{-1} + k_2$ minus $[E]$ plus k_2 $[E]$ $[S]_0$. Then, it will be with a minus sign. Then $[S]_0$ into $[E]$. So, that means, that means if you transpose this to this side, then this will be your $k_1 [S]_0$ $[E]_0$ $[S]_0$ divided by $k_{-1} + k_2$. This one, then $[E]$ plus $[E]$ times $[E]$ will be equal to k_1 by $k_{-1} + k_2$ $[E]_0 [S]_0$. This one is for this one and this one has been transported over here. So, times $[E]_0$, so, that means, what we got? What we got is, $[E]_0 [S]_0$ will be equal to $[E]_0 [S]_0$ like $k_1 [S]_0$ $[E]_0$ $[S]_0$ plus k_2 plus k_{-1} divided by $k_{-1} + k_2$. So, you will be having k_1 divided by $k_{-1} + k_2$ $[E]_0 [S]_0$ times. We will be getting another term that $k_1 [E]$ is there. So, that means, k_1 $k_{-1} + k_2$ $[E]_0 [S]_0$ $[E]$ $[S]_0$ in the numerator and in the denominator you will be having $k_{-1} + k_2$ plus $k_1 [S]_0$. So, these two are canceled. So, therefore, it will be $k_1 [E]_0$ divided by $k_{-1} + k_2$ plus $k_1 [S]_0$. So, this is the enzyme-substrate concentration, enzyme substrate complex concentration.

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The Final Equation

- Substituting into the rate law, v_p

$$v_p = k_2 [ES]$$

$$v_p = k_2 \left(\frac{k_1 [E]_o [S]_o}{k_{-1} + k_2 + k_1 [S]_o} \right)$$

$$= \frac{k_2 [E]_o [S]_o}{[S]_o + K_M}$$

See, if we substitute, then your rate expression will be **will be** basically you know, your rate expression. Let us write down the rate expression that is, rate of formation of product.

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The image shows a handwritten derivation of the Michaelis-Menten equation. It starts with the rate law $v_p = k_2 [ES]$ and substitutes the expression for $[ES]$ from the previous slide. The derivation is as follows:

$$v_p = k_2 [ES]$$

$$= k_2 \left\{ \frac{k_1 [E]_o [S]_o}{k_{-1} + k_2 + k_1 [S]_o} \right\}$$

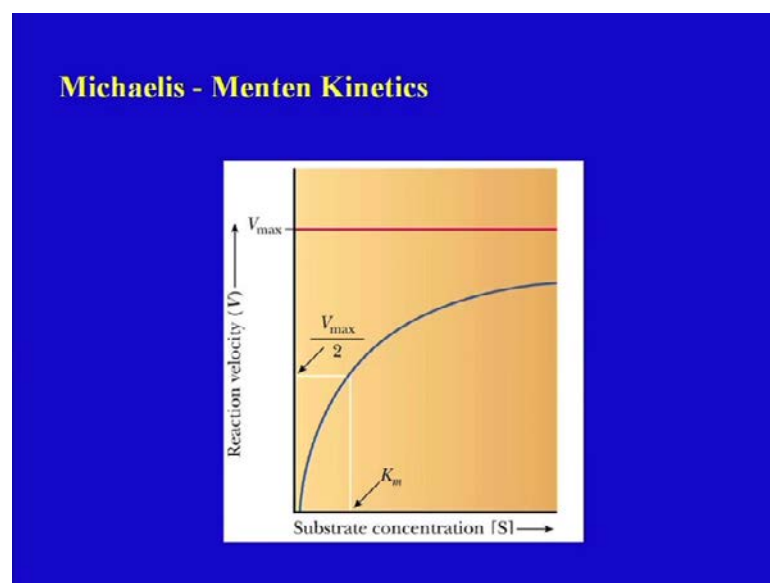
$$= \frac{k_2 [E]_o [S]_o}{\left(\frac{k_{-1} + k_2}{k_1} \right) + [S]_o} = \frac{k_2 [E]_o [S]_o}{K_M + [S]_o}$$

Below the equations is a graph of reaction velocity v_p versus substrate concentration $[S]$. The curve is a rectangular hyperbola that approaches a horizontal asymptote at v_{max} . A vertical line is drawn from the point where the velocity is $v_{max}/2$ to the x-axis, indicating that at half-maximal velocity, the substrate concentration is equal to the Michaelis constant K_M .

So, v_p velocity of product formation is k_2 . **so** That means, your $k_2 k_1$ divided by $k_{-1} + k_2 + k_1$. So, this is ES , therefore, this is k_2 divided by k_1 . **so** Therefore, it will be k_2 , if you divide this. **means** I mean, in the numerator if you divide. Numerator is divided by k_1 and also denominator is divided by k_1 . So, it will be $k_2 E_0 S_0$ divided

by your $k_{-1} + k_2$ divided by $k_1 + S_0$. So, let us call **so** this is, basically you know, combination of three rate constant terms. So, this is for the forward and this, these two are for the equilibrium state. That is, **that is** equilibrium state of formation of **of** you know, equilibrium states of formation of k , you know, this ES formation and then dissociation of ES to E plus S. So, therefore, let us write this as a new. Giving a new name, $K_M + S_0$ which is, called the Michaelis constant Michaelis-Menten constant with $k_2 E_0 S_0$. So, this is the **this is the** rate, I mean, velocity of product formation velocity of reaction. You can write in this way.

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So, if you plot the reaction velocity as a function of substrate concentration, then this will be the graph. **will be** Like you see, that it will show, like this. Initially, it will rise and then ultimately it will be close to saturation. So, it will go to this direction. I mean, it is not shown this portion it is still going up and saturates at some other point. So, that portion is not shown totally. So, only little portion is shown. So, that is why V_{max} . So, V_{max} is, **this** where the maximum velocity of this plot. So, this is your V_{max} . So, it is like a non-linear, I mean, looks like you know, parabola, non-linear one. So, parabola like dependence not exactly you know. Parabola like dependence means, it is not very clear from here. Anyways, but, it **it** is like this. So, it is V_{max} and this is your, may be V_{max} by 2 **by 2** and this is your substrate concentration.

Now, what is this? Why I have written V_{max} by 2? So, **so** this is your v_p , this is your V_p , V_p is equal to $k_2 [E]_0$ divided by K_M plus S_0 . Now, if your substrate concentration is **is is** very high **if your substrate concentration is very high** like this, then what is going to happen, that you can neglect this K_M compared to this.

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The Maximum Velocity

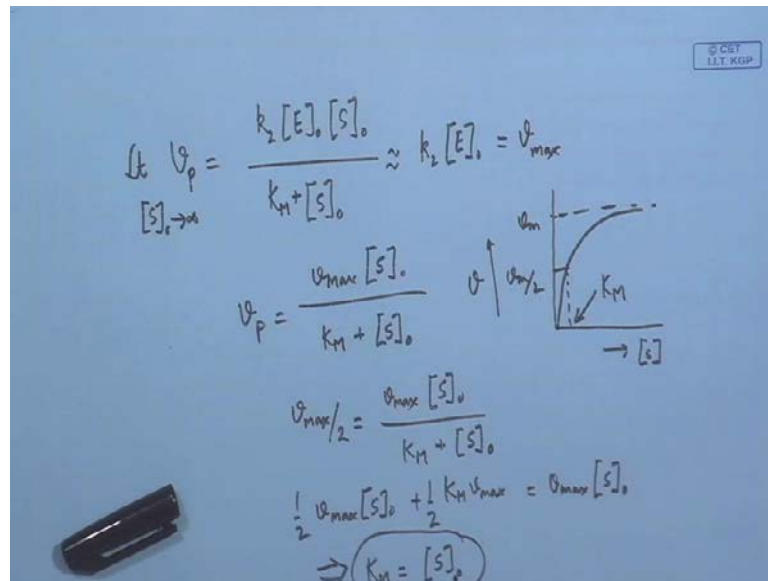
- As $[S]_0$ gets very large.

$$\lim_{[S]_0 \rightarrow \infty} v_p = k_2 [E]_0 = v_{max}$$

Note – v_{max} is the maximum velocity for the reaction. The limiting value of the reaction rate high initial substrate concentrations

Therefore, this equation reduces to v_p when, substrate concentration limit, substrate concentration S_0 is very high. Then, this equation becomes $k_2 [E]_0$. So, that means, you can write in place of $k_2 [E]_0$ for your Michaelis-Menten equation, like, your V_p is equal to your $k_2 [E]_0 S_0$ divided by **divided by** K_M plus your S_0 .

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So, if this is neglected, then, these two will **will** just cross out. So, this will be you know, k_2 into E_0 . In that case, this V_p , we will call as V_{max} . So, when limit **limit** concentration of this is infinity, so, that is equal to given name V_{max} . So, if you write this V_p is equal to your, this one, $V_{max} S_0$ divided by K_M plus S_0 . So, in terms of V_{max} , it is like this. Therefore, **therefore**, why a half height has been taken? Like this?

So, this is your half height, v_m by two v_m . This is V_{max} , this is your velocity, this is your substrate concentration, **so** this axis. So, if we put V equal to V_{max} by 2 then, what happens let us see. So, V_{max} by 2 is equal to $V_{max} S_0$ divided by K_M plus S_0 . So, you just transpose it. That means, $V_{max} S_0$ half plus $V_{max} K_M$ V_{max} into half is equal to $V_{max} S$ not. So, that means, if you **if you** just cross out V_{max} from **from** both the sides, you will be getting **your you will be getting** K_M is equal to. **K M is equal to you know** You see that, if you remove it, then this gives you S_0 because, if you transpose it here, **it it here** therefore, it becomes half $V_{max} S_0$. So, V_{max} **V max** gets canceled. **so** And, this happens when? With this condition that, when **when** means, your **your** v is V_{max} by 2 then corresponding substrate concentration is nothing, but, **is nothing, but,** your **your** K_M . So, K_M is nothing, but, substrate concentration at half maximal velocity. That is why, this v_m by two, I have chosen. So, it is S . I mean this, you can write as K_M , so, substrate concentration at half maximal speed.

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The Michaelis Constant and the Turnover Number

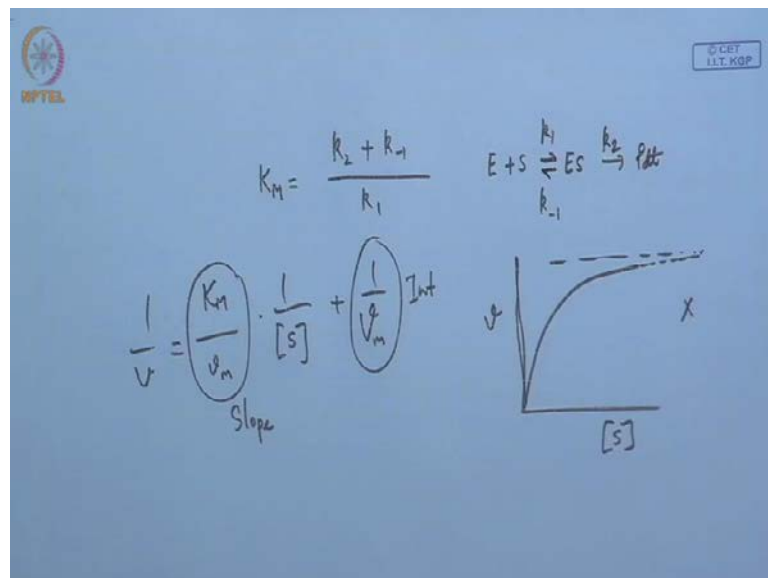
- The Michaelis Constant is defined as

$$K_M = \frac{k_2 + k_{-1}}{k_1}$$

- The rate constant for product formation, k_2 , is the turnover number for the catalyst
- Ratio of k_2 / K_M – indication of catalytic efficiency

So, the Michaelis constant, what is this Michaelis constant? Michaelis constant is defined as, like, what is that? Let us see. K_M is equal to k_2 plus k_{-1} by k_1 .

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So, what is this? This is, you know, you again write enzyme plus substrate, enzyme substrate. Then, product k_1 k_{-1} k_2 , so, this is basically you know, in the numerator. So, high means when it is, this is high. High means, it may be that **it may be that** this is very high **this is very high** or **or** may be you know, this could be very high or in other words this **may** may be **maybe** slow. I mean this may be low. So, that this K_M

becomes high. So, in that way, **means** relative weightage of which step is, you know is more **more** pronounced or which step is important? I mean, whether this k_1 is more, k_{-1} is more or k_2 is more?

So, their relative you know, magnitudes will determine whether you know, Michaelis constant will be **will be** high or low. Now, the rate constant for the product formation k_2 is called the turnover number **for** the catalyst. And ratio of k_2 by K_M is called the, you know, catalytic efficiency. So, these are the, **these are** you know, defined terms. Now, you can come back to this slide, that when **means** V_{max} slide, that V_{max} is the maximum velocity for the reaction and limiting value of the reaction rate at high, initial substrate concentration is called the V_{max} .

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Lineweaver-Burk Equation

- Plot the inverse of the reaction *rate* vs. the inverse of the initial substrate concentration

$$\frac{1}{v_o} = \frac{1}{v_{max}} + \frac{K_M}{v_{max}} \frac{1}{[S]_o}$$

Now, again, let us do the plot **plot** of the inverse of the reaction rate versus inverse of the initial substrate concentration. If you **if you** do the double reciprocal, that is called the double reciprocal, then this **this** graph you know, linearises. So, that means, here you have got you know, **you have got** this rate means you know, inverse of rate versus inverse of the initial substrate concentration will give you these numbers K_M by V_{max} and in intercept will give you 1 by V_{max} .

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Lineweaver – Burk Double Reciprocal Plots

- It is difficult to determine V_{max} experimentally
- The equation for a hyperbola can be transformed into the equation for a straight line by taking the reciprocal of each side
- The formula for a straight line is $y = mx + b$

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$
$$y = m \cdot x + b$$

- A plot of $1/v$ versus $1/[S]$ will give a straight line with slope of K_M/v_{max} and y intercept of $1/v_{max}$
- Such a plot is known as a Lineweaver-Burk double reciprocal plot

So, you know, this equation, as I told you that, it is a hyperbolic equation. That is, I told you that, it is non-linear, different from parabola of course. And, it is a hyperbolic equation. So, it is **a it is a so** therefore, difficult to determine V_{max} experimentally. Experimentally means, just finding out, you know a velocity of reaction and it is difficult you know, since **where is this you know, since** you know this.

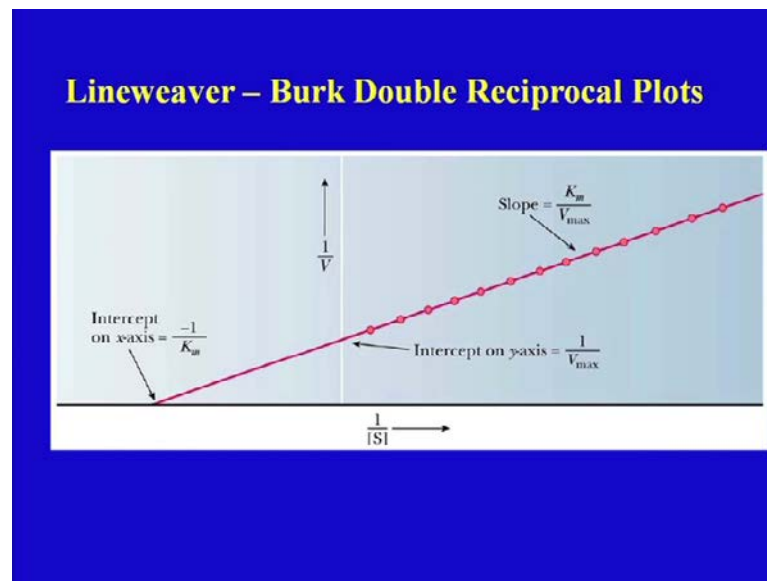
One, if you look into this graph, then it is some you know, asymptotic going and going, rising and rising. So, **so** experimentally where **to** you know, from where you have to draw this line, it is difficult. So, that is why to get you know, more information **to get more information** it is always better to express this complicated equation in a **in a** linearized fashion. That is why you know, this double reciprocal plot. That is, inverse of reaction rate versus inverse of initial substrate concentration that is plotted. And you see, if you do this, two inversion plot then, **two inversion plot** then you see that, it **it** is just like a linear equation. That your y will be $1/v$ and x will be $1/S$ **not so**, it is easy to handle rather than you know, doing it in a non-linear fashion.

So, **your** this is the velocity and this is your substrate concentration. So, if this is not a good choice, therefore, we do not like this, we do not use it in general. So, here you have to use you know, this Lineweaver-Burk plot. There are other plots also, but, this is the, you know, celebrated expression. So, **the** it is difficult to determine V_{max} experimentally and the equation for a hyperbolic can be transformed into equation for

straight line by taking the reciprocal of each side. That is why, you have done this double reciprocal plot and the formula for this straight line you know, it is y is equal to $m x$ plus b . So, b is the intercept and m is the slope. So, just comparing these two, 1 by V is equal to K_M by V_{max} into 1 by S plus 1 by V_{max} like this.

So, **so** slope will be K_M by V_{max} and intercept will be 1 by V_{max} . So, intercept will, inverse of intercept will directly give you **directly give you** the V_{max} . So, it is easier to **to** deal with. So, that is 1 by V is equal to K_M by V_{max} into 1 by S not or S initial-substrate concentration plus 1 by V_{max} . So, this is your intercept, this is your slope. So, this will give you, **this will give you** you know, easily. Means, easily **easily** you can, you know, deal with the data rather than dealing with non-linear fashion. It is in a linear fashion you can **you can** manage the data easily. So, this plot is known as the Lineweaver-Burk plot or double reciprocal plot. So, let us go. How does it look? This graph how does that look?

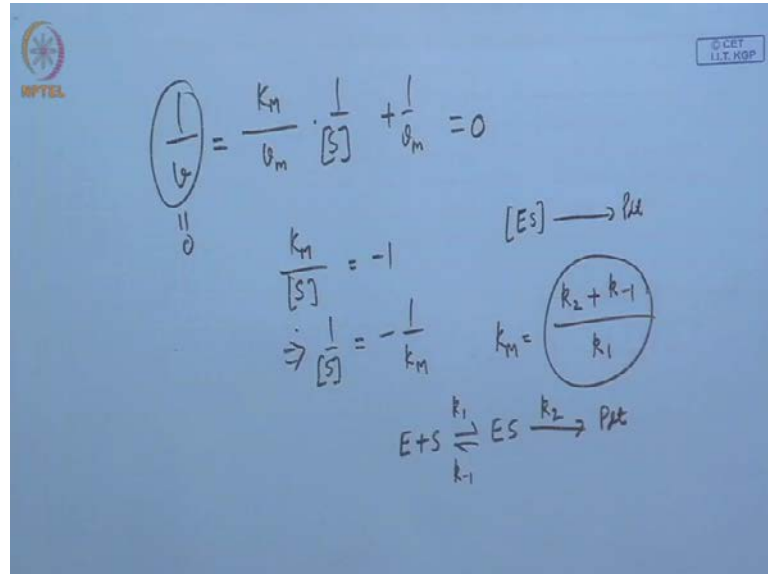
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So, you see that, this is your, this is the plot. So, these are the experimental data because, you **you** may not need to **you may not need to** go to this concentration. May be, you do this concentration slope is K_M by V_{max} . Intercept is this. Intercept will be giving you 1 by V_{max} . Now, if you keep on, you know, **you know** going to the other side of 1 by S then, what would you get? Let us go back to the earlier slide that, you know, this just put

your y value to 0. So, if this is 0 if this is 0 then what happens? Let us let us try that. So, we have got $1/V$ is equal to K_M/V_{max} by $1/S$ plus $1/V_{max}$.

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So, now, put this to be equal to 0. Why this to be equal to 0? Because, we want to extrapolate further. So, that this line touches your your x axis and under that condition y value is 0. So, that is why, we are putting this to be equal to 0. So, that means, this is equal to 0, means you put this equal to 0 under that condition. So, so your. So, what do you get? K_M by S is equal to minus 1. So, so what is happening that, your this this is basically, you know, from this. You write 1 by S at that condition, so, 1 by S S , when it is touching the x axis. That is your minus 1 by K_M . So, you can directly find out K_M .

So, it is it is a nice way. It is a is a it is a real nice way of finding out the, you know, individual things like $1/V_{max}$ and I mean, V_{max} and K_M . And also, slope will directly give you, although slope will be giving K_M by V_{max} , but, even if you do not want to find out the slope, then, just keep on extrapolating until, the line reaches your x axis. That is 1 by x axis, that is inverse of your your substrate axis, inverse of substrate concentration axis. So, it is a it is a nice way, it is a really nice way of dealing with enzyme enzyme substrate reaction you know, kinetic data. so And, you just you you have to remember that V_{max} is nothing, but, the maximum velocity that is, this one. When substrate concentration is very high, then your expression is simplified and the corresponding corresponding velocity is your V_{max} . Because the reaction, you see the

step where you know, in which this product is formed, that is your ES to product. So, more the substrate, **more the substrate** more of you know, substrate will be, **will be** you know associated to with enzyme to produce ES. And then, **the** more the concentration of ES, **the** more is the possibility for the product formation. So, that is why you know, this is your V max and KM is your this 1. KM is $k_2 + k_{-1}$ by k_1 . So, this combined k means combined constant.

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Significance of K_M

- K_M is a constant
- Small K_M means tight binding; high K_M means weak binding

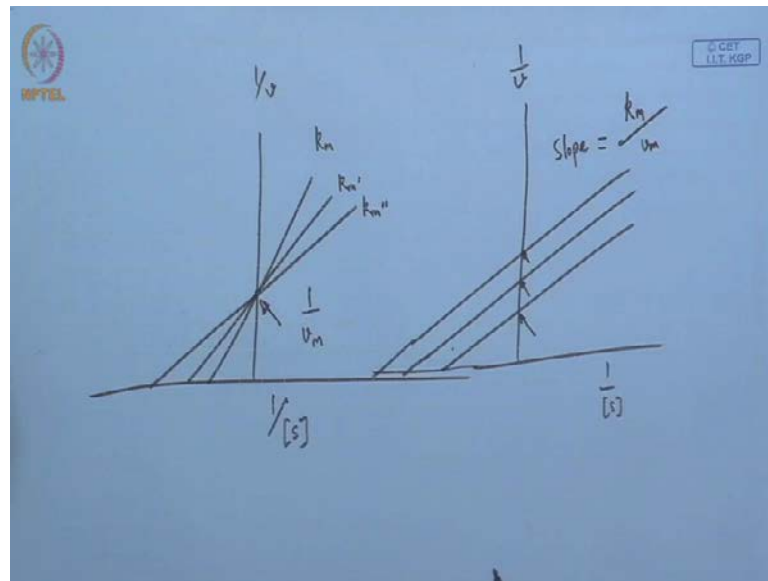
So, **so** what is the meaning of small k? Means, small value of k and what is the meaning of large value of k? That means, **that means** if you go back to this plot, how this will you know, this will be affected, this plot will be affected? So, 1 by K_M intercept. So, say 1 by K_M means **1 by K_M means** large value of K_M means it will be **it will be** somewhere you know, somewhere over **over** you know, large value. Means, this will be minus 1 by large. **large** Means, it is a smaller, you know, here it **it** this will be, since it is minus 1 by large means, small **small**. Means you know, with a negative sign means it will be this way, like 1 by 2 and 1 by 3 **1 by 3** will be here. 1 by 2 say may be here, 1 by 3 will be somewhere. It is less, still less with **with** minus sign. So, you know large K_M . So, this graph large or small, k_m will shift to this point. May be this way or that way and if K_M is high again, **is** K_M high means your slope will be high. Slope will be high means; this graph will be like this. So, graph will be like this **this** way may be. So, K_M high means, your **your** this value will be high. This value will be high means, you know, it will be coming close to this **this** origin. **So, and.** So, let us again go back to next slide that.

So, what is the significance of small K_M ? There must be significance. So, what is the significance of small K_M ? Now, you just again recall this expression that, K_M is equal to $k_2 + k_{-1}$ by k_1 . So, that means, this step enzyme plus substrate giving rise to enzyme substrate, giving rise to product k_1 k_{-1} k_2 . So, small K_M means small value of k_{-1} . What is the meaning small value of k_{-1} ? Means you know, this is k_1 . k_1 means, this step this is large. This is large means, this step is pronounced. So, back reaction is possibly less and high K_M . So, small K_M means this is high and high K_M , means, this is small or this is high.

In other case, you know small K_M means this is high or this is small. This is small means your possibly this step is also may be less. That is why, this is small and also back reaction is less. So, you can think of the magnitude in terms of numerator and denominator. So, when denominator is high means, this step is stronger. I mean, this is fast. I mean it is a strong bond formation. And, in the same way, if this is high or may be if this is low means, this maybe low if this is low means this reaction is less. So, tight binding condition and therefore, when it is tight bound, slowly it will dissociate to give you the required product. So, that is why it is you know, it is a tight binding situation and when K_M is weak, means K_M is, you know, K_M is high means, weak binding. Why K_M is high? Means this is high, this is low, this is low means this step is low. So, this is high means, this step is high and that step maybe high. So, if that happens; that means, easily it will dissociate to give you $E + S$ or maybe it will eventually means very fast go to the product side. So, in this way, you can think of the significance of K_M .

So, that means, small K_M means Michaelis constant is small. That means, it is a tight binding, Michaelis constant high, weak binding. So, you can also rationalize in terms of, this you know, this in terms of this, Lineweaver-Burk double reciprocal plot. You can just think of when what happens to this graph? I mean plot, when K_M is high. K_M is high means, slope will be steeper and the you know, this minus that is you know, this cutting point on x-axis or the $1/S$ axis will be here. So, this is one point and also suppose, we have got same you know, K_M means like suppose the situation is like this, what is the reason for this? Let us have this plot.

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So, we have got, like this plot. Maybe, another plot maybe another plot like this. So, this is your intercept intercept. Means, $1/v_{max}$ and you see intercept is same, but, K_M is you know, same maximal velocity, but, with different K_M . So, this is you know, K_M is more, this is less K_M . So, K_M , this K_M , this is another K_M prime. This another K_M . K_M double primed. So, changing the value of K_M will just you know, and if this is remaining same, we will be seeing that, it **it** will pass through the same you know, v_{max} point, but, you know, this is coming close to each other. Now, in other situation what happens? Say, you have got like different graphs like this. This is your $1/v$ and this is your $1/S$, $1/v$ and $1/S$; that means, your K_M/v_{max} is same. But, your intercept is changing here less. This is little more, this is more. And so, as a result of which your this **this** side is going you know, farther. So, this is because of, you know, because of the slope is same.

But, the relative, you know **you know** weightage of, I mean, relative magnitude of K_M and v_{max} will determine means, where this you know, this one I mean, v_{max} is changing. Although, maybe K_M is remaining same, I mean slope is remaining same, K_M/v_{max} maybe you know, v_{max} . So, it is slope. Slope is same. Slope is remaining same means maybe, this ratio **this ratio** is changing in such way that, slope is same. So, that means, both are maybe changing. One is increasing, means this is also increasing. This is also increasing means, you know, this way **this way** it is going or maybe that way it is going. So, you can check it directly from this **this** expression. From

this expression that slope is, K_M by V_{max} and intercept is $1/V_{max}$. So, it is **it is** very difficult to conclude. Means you know, **I mean** very easy to conclude the fate of this K_M and V_{max} when such graphs are obtained **such plots are obtained**. So, these are **these are** a bit detailed things with respect to, you know, Lineweaver-Burk double reciprocal plot.

So, today what we learnt is; your enzyme kinetic, kinetics and it is a, means it was **used to be** thought to be like the **mechanism is a** lock and key mechanism. But, the popular thing is that, now a days it is **it is** no longer used. This lock and key idea now, it is thought that there is an enzyme substrate complexation and that complex is then responsible for giving rise to, **giving** giving the product. And you know, this **this** K_M is nothing, but, your, we have just shown that K_M is nothing, but, the substrate concentration at half maximal velocity. And, this plot is this V , versus that is, velocity of reaction versus substrate concentration, is a hyperbolic plot. So, this hyperbolic plot is **difficult to** sometime difficult to deal with. So, therefore, we have to **have to** have a double reciprocal modification of this. So, when you do a double reciprocal plot, then this **this** becomes a linear plot and from that, we can find out from slope intercept and you know x , x cutting point. We can find out the value of you know, K_M and V_{max} . So, that is all about **that is all about** this enzyme catalyzed reaction.

So, in our next lecture we will **we will** deal with you know, again some specific reaction and then we will switch onto normal catalyzed reactions in a bit detail. So, till then have nice time. Thank you.