Biochemical Engineering

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Module No. # 01

Lecture No. # 09

Enzyme Kinetics: Michaelis-Menten Kinetics

Welcome to the second lecture in the series of biochemical engineering. We will start off today from where we left and as a title of the lecture suggests, this is going to be concentrated to a kinetics of enzyme enzymatic reactions.

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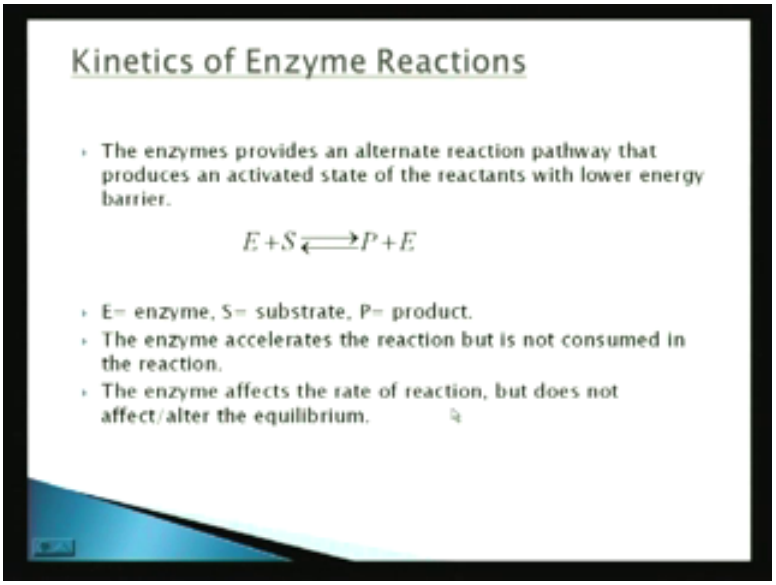

So, we will just quick wrap up from where we left. In the last class so the enzyme essentially as I said is catalyst which does not participate in the reaction but, accelerates the reaction by reducing the threshold activation energy.

So, if we are to denote the reaction of S being substrate going to P by reversible reaction, S reversible going to P; then in the presence of the enzyme it turns out that E plus S goes

to P plus E where E is enzyme that reacts to the substrate to generate the product P and the enzyme is regenerated back.

So, this is the basics basic of this basics of the kinetics. But, now what we are going to do it in today's classes; there will be little deeper in to the kinetics and try and understand how this really happens. How the enzyme catalyzes or accelerates the reaction and things like that?

And as I said, the the two point here to note as the the enzyme is not consumed in the reaction a very important point note to actually, it only accelerates the reaction and it does not alter the final equilibrium state. So, both of these are important points which will come into play while we do our calculation. Today, I would like you do have your exercise books and pens ready because we are going to do some small calculation as we go along.

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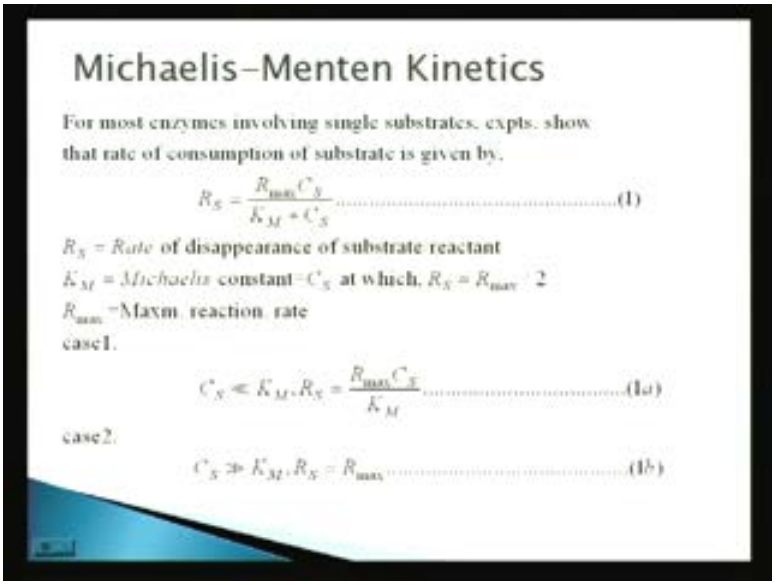

So, just quick wrap up you know, from yesterday the RS, the reaction rate for the Michaelis-Menten kinetics which is most popular kinetics use in my all of biochemical engineering and much of this course that we are going to do is, going to be concentrated to towards Michaelis-Menten kinetics is given by the reaction rate is given by R max which is the constant and the maximum reaction rate possible time C S divided over K M plus C S. Now, a C S being the concentration of the substrate, K M is called the Michaelis constant and it is its value is evaluated by finding out what is the R? What is

the C S at which R S goes to half of R max? right So, let us lets lets go little beyond this and let us try and understand how this reaction kinetics comes out of the reaction between the enzyme and the substrate right. So, this we cannot except this reaction as it has been given us.

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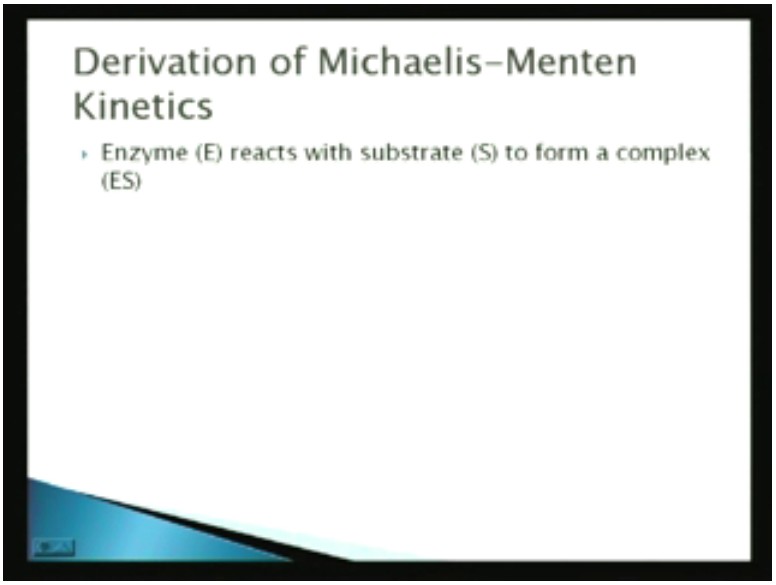

So, what we will today is derivation of the Michaelis-Menten kinetics and I would like you to participate in that as I said by you know going through the derivations with me while we go through this lecture. So, the first in so if I have to thing I am to ask you to split up the last last reaction that we have which is E plus S go going to P plus E intuitively

So, there will be lets us think of it is a two step process. So, the first step in this process is going to be the enzyme E reacts with substrate S to form a complex E S. So, I want you to write down in your copies very quickly what that reaction is going to be. It is a very straightforward thing just as the enzyme E reacts with the substrate from the complex E S.

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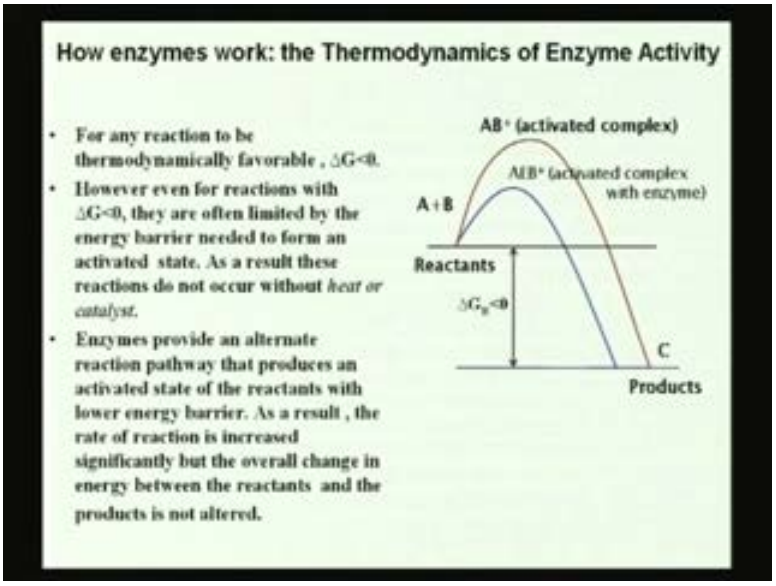

Because if you remember when we did the thermodynamics of it, here in the presence of the enzyme, a complex is formed which is a E B plus. So, here we not dealing with two substrates this diagram is for two substrate A plus B. But, now we are dealing with only single substrate. There is a result which what will happen in S is going react with the enzyme to form a complex A C right which will then go to the product. This this has to be clear. So, this is the mechanism of the Michaelis-Menten kinetics right. That you first form a complex the enzyme, first reacts with the substrate to form a complex and then from that complex you form get to the product right.

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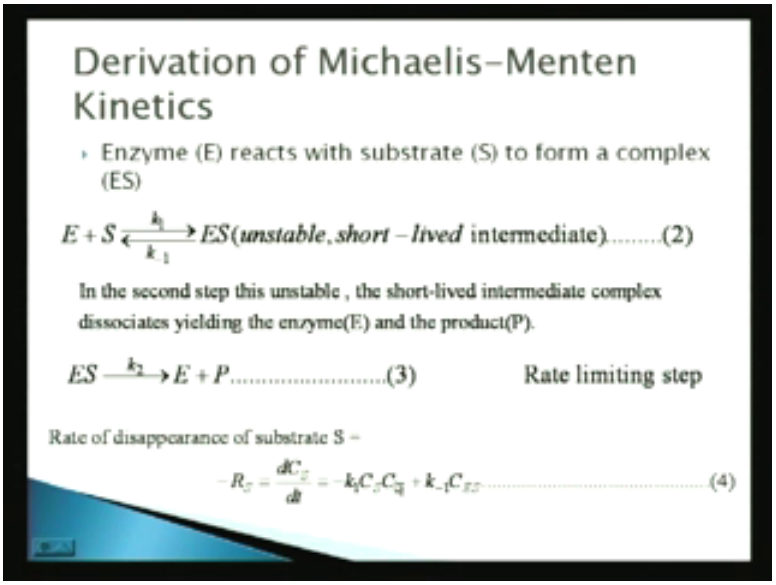

So, now let us get your pen down to the paper and turn and write this reaction. Done? So, this is the reaction as it turns out. So, which is that? E is the enzyme reacts with the substrate reversibly excuse me reversibly to form the complex E S and the forward rate constant here I put as k 1 and the backward rate constant I put as k minus 1. Important to note it what is given in parenthesis is that this complex E S that is formed is a unstable complex that is short-lived. It does not live for very longtime. It degenerates or degrades or you know change it over to something else and so the next step and this is the intermediate species right. So, the next step is this. In this second step, this unstable complex that is E S it is which is short-lived complex dissociates to form the enzyme and the product so write that reaction down. Fine. So, what is it Sahil?

E S gets P plus C (( ))

What kind reversible or?

Reversible

Huh

Reversible

So, all of you wrote reversible? Actually, it is irreversible. As I said that in the class that all your reactions are you know typically reversible but, in this reaction you have to have it irreversible otherwise not enough product are going to be formed. And if you look at the overall reaction, the overall reaction is actually reversible.

So, it is obvious that this reaction also has to be reversible. But, we take it as a irreversible reaction because the backward reaction is very slow typically and this it turns out this is rate limiting step. right I hope all of you are are aware of the concept of rate limiting step, which is that if you have multiple reactions is the rate network of reaction then the rate of final product formation is governed or dictated by the rate of the slow step right.

So, I give this example in class you know that for example, if you walking somewhere with your little sister and she walks much slower than you then the speed at which you can walk is governed or dictated at the speed at which she can walk can and not so speed at which you can walk. So, you know this kind of examples will probably impend this

concept of rate limiting step and this is very important concept in all of chemical engineering.

So, whenever you have a network of reactions you have to go down and figure out which is the, what is the rate limiting step. And in this case this is the rate limiting step. Why are we so worried about what is the rate limiting step? Because the rate of formation of product is going to be dependent on the rate limiting step so, this is the rate of disappearance of the substrate.

So, if you look at reaction two over here, the substrate is a P S in reaction two from the reversible reaction. right Is that clear? So, these are the two terms that come in from equation two. Look at equation. If there is forward and backward reaction and all are written in equation four is simply the rate of so the disappearance of the product d C S d t S, the rate of disappearance due to the forward reaction minus the the rate of disappearance due to the forward reaction plus rate of formation due to the backward reaction. Fine, now can you write what would be the rate of product formation? Product is here and product is formed from equation three. right This should be very straightforward. What is that?

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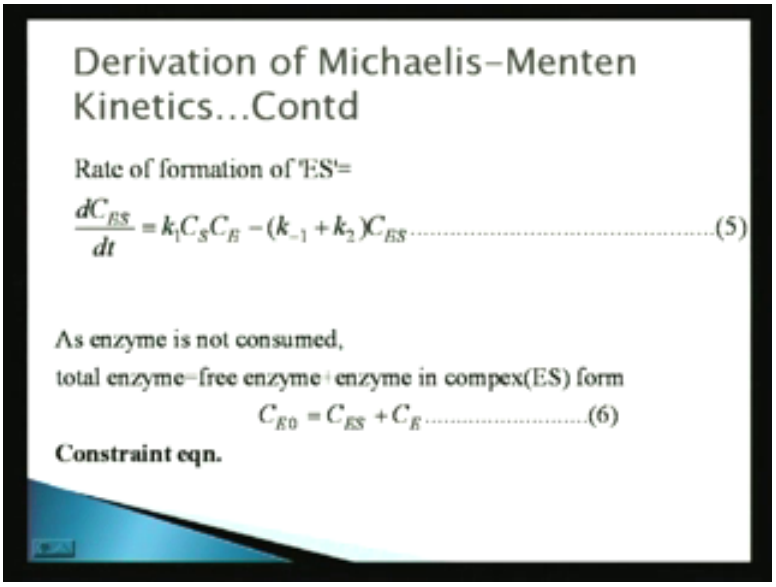

(( )) constant (( ))

YeahWe will come to that little bit. So, what I now want you to look at is this is the intermediate species right which is unstable and short-lived. What I now want you to look at is what is balance for this intermediate species, that we wrote a balance for the for the substrate. We can write balance for the product which you already have written. What is a balance for the intermediate species?

And I want you to write that down is very straightforward because intermediate species is part of two reactions; one of them reversible one of them irreversible. So, there will be just an additional term there. So, just use equation two and three to write what is your d d C E S d t. Fine? So, this is I hope what you got right. One for the forward reaction, one for the backward reaction of equation two and one for the reversible reaction of equation three.

So, I want to go back a little to this now one the important things in all of this biochemistry and biochemical engineering is a fact that there are some constrains on the system. What are these constraints? It is the mass balance constraint or in other words when you talking of an enzyme, these enzymes as you know participate in the reaction do do not participate in the overall reaction, participate in the intermediate steps of the reaction but, not in the overall reaction.

So, at the end of the day the enzyme, the amount of enzyme remain unaltered and this is a very important thing physiologically. Why is that? Because these enzymes are typically available in trace quantities in the human body so you cannot afford to waste them. So, one of this this physical constraint boiled down or you know give rise. What is a mathematical constraint on the amount of enzyme that is present in the system and if you look at this and we done this before I know if you look at this what is the amount of enzyme that is present in the system? What are the different forms in which the enzyme is present?

(( ))

Absolutely So the free free enzyme and in the enzyme in the complex form. So, if you look at this, so C look at equation six. C E O or C E not, is a total amount of enzyme present before the reaction starts. That equals the amount of free enzyme and the enzyme in complex form form I am sorry in here I wrote it in the other way.

So I should have written C not equal C E plus C A C S right because here free enzymes come first. So, the free enzyme plus so which is C E and the enzyme in the complex form which is C E S right. So this is this is this my equation. I want you to make a note of that. This is known as the constraint equation.

So, whenever this is very simple system that we are trying to derive and we will try and derive much more complex systems later on in this course and in the you know and in the assignments and test probably give you lot more complicated system to solve. One of the things that is important and to make a note and always remember and and put it in to practice this is the fact that you have a constraint equations so these systems are all biological systems are governed or dictated by certain constraints and to figure out what that constraint constraint is. So, this is known as a constraint equation.

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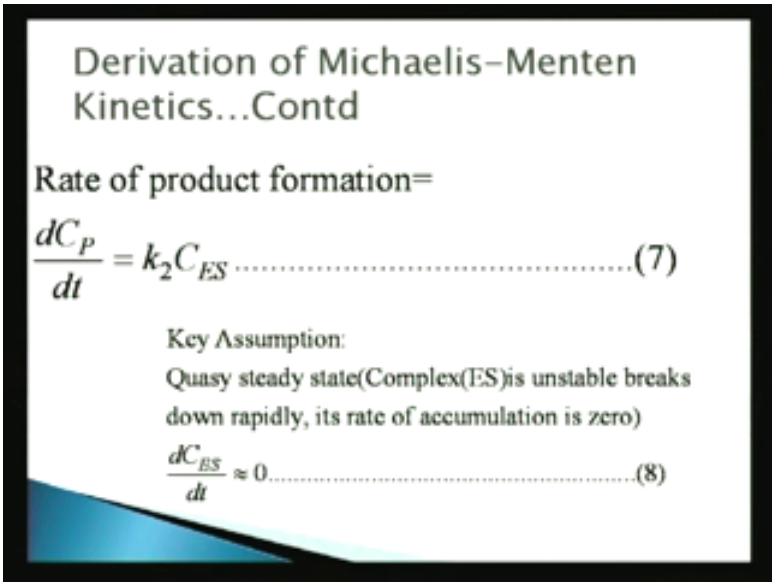

So, the rate of product formation is something that you have already written. Now, I give you few minutes, couple of minutes. What to do is that this rate of product formation is written in terms of C E S the complex? Write the concentration of the complex. What is the problem with the concentration of the complex? It is an unstable compound as I said intermediate unstable compound and it is not measureable, value of that is not measurable.

So, what we have to do is we have to calculate this the value of this concentration intermediates, of the intermediate species in terms of parameters and variables that we

can measure right and then we have to put it back in to the rate of product formation to be able to get what the final rate of product formation is right. So, this is what I want you to do quickly in the next couple of minutes is calculate what is C E S? So, to help you I will just give you some hint. So, what do you think Pallavi, would be would be the first thing that you need to do here.

We will be using the constraint equation

Constraint comes later before that what? What is the

(( ))

Yeah

(( )) intermediate is shortly so

So yeah right

So that d C S by d t equation if we have

Is going to be

(( )) zero

That is going to be 0. So, this is very good. So, that is the key assumption that we have to use. So, look at equation number eight I put up there. So, the quasi state, this is known quasi-steady state assumption. There is a complex is very short-lived, it breaks down very quickly and as a result its rate of accumulation is 0. So, d d t of C E S is 0 so what you need to do is look at equation five, where you where you give the rate of formation of this d d t of C E S right put that equation to 0.

So, that is what you need to do and then use the constraint. So, couple these two to now write C E S. Express C S is very straightforward. Express C E S in terms of the other variables. So, from equation five you will get C E S equals k 1 over k minus 1 plus k 2 C S C E right right.

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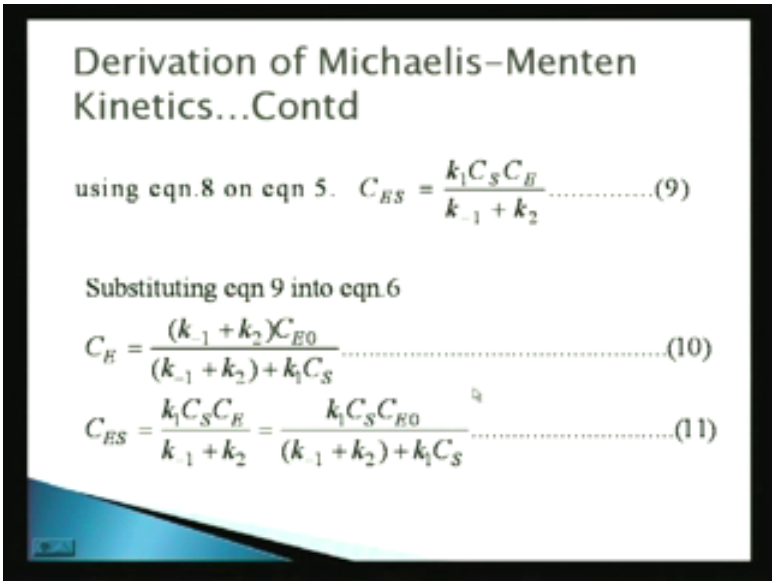

So, I have it on this screen now that the d d t once you put the d d t of C E S equal 0. You get C S equals k 1 C S so time C E over k minus 1 plus k 2. Clear Liza? Okay. Now, what you need to do is express use equation nine which is equation I got here on the constraint equation over here because you have now got C E S. So, what you need to do is substitute C S over there. You see Arush. See equation nine. You got over here substituted back to equation six. And then you can express your C E as a function of C E naught and C S. Why are you trying to do this Krishnapra? Why are we trying to express C E as function of C E naught and C S?

(( )) C S (( ))

I know why why

(( )) we have to find the rate of reaction

I know but, why not keep C E there?

C E is not (( )) we do not know C E

C E as an evolving quantity. We are not aware of what C E is. We are aware of C S because C S is something can measure at C C S is also dynamic quantity remember but, C S is something we can measure at any point of time. C E naught is known quantity because this is something that we started with. So, we want to express everything all our

parameters and values in terms of C E naught and C S. You got it? What you got? You got the same as what I have on the board? Equation ten and then C E S now is given by so, once you got C E as a function of C E naught and C S. Then you can simply replace that in the C E S equation and you get slightly more complicated relation but, you get C E S as k 1 C S C E naught k minus 1 plus k 2 plus k 1 C S. Fine? What shall we do now? What is the next step?

C S (( )) rate of reactions

Which one? Which reaction?

This (( )) goes to k 2 (( ))

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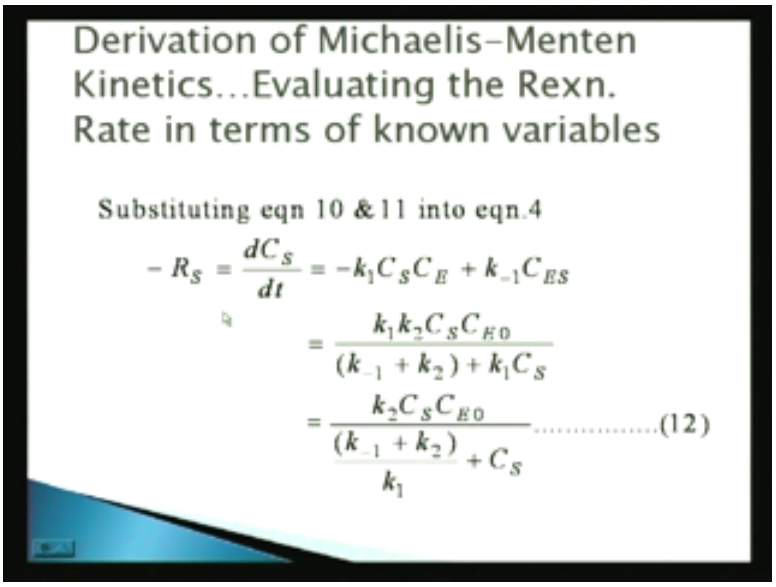

Very good. yeah So what we need to do now is, figure out the what is the rate of product formation? The rate of you know reactions so to say. So, the rate of substrate, this is the rate of substrate, the rate at which that the substrate is taken up and actually we will see that this is this is equal to the rate of product formation. Why is that? Which one?

(( ))

Yeah I know. That is not the final answer. That is an easy answer but, I am evaluating. Question is I am evaluating the rate of substrate production formation right but, what do I find? Actually want to evaluate. I want to evaluate the rate of product formation but, as

I said the rate of substrate that is taken up consumption equals to the rate of product formation as intuitively it is very clear right because S going to P.

So how much equimolar things so if x amount of S is taken up x amount of P is going to form intuitively it makes complete sense. But, what I am trying to ask you is mathematical can you prove that to me? This is there right in front of your eyes. You just have to tell me which equation to use.

Its overall reaction

No what is that which equation to use to get that?

(( )) d plus

No (( )) straightforward equation five and equation eight. Equation five is this. The d C d t of E S equal that that you put equal to 0 then, you will find that the rate of product formation equals to the rate of substrate consumption right. So, essentially what I am trying to say here is that in this in this slide is that, if you look at R S R S is d C S d t which equals minus of d C P d t. Fine? So, I want to go through this, want you to go through this calculation quickly. You can do it this way or there is another easy way is k C C d d t of C P is equals k 2 times k 2 times C S right.

So, you simply need to multiply this by k 2. Clear pallavi? Everybody liza? (( )) This simply multiply this by k 2 then you get the d del del t of C P equals this fine and this is what we get here. k 2 simply multiply this this same thing multiplied by k 2. That is my the rate of product formation equals to the negative of the rate of substrate consumption fine good.

So, now the question is, so this is what we got. Say this is the rate of product formation or this is the rate of substrate consumption, this is what we got. Now, my question is that, how do you recast into the Michaelis-Menten form because what we started (( )). These are two objectives we said for S that it is a Michaelis-Menten form. It has been told to us that this is Michaelis-Menten form but, we do not accept that as scientists as you know as something God given.

So, we started off with the very basic and try started to started to derive that. So, there is no assumption or in built assumption that is Michaelis-Menten form. At any point of

time after we have got this rate expression given by equation twelve what we are trying to figure out is this this Michaelis-Menten form? So, what we do to that the what we do is the Michaelis-Menten form was given to us here here equation one. And what we need to do is we need to compare this form with that form and figure out that how can be recast this form to that form so equation twelve that I got. How can I recast equation twelve in to a form that looks like the Michaelis-Menten form?

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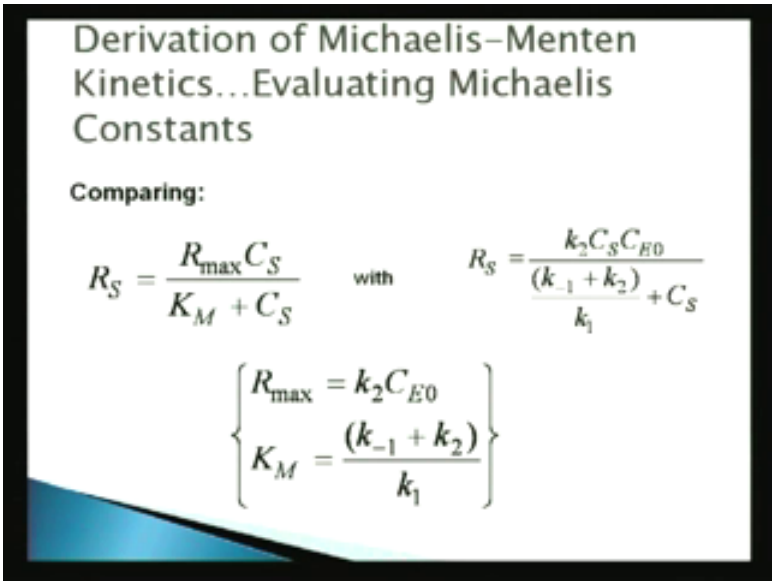

So what I need to do so this is the Michaelis-Menten form I need to compare this with what I got. So, if when we compared this it is very straightforward what turns out is, k C what turns out what turns out is R max equals k 2 C naught and K M which is known as michaelis constant is k minus 1 plus k 2 k 1 and R max is a maximum rate. Can who will tell me will you tell me Krishnaprada, why R max is intuitively why R is max is k 2 C naught?

It is maximum maximum rate (( ))

Yeah way is that

All the enzyme (( ))

Right If all the enzyme had reacted and form the product then, the C naught see if there is no free in enzyme left then, C naught is the total amount of enzyme that will be in the

complex form and C naught is the is it is a total catalyst so there will be no free enzyme left in which case my R max is the max.

So, the maximum reaction rate possible this is something that you need to understand intuitively that because you know despite all the reactions and the equations we write there might be times when I might have to just figure out in a problem that what is a the maximum reaction rate intuitively? So, what I am trying to promote is not just a mathematical way of looking at it but, sometimes intuitive way of looking at it as well. So, R max is k 2 times had the total amount of enzyme reacted so that is the maximum possibility and K M just comes out of the of the calculations right. Is it physical understanding clear to all of you?

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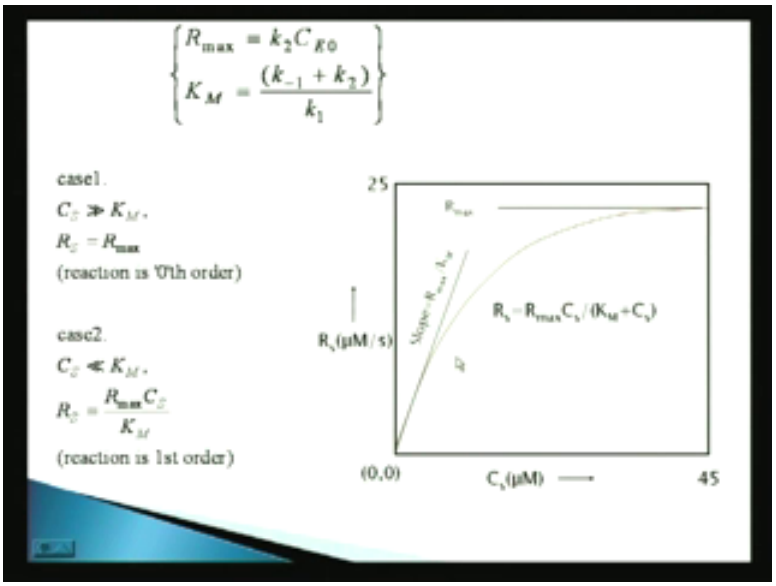

Then move on now what will do is how to evaluate these constants? So, these are these are the two are R max K M R now my two constants. What have been have I been able to achieve through this in the by recasting is in the in the Michaelis-Menten form, can you tell me that? Why did I say when I once I recast in to the Michaelis-Menten form? I had no reason actually Michaelis-Menten they right the form good for them but, I do not have to necessarily recast my equation in this that platform why would I be motivated to do that? What do I gain by doing that?

We only have two (( ))

Very good

(( )) calculated that why

Very good not variables constants

(( ))

Parameters yeah very good so, what we can manage to do or manage to achieve by recasting in the Michaelis-Menten form you see in this first form you have one, two, three four variables, four parameter; C naught, k minus 1, k 2 and k plus 1. When you recast in the Michaelis-Menten from, you end up with just two parameters. Why is that good for us? Because we have to finally, at the end of the day evaluate these parameters experimentally right. So, once we recast in the Michaelis-Menten from its much easier to evaluate two parameter experimentally. Then four and some of you if you are doing experiment you know how hard it is to you nourish, you probably know how hard it is to evaluate these kinetic constants experimentally.

So, if you reduce the number of kinetic constants as much as possible it is just make your day easier right. So, what will look at now in the next few minutes is how to evaluate these kinetic constants? So, what do you think you know, give me a sense this is your rate equation for example, the Michaelis-Menten. What do you think would be an easy way to measure these constants?

(( )) performance (( )) at a very constant at a very low concentration of C S

Hm

(( )) perform (( )) very high (( )) of C S

So we have two rate ah

You right that is the one way. yeah That is a very good way actually but, that is a clever way actually of doing it. But, one things you can do simply is measure the rate that various concentration straightforward way. This is not a first order reaction but, I will show you how to how to convert it to something similar to that. So, very straightforward way would be to just measure these rate rates and various concentrations and what happens is once you measure these rates at various concentrations you get a plot like this.

So, these numbers that you see here are actually numbers corresponding to certain experiments and R S is a rate versus C S the concentration and this is in micromolar the concentration in the x axis and the rate in the y axis in micromolar per second. So, what happens is that what this is what you said the R S is R max C S over K M plus C S. You plot the whole thing because it is not a good idea to do what you talking about is knows as asymptotic analysis. What you have on the left on the if you see on the left case one and case two these are known as asymptotic analysis. And I think I mentioned this yesterday's class the asymptotic analysis is very important in all kinds of engineering analysis. You do because you need to figure out what is the maximum, what is the minimum and then your final solution is bounded by these two right. So, if you look at the plot over here this is asymptotic solution.

So, case one is case two. Let us look at which C S is very, very small. C S is much much trace quantities of C S you get and the R S you that you get the reaction rate is R max C S over K M. I explained this yesterday but, I will I will show it one more time today. So, if C S is very, very small then this term in here is now not present. Negligible as compared to K M and you get R max C S over K M if C S is very, very large then K M is negligible over here and C S in the numerator and denominator cancel out and you get simply R R S equals R max over K M. Fine? No R x equals ah

(( ))

R max yeah right so if you plot this this is what you get in the two asymptotic cases; one is turns out to be zeroth order case, this one and one this is zeroth order case on the top and one is the first order case which is near before small concentrations of C S. Now what do you do for small concentrations you know the slope.

So, you measure the slope and the slope you know is R max over K M for large concentrations you know that, the slope is R max. R S is R max and so straight away you can evaluate R max from the from the asymptote the saturation limit it reaches excuse me and once R max is evaluated then, you can use your slope to evaluate one over K M. Fine? This looks good and easy now? I am going to quiz you little bit here. At large concentrations of C S, small and large concentration of C S intuitively, physically tell me why does the system approach first order and zeroth order? Let us start with the easy one

which is at large concentrations of C S why does the system approach zeroth order solution?

(( )) limited by concentration of (( )) concentration of enzyme (( ))

So R max essentially contains the enzyme concentration the initial concentration of the enzyme. So they the react, substrate is no longer limiting reactant. It does not matter how much substrate you are putting in because you are putting in less amount of substrate. The substrate is no longer limiting reactant. So, as a result so the rate is independent of the substrate. Is that clear? So, that is what zeroth order reaction is essential you know some these questions often asked in quizzes. That is zeroth order reaction what does zeroth order reaction essentially mean? Does it mean that the the rate does not depend on the reaction rate at all? Then it mean it will mean that you know it does not matter how much substrate I put in, the reaction rate is still the same right.

What is the zeroth order reaction mean? These are like if it my mean that if there is no dependence on concentration, if I put a large amount of substrate and small amount of substrate it makes no difference right. Is that so? In a zeroth order reaction if the rate is independent of concentration does it mean that it is immaterial how much material I put in?

(( ))

Hm

(( )) concentration (( ))

No, if I am saying the reaction order is first order itself this is zeroth order itself. Does it mean that it is independent of the amount of substrate that I put in? It means it is the independent but can I put in a very small amount of substrate?

Hm

This way

No. That is the point you know that is what I want you to realize. It is independent but, at a large asymptotic value of the substrate so what is a concept whole concept of the (( )) reaction mean that you are giving enough substrate for the system to be independent of

the concentration? There are very very hardly very very few systems in the in this in the universe in reaction engineering universe where the system is actually independent of concentration.

So, if there is zeroth order reaction mean that is independent of concentration. No, not really. It looks as if it is independent of concentration. But, it is not real independent of concentration. What it means is that, for large amount of substrate being present there the system behaves this is as if it independent of concentration. Now you decrease the substrate concentration. You will figure out that it has to be dependent and concentration and that is what happens at the at the at the small concentration. What you find it is linearly dependence on concentration. Which mean that its limited actually by the amount of substrate that you put in. So, this is the physicals. You know we it is very easy to and good to do the mathematics but, at all points of time we should have the physics of the, what really is happening at the back of our mind.

So, we will go on from here. the this is This is clear to you? So, this is how we evaluate evaluate the R max from the top asymptote and the K M K M K M over the bottom asymptote, from the bottom asymptote and figure it out. Let us see one way of throwing asymptotic analysis. Can you tell me what other ways could be there apart from asymptotic analysis if I want to use the whole whole range here. I am not using the whole range. Plot one way R process over C

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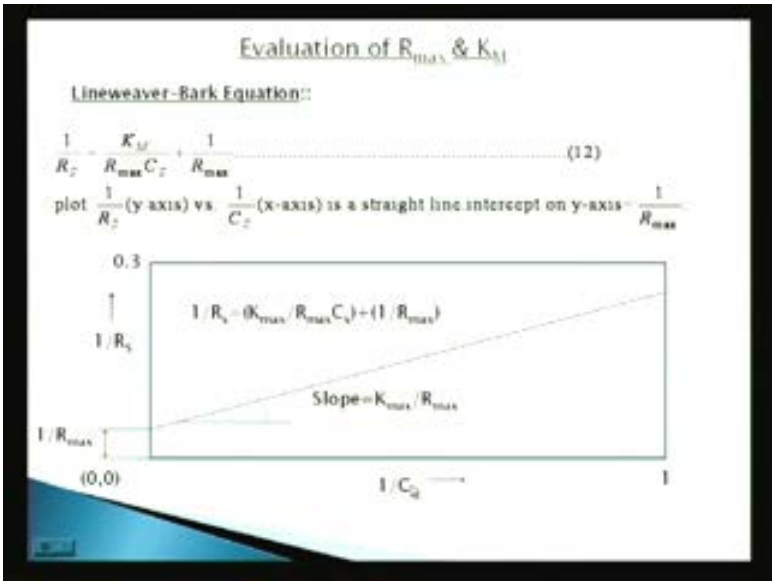

Huh Very good. So, the this is known as a Lineweaver-Burk equation. So what you do is, look at this. So, what you do is you had R S given by that formula that we had here here here so if you take the inverse of this. Just take the inverse of this one over R S then what will happen is 1 over C S will come in the denominator and this is what happens K M over R max 1 over C S plus 1 over R max. Now if look at it, what is what is what happen Lisa is that, 1 over R max 1 over R is and you plot 1 over R S against 1 over C S. Then they linearly related right. So, then you can use the whole range of data. Why am I, why do you think Krisnaprada did sometimes better to use this this equation rather than the asymptotic one?

Sir error is less

Hm because in in in experiments when you actually use experiments you are not sure if your data in the two asymptotic limits necessarily correct right because it is and those of more prone to errors. The When the concentration is very small of the concentration is very large, those are more prone to errors and you actually want use your data through the entire in task over the entire spectrum which you cannot use if the, if you are just doing an asymptotic analysis.

So, that is why this is sometimes better because in this way you can use your data over the entire spectrum. And this is how plot looks like. This is a this is a representative plot of the system so, if you look at this so your slope is here look at equation twelve. We have slope is here 1 over R max sorry your your intercept is 1 over R max and your slope is came over R max. So, once you evaluated intercept, you can directly evaluate here slope from this. Just give you few seconds to if you want to make a note this, make a note of this So, 1 over R is linearly related with 1 over C S. Shall we move on?

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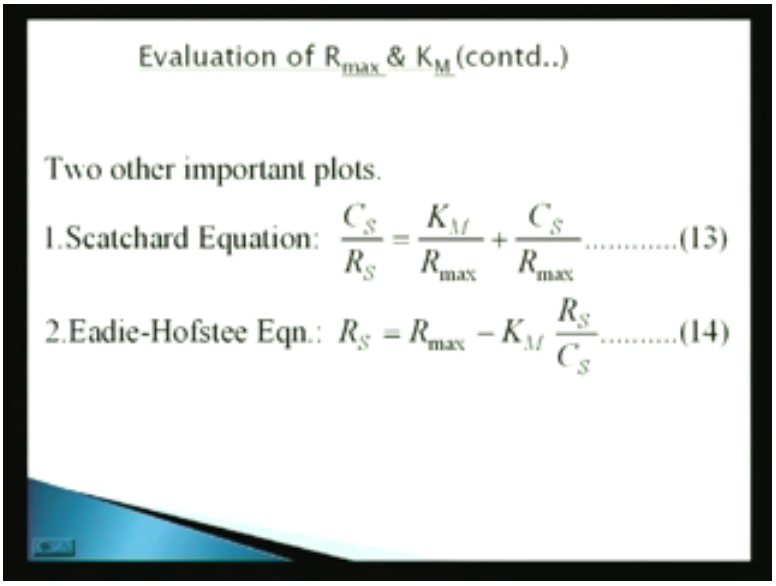

So, there are other the couple of other relations or equations that are used. So I just showed you the lineweaver-burk and the asymptotic method and the two Scatchard plot Scatchard scatchard equation and Scatchard plot and the Eadie-Hofstee equation and the plot, respective plot. These are also use to obtain the, these, why are why do we go in to different forms of these plots? The reason is you know, when you evaluate these experimentally the data may be available in different forms at different points of time depending on what kind of experiments you are doing. So, here what you have is C S over R S is K M K K M over R max C S over R max in the scatchard equation. So, if your data is available as C S over R S may be your one of set of data is available C S over R S versus C S. You know it is possible. It does not, we not aware how you did collect the data. So, depending on how you collect so there are these several options are available to you and depending on how you collect your data, you actually decide what form of the plot you want to use. So, the equation thirteen uses C S over R S.

So, you measure C S over R S. That is your y axis and your x axis is C S. The equation fourteen uses R x equals R max minus K M R S over C S. This is very straightforward. So, your intercept is going to be R max and again you use your R S instead of C S over R S you just use R S over C S.

Now, till this point is very good and wonderful by the thing is that all our all our analysis that we have done. We based it on an assumption and a very important assumption which

is the quasi-steady state assumption. Which is that the C E S that you have the the intermediate species it short-lived and it accumulation is 0 d d t f C S is zero. That was assumption we made. Now, what we have to do is, you know as not just work as a engineer but, as a scientist we have to go back and evaluate that whether that assumption is correct or not. So what we do about it? Sahil any thoughts?

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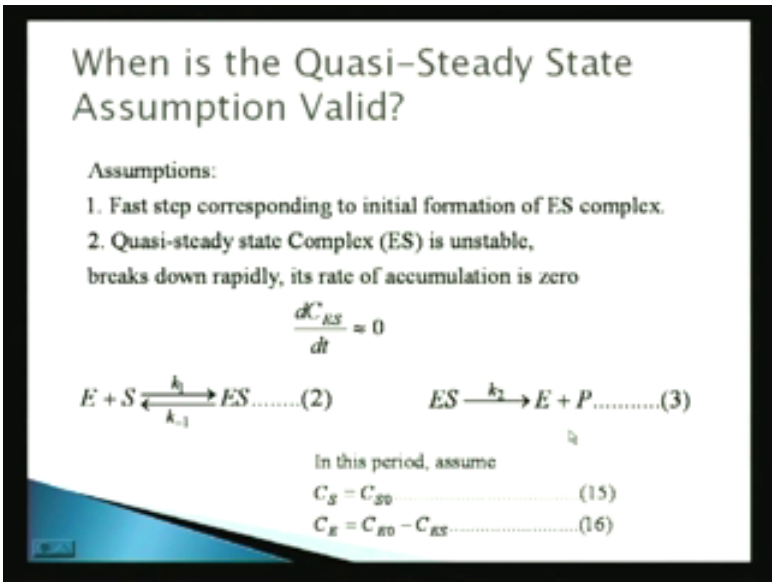

Quantitatively (( ))

Harish

Experimentally

Hm well experimentally you can but,

(( )) in the intermediately (( )) we do not have (( )) at different points while conducting the experiment

Yeah You can use fluorescent stuff like that. That is the possibility. What we might do is theoretically is tha,t you know we can actually go and not make that assumption we met that assumption and did the calculations and we can actually go on not make that assumption and then see based on a rate constants whether that assumption is valid or not. That is a possibility so we can do the same analysis not making that assumption

makes that calculation little complex. But, we can do that and then compare that with the system very make that assumption.

So, that is always you know in when you validate something in a, validate a model for example, if your lower dimensional model is an average model and you want to validate your average model what is the procedure? What you do is you take the full three dimensional model. Compute the solution of that. You take the average model compute the solution of that and compare the average solution with the full solution and if that error between the two is not high, there is obviously going to be an error because once you make an assumption that is always going to be an error. But, if the error between these two are not very is not very high then, you then we are say that your assumption is more or less correct. So, that is what we are going to do.

So, this is an assumption that we made the complex formation. That is the fast step and you know it as a result of quasi-steady state is unstable and there is no accumulation and this was an equation that was related to that. Now, if I ask you to I we actually wrote that equations these are the equations.

So, if I ask you to go back to that, I think equation five or something if I remember. Just give me a minute yeah equation five. So, this is my this is where we put the assumption in this. I equate it to 0. If I do not equate this to 0 and I want to solve this in consumption with others, we can do that one of the assumptions that we make while solve and that is exactly what we are going to do. We are going to solve equation five. Is that clear? We are going to solve the equation five, making some some small assumptions but, not make the big assumption that it its accumulation is 0. yeah So, the assumption that we make is C S at this period is equal C S naught. That is only assumption we make. What it means is that the it is at the initial stage of reaction. As a result that would amount of substrate that is found that is present equals the total amount of substrate that was initially present. That is the small assumption that we make and once we make that assumption we can go ahead and solve this.

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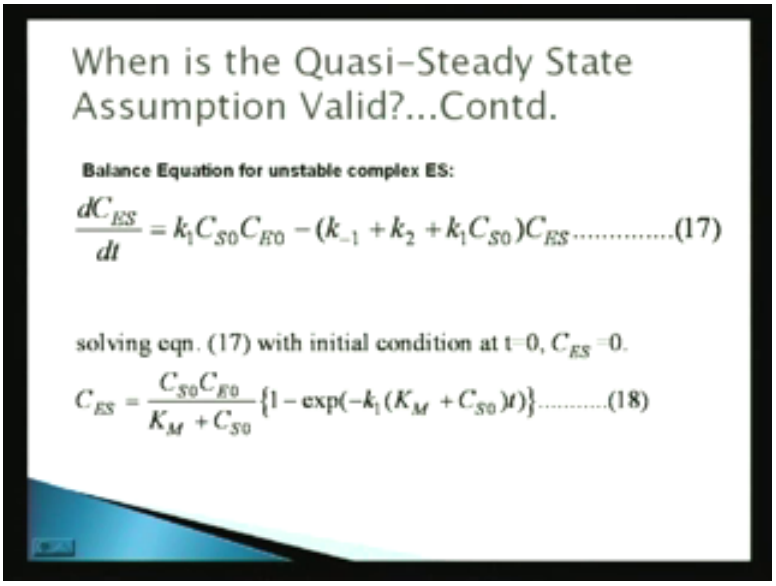

So, this is my balance equation right and all I am doing over here so how is the different from equation fifteen is that, all I am doing over here is I am substituting C S by C S naught right. So, you need to, who will tell me how to solve this equation? Method, the method I am not asking for the solution but, what will be the method? That is straightforward right. There is no method involved is d y d t equals some constant minus a minus b y right. So, you can go ahead and solve it is b exponential solution.

So, this is what you get. Once the solve it what is the what is assumption at equals so what is the initial condition at t equal 0, there is no substrate no complex. That is safe fine. So, you write you can write down equation 18 which is a solution of the system so it is an exponential solution equation eighteen. Now, from here you have to come up with an idea that so the solution is good. But, you to come up with an idea what to do with it, where we go from here?

To begin have a plot (( )) C S (( )) this k 1 in to k 1 plus C 1 C S naught this (( ))

What do, anybody else what do you think? So, this is the first order reaction right. And so, when you do a first order reaction what is important is the time scale for reaction. What you trying to measure, how fast the reaction is you know, what you said you trying to measure. How fast the reaction is? So, what how do you evaluate the time scale for reaction?

Time constant

Yeah The time constant, not the time constant but, the inverse of the time constant right. So, if you time (( )) constant which is a prefactor of t is something you know. It is the inverse of it. Essentially, the time constant now so what will you do from here? What you have to figure out? What the time scale for the three actions is right or in other words what is the effective rate constant for this reaction is? And if you look at it, what he said you know, just Krishnaprada said that is very correct that if you look at this equation eighteen.

What is the term that matters when it comes to the dynamic? See, we are trying to understand the fastness of the process, how fast this process is. If it is fast enough, then our assumption is pretty good we are good with it. So, we are trying to evaluate the fastness of the process. What is the part or the term that has to do with fastness of the process? The term when the exponential right because that is the one that has a time unit, so you have to take that prefactor of time out and we have to compare it with the time constant for the other reaction.

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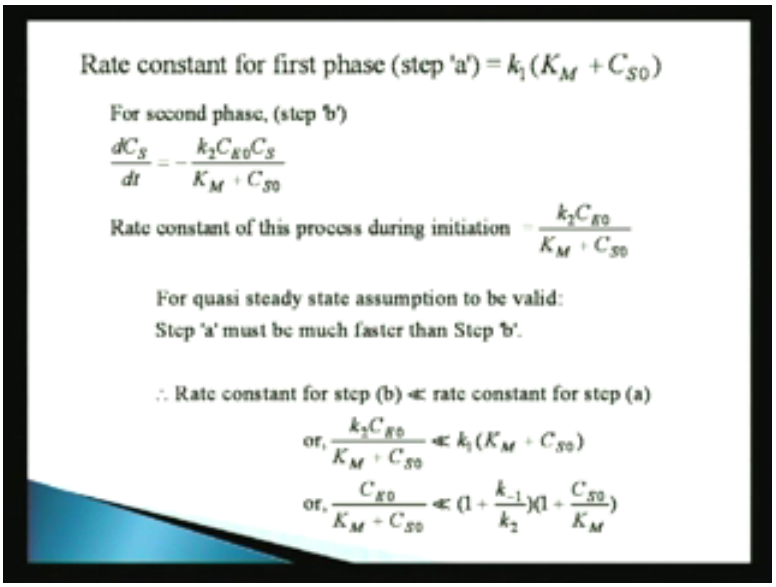

So, if you look at this, this isthe rate constant for the first step. k 1 let us go, let me go back so k 1 yeah this one k k 1 K M plus C S naught. This is a merit constant for the first step. Now, the second step second step is I will go back again. my This equation three E

S giving E plus P so, that is the gain of first order reaction with respect to E S and you can evaluated its rate constant clear.

So, what we have to do is we have to compare the rate constant or time time times time constant or rate constant whatever the time scale of first of equation two two or reaction two with the time scale for reaction equation three. Or in other words what it means is that equation two has to be much, much faster than equation three. If I am talking in terms of reaction time scales, how does it translate the reaction time scale for reaction time scale for?

Two is very less

Two is very, very less as compared to reaction time scale of equation three. Is that clear? Reaction two has to be much, much faster than reaction three which means that the time scale for reaction two has to be much much smaller than that of reaction three. Clear? So, we calculated our time scale for reaction one. Now, I wanted to do is calculate the time scale for reaction two. I can help you with the, I can you give reaction two here. So, there is equation three. ((no audio 44:34 to 45:20)) What is it going to be?

(( ))

Hm No idea? See this is going to be same as you know, what you see on the board reaction twelve, equation twelve with the exception that C S can be changed to C S naught because that that was initial idea that we can keep it as C S naught. Because it is a pseudo pseudo first order approximation we are making over here. Because when you couple see what we have trying to do is, we trying to decouple the phase one from phase two. But, in reality they are coupled right unless you evaluate what you are E S is you cannot so what should you do ideally what should you do here you have if I ask you to solve this, we can do that is an assignment. I do not have the time in this class do it. This is your C S C E S right. So if you are, if you write your next equation what should it be? Next equation is E S giving E plus S E plus P right. So your C E S is given here. You know C E S is as so I write that equation quickly. Write the balance equation here. I have it here. So, write the balance equation for the second reaction formation of P right.

C s

C S, now we know C S. Put the value C S. Is that clear k 2 in to C S and all in to have do is you have your C S over here and this is your C S. You have to just put the value of C S over here. So, the rate constant for that turns out to be something like this and for for this for the second phase k k 2 in to this part. Now, what we are doing here this is an assumption in place it over here. Which is that the What is assumption here? Tell me.

(( ))

K2? if I just multiply this by this k 2. Then we have k 2 C in to C naught in the C S naught E naught K M plus C S naught fine. But, when I put the rate constant over here, I am just putting k 2 C C naught K M plus C S naught and the this part is missing over here. The time part is missing. Why am I, what is the assumption implicit in that? There is obvious an assumption that is because that is not the right answer. You know that right. Is that clear? The right answer is you have Del C P del t equals k 2 in to C E C S and C S is given by that. So, your Del C P del t is essentially k 2 in to this part time say exponential minus time.

So, the rate itself is now a function of time. You see the difference in equation seventeen, the rate itself is not a function of time. Only after integrated, it becomes a function of time. But, here in equation eighteen, not equation eighteen when you do the second part the rate itself becomes a function of time right. But, I am trying to do pseudo first. So, this is not a first order reaction. But, I am trying to do pseudo first order approximation of that and as a result you convert this in to first order and say there is a rate reaction rate is simply k 2 C E naught K M plus C S naught. Is that clear? Let me go through one more time. So, this is my C E S (( )) C S naught E naught C E naught over K M plus C S naught times this. Now, in when I write would like this this is what I get k 2 C E S you can you can change it to C S times what I miss over here is, this times the exponential part one minus exponential part is there. But, I neglect that exponential part. Why? Under what circumstances do I do that?

(( ))

(( )) Right, very good. So, the the assumption the whole idea or the the concept behind that we are analyzing the first part at initial times, small times whereas we are analyzing the second part it large times. And if you look at this, it is a little complicated. So, I am giving a little bit of time with this. So, if you look at this so what happens is exponential

minus k 1 K M plus C S naught. This part will go to 0 because at large times this part will go to 0.

So, only the prefactor the the the part that you see over here is going to remain and that is going to be your rate constant right. Fine? Clear to everybody? Ashwin, Liza? This is little complicated so that is why I wanted to give you enough time for this. Now, what we do is what we had discussed which is that step a has to be much, much faster than step b. Or in other words two ways of putting it, the rate constant of step a has to be much, much higher than the rate constant of step b. Or the reaction time for step a has to be much, much smaller than the reaction time for step b. Fine? So, as I put here the rate constant for step b has to be much much smaller than the rate constant of step a and when you put that over here you find the rate constant for step b something we got here k 2 E C E naught over K M plus C S naught has to be much much smaller than the rate constant for rate step a which is k 1 times K M plus C S naught.

Fine. So what we will do is you know, so this is you done with this. Now, you essentially have what you have the is a relationship between your enzyme concentration and the substrate concentration. You see what is happening? The last equations that you see essentially see your k 1 k minus 1 K M. These are constant for the system. You have no control over these constants right. What you have control over the substrate concentration and and the initial enzyme concentration.

So in order to make sure that Michaelis-Menten kinetics is satisfied; you have to make sure that this relationship between the initial enzyme concentration and in the initial substrate concentration is followed right. Or in other words let us not say that in order to make sure and in other words if the relationship between C E naught and C S naught, this relationship between C E naught and C S naught is maintained then Michaelis-Menten kinetics is valid otherwise it is not valid.

So, let us not you know decide forever that Michaelis-Menten kinetics is always valid given you know whether it the sunshines or whether it rains. Exacts not the thing so only under certain conditions it is valid. And these are the conditions what I you know, I think some of you might have to go. But, what I wanted to do is there is any question on this? Let me address that if there is.

So what I will quickly go to do is you know, we just have couple of minutes more. Summarize for you what we did today very quickly and in the next class we will start with regulation of enzyme activity and I will give the introduction later. But, very quickly I will just summarize for you. So, this is where we started the Michaelis-Menten kinetics.

And we said that the Michaelis-Menten kinetics is given by R max C S divided by K M plus C S. But, we do not want to take it phase value and we want to derive it. So, what we did was we said that the enzyme reaction that happens is the two step reaction and the first step the enzyme reacts with the substrate to form unstable short-lived intermediate E S which has a short life and but, reduces the threshold energy barrier. So, that the reaction happens and it accelerates it fine. In the next step what we said is, that this complex now breaks down to form the enzyme and the product and this reaction is more or less irreversible. In the sense that, if and if there is a backward reaction possible it is very, very slow as compared to the forward reaction. So, we could write the rate of disappearance of the substrate which equals which we will see in a minute, equals the rate of formation of the products. So, this is the rate of formation of the complex E S given by given on the screen by equation five. And our another equation that we have is a constraint equation which says the total amount of enzyme beat in the free form or the free form plus the form in the complex together is a constant which equals what was what we started with right.

So, once we do that, then the rate of formation of product is d C P d t is k 2 times C E S. Now, very very important assumption that we make which will which we test in the next few minutes is a quasi-steady state assumption that the rate of disappearance of the complex is 0 because it is a short-lived intermediate. So, the rate of accumulation of the complex rather is 0 because it is short-lived intermediate compound. Now, once we make that assumption, what turns out is the equation seven, that is the rate of formation of the product is negative of the rate of formation disappearance of the substrate right. So this is all fine.

Then, we do a little bit of algebraic manipulation. Let us forget all of that and we get rid of C E S, the complex some concentration of the complex and we get rid of C E E the concentration of the enzyme. Why do we do that? Because, these are not measureable quantities. We want a solution in terms of measureable quantities which is the

concentration of the substrate and the initial concentration of the enzyme. So, then we go through these calculations.

Let us forget all these calculations and then we come up with reaction rate which is given by equation twelve. Now, we do not know if this reaction rate is resemble in Michaelis-Menten form. So, what we do is, we simply compared it with a Michaelis-Menten form and figure out yes it does does look like the Michaelis-Menten form. And then we converted in to Michaelis-Menten form. The reason we do that is we come down from four parameters which are hard to measure to two parameters which are easier to measure experimentally.

And then we go ahead and measure it experimentally. We should talk about four different ways of measuring it; one is the asymptotic analysis, the second one is a linear curve where it take the inverse of that and it is easier to measure and then there is scatchard plot and the eadie-hofstee equation and the final thing we tested is the quasi-steady state assumption. Whether that is valid or not and what we figured out is that it is not necessarily valid under all conditions and the way we did it to split the entire reaction, the two reactions that happen in two parts. So, the first reaction is supposed to be fast and the second reaction is supposed to be slower. And during the first reaction which happens we assume that the concentration of the substrate equals the initial concentration and taking that and taking an initial condition that there is not complex present we integrated that we found the dynamic solution to that. And we use that to get to the second part.

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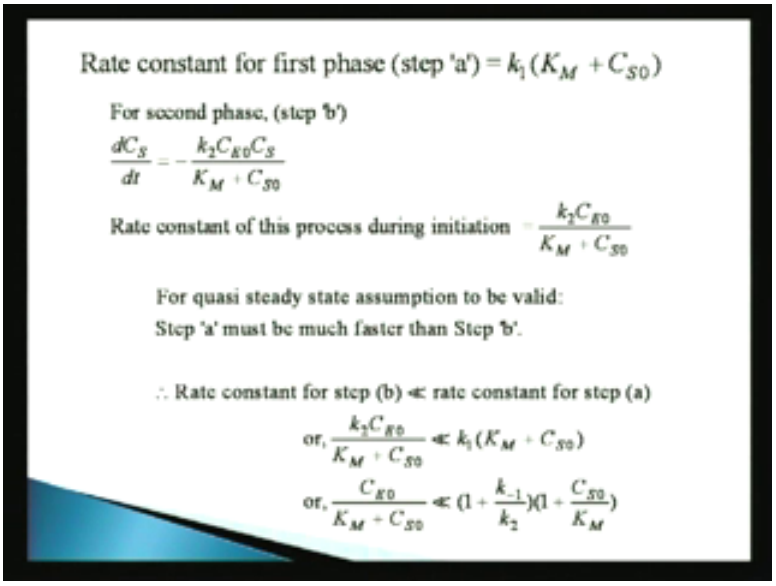

And then, we compared the rate constants of the first and the second part and we figured out that if rate the second part is much, much slower than the first part. Then only it is possible to assume as quasi-steady state relationship and then only can be get to Michaelis-Menten form right.

So that was and so what we figure is that yes if there is a this this possible if a certain relationship between the initial enzyme concentration and the initial substrate concentration is maintained. So, I guess we will stop here today and the next week we will continue with how to regulate these enzyme activities which is a different section. So, thank you.