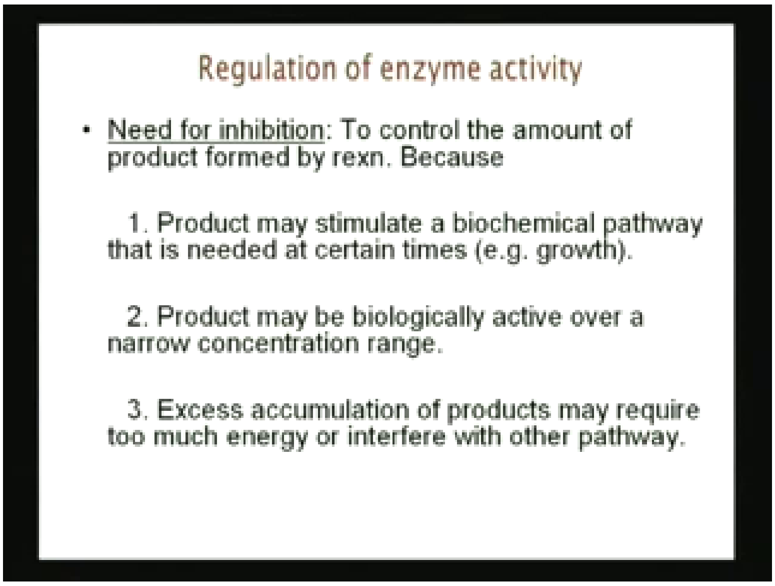
Biochemical Engineering
Prof. Dr. Ritu Banerjee
Department of Agriculture and Food Engineering
Asst Prof. Dr. Saikat Chakraborty
Department of Chemical Engineering
Indian Institute of Technology, Kharagpur

Lecture No. # 10
Regulation of Enzyme Activity: Inhibition

(Refer Slide Time: 00:29)



And this lecture is essentially on regulation of enzyme activity. So, we will start with you know regulation of enzyme activity, and one of the things that we might want to discuss for us it is why do we need to regulate enzyme activity at all, before we try and understand how we do that. And the way and one of some of the reasons that we might want to regulate enzyme activity are things that I talked about in the first lecture, which is these enzymes are essentially used in the biological pathways in very trace quantities, and they are specific.

So, the definite by enzymes by definition are specific because of specificity and because of fact that they are used typically in very minute quantities, regulation is important. And list for you the reasons for regulation. So, first the thing, the need for inhibition is to amount control the amount of product formed by the reaction. So, that is the first need for inhibition to control the amount of product formed by the reaction. So you do not want to lot of lot of the product formed the reason is that a certain product in a biological pathways the certain activity and you want a specific amount of product to attain that activity not less not more, because the body is the human physiology you know we have done a course on that before and as you know is very carefully so, it only use once to use as much as it should use.

So, why is that, because why do you want to produce products only in up to a certain quantity and no more than that, because what happens there is the products can stimulated by biochemical pathway that is needed in certain time for example, you in a wave if the if the if the product is present in a certain specific quantity it stimulates the certain biochemical pathway if it exceeds that quantity or lesser than it can stimulate some other biochemical path which is which is may be undesired.

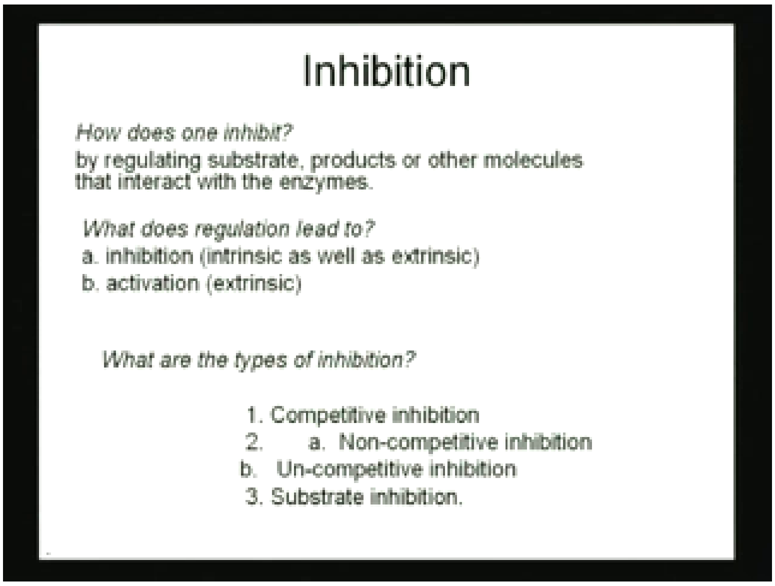
So, that is that is reason number one. Reason number two is that, the products typically or biologically active over a very small concentration range, and some of you who you know who have studied or interested or have done anything learnt anything on drug delivery would know that when we deliver drugs for example, the your doctor tells you to take the medicine as the certain quantity not more not less. Why is that? because if it is less then that particular quantity then the drug is not effective, if it is more than that particular quantity it is no good either, because it can have start to have side reactions.

So, what happens is I am if we do you know if you get any time to do one lecture on drug delivery in this course you will see that the drugs that typically active within a very small region or narrow zone in concentration same it is with enzymes. Enzymes are active within a small narrow zone, and you have to regulate the enzyme such that it is it remains within that particular zone. So, if the body is producing more of enzymes it is not good either, which producing less it is not good either, so you want to maintain at such that it is within that particular zone.

And the third reason is, a very thermodynamic reason as it is you can see is that the excess if you if you produce excess enzyme what will happen you generate excess products, and excess products may require too much energy first too much energy for generation, whatever is the amount of enzyme if you if you use excess enzyme you will form excess products, and each of these mole of excess product would need to cross the threshold of energy. So, in the process the body is going to use of more energy than it should and an as I said the body is the physiological body is a very my miser you know very calculative entity.

So, it tries to always minimize the amount of energy it expense. So, you know you can given think of yourself you know given that you can sit you would not stand even that you can sleep you would not sit. So, it is as simple that so your body would like to use energy with as miserly as possible as a result it would never like to generate products that it is not going to use because it is going to waste energy A, and the other option is that if you generate more product, and that is the one of the things that is the said at the start of this lecture it can interfere with other biochemical pathways it can trigger off other biochemical pathways which are not desired.

(Refer Slide Time: 04:56)



So, you do not want any of these so to do this you regulate and the way you regulate is essentially by using inhibition. So, the question is how does was one inhibit. So, one inhibits by regulating the substrate products or other molecules that work or interact with the enzymes what does this mean? the enzyme is an I gave you clue to this a little before also I mean not in today's lecture one of the earlier lectures is that an enzyme is specific, and it is supposed to interact or react with a certain substrate, now if you can mimic another substance or another substrate which resembles stereochemistry of which resembles that of the substrate that the enzyme is specific towards then the enzyme will deceptively you know it you kind of deceive the enzyme to start an start to interact with that substrate.

So, that is the that is a way you do that there is there are ways you can use a product also to inhibit and we will come to that in the if not in this lecture may be in the next lecture. So, let us try to see what does inhibition lead to, inhibition what does regulation leads to regulation leads to, inhibition it could be an intrinsic inhibition or an extrinsic inhibition that is you know in a bit you know intrinsically or in extrinsically from outside. It leads to activation or lowering the activation energy the amount of energy we talked about these things you know the amount of energy that you used to lower that.

Now, what are the types of inhibition? So, just for your record these are the three different or four different rather types of inhibition. So, the first type of inhibition is known as competitive inhibition, the second type is split up into two it is called non-competitive, un-competitive, and the third one is substrate inhibition. So, at this point you know I am going to discuss all of these in great detail but, let me test your intuition a little bit, what do you think would be a competitive inhibition so, it is just intuitively I am not even asking about reactions I will give you the reactions in a minute but, intuitively what do you think would be a competitive inhibition.

(( ))

Then, what will happen?

(( ))

Will compete with the same enzyme that is right. That is competitive inhibition, what would be non-competitive or un-competitive inhibition say non-competitive.

We reduce a (( )).

No, not really what would be a substrate inhibition?

(( ))

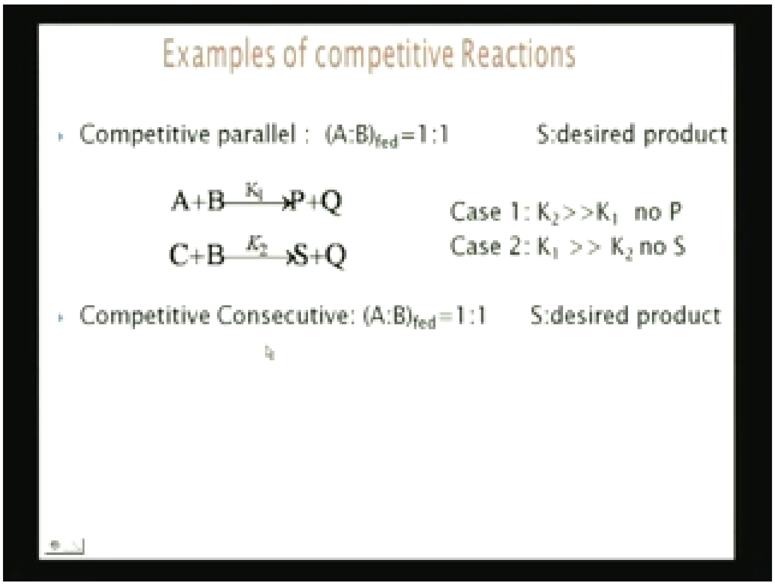
No, not really any other thoughts on the last till the first one is correct but, you said what competitive that is correct, but any other thoughts on what could be a non-competitive?

just some guess I am going to discuss this in a in few minutes so that is not the question but, what just some guess what could be non-competitive, what could be substrate? In non-competitive a I will give you a hint the non-competitive the enzyme the inhibitor so essentially in the competitive what happens inhibit as question put that outside in inhibitor competes with the substrate for the attention of the enzyme.

So, the enzyme goes and reacts with the substrate and then the inhibitor also will go and react to the substrate parallelly. So, the inhibitor competes with the enzyme for the attention competes with the substrate for the attention of the enzyme that is competitive inhibition. In non-competitive what happens? it does not compete directly with the substrate it does not compete directly with a substrate it goes about some roundabout way for example, what it does it can bind to the complex of the substrate binds to it is getting too complicated I do not want into the this moment will do it in a few minutes.

Substrate inhibition is again simple there is a system and these are very specific special cases where it happens it is the system where the substrate itself too much of the substrate, because the other way around actually from what you said too much of the substrate if you put another excess of the substrate if you put and the substrate itself can inhibit the reaction the binding. So, these are the four different kinds of inhibition that we are going to do in today's lecture, and the next day's lecture.

(Refer Slide Time: 09:06)



So, what I will start with an examples of competitive reactions. So, there are two kinds of competitive reactions; one is called competitive parallel, and the other one is called competitive consecutive. So, let us assume that A and B are your feed and S is your desired product. Now why I did it this put it this way is I want you to tell me what would be a competitive parallel reaction to start with? You have studied this in your chemical reaction engineering, I just want to you to tell me what could be a competitive parallel reaction.

They use enzyme they use this (( )) parallel (( )).

No but, A and B are reacting it is a second order reaction, A and B are the substrate not a single substrate.

(( ))

A plus?

(( ))

Not exactly any more thoughts?

Elements (( )) two quadrants of a (( )).

And you are close you are close so, A plus B getting two products say one is there so even a one product one of this, and what happens what did you say Krishnaprsad repeat that one more time.

(( ))

Well.

(( )).

No not really but, thought of you know you are quite close but, not really one reaction is A plus B giving P, and the other reaction is either A or B one of these reactions reacting with something else not A.

(( )).

Some other reaction you know some other reaction that is the product some other substrate that is in the system or some other product or some other reaction that is in the system reacting with that to form as say. So, this is how it looks like A plus B giving P, P plus Q or P does not matter, and then B reacts simultaneously with some other reactant C which is present in the system to generate S, and S is your desired product. Now what is interesting about this what is interesting is if you look at the case 1 and case 2.

A and B are fed equimolar quantities as I have written in clearly A and B are fed in equimolar quantities what is the interesting is think about this K 2 is K let us talk of case 1 first which is the easier, case 2 is first which is easier which is K 1 is much greater than K 2 the first reaction is much faster than the second reaction. So, what will happen because A and B are fed uniformly and are fed in the ratio of one is to one what will happen.

(( ))

Very simple, all of B will react with A, because the first reaction is much faster than the second one so, all of B will react with A to form P, and no S is formed so S is that desired product which is not going to be formed. In the second case, which is case 1 we written over here is K 2 is the second reaction is much faster than the first one. In which case A would not get a chance to react with B, and C would react with B to produce my S which is a desired product. So, if S is my desired product what I would really like it is I would really like my second reaction to be much faster than the first one, but well the world does not run the way I want it to, and in most cases what happens these are these are ionic reactions these ionic reactions many of the ionic reactions comes under this category and what happens is that the first reaction is actually faster than the second one.

In many cases it happens so I give you little crisp question here tell me as an engineer and you have all studied reaction engineering some of very basic reaction engineering principle I am feed, feeding A and B uniformly in a say continuous turn tank reactor, and A and B are fed in the ratio one is to one, and the first reaction is much faster than the second one, which means that no S is going to produced but, I want my S because that is my desired product, so what should I do?

(No audio from 13:36 to 13:53)

(( ))

Vary now I already said that As A and B are given is the ratio one is to one if I if I could vary that then I am in business, what I will do is that I realise that B is reacting very fast and I am going to change the ratio feeding ratio of B is to one A which I am not allowed to do I am already feeding in one is to one ratio and S is my.

(( )).

Increase the?

(( )) of P.

How will that help? P is the product. It is a

(( ))

That is a that is a reaction here reaction way of doing it you know reaction engineer way of doing what could be a very simple going a some sometimes the simple solutions escape you and you are thinking of something too complicated that is simple.

Understanding (( )).

Making?

(( )) presence of (( )).

That still do not help, because see you have studied this in reaction engineering what will happen is it will produce only in the ratio of S, S is to P the yield of S is to yield of P is going to be in the ratio of K, K 2 is to K 1 it does not matter it dependent of the resistance time.

Yes, Pallavi what is going to happen? no clue, what should you do? very simple engineering solution is there. What you should do is instead of fiddling around with reactions what he said is possibility that you introduce another reaction which is going to react with a that is a interesting way of doing it but, if you do not that is.

(( )) of C.

But, these still limiting the reactor they do not have you can in to put as much C as you want you can flood your reactor with C but, B is still your limiting reactance so it going to be dependent on B so, yes one of the things you said that is possible introduce an inhibitive which is going to react with A, but that is not the only solution there is an easier solution which is that very simple you would buy your time if I tell you which is that slow down the stirring rate, just simply slow down the stirring rate. So, you are doing it in a CSTR continuous third tank reactor and your stirring at a the particular RPM simply slow down your stirring rate decrease your stirring rate to lot so if you stirring at say 300 RPM you start at say 50 or 20 and so I gave you the answer. So, my question to you is that explain.

(( ))

No what is just say saying key word what is going to happen I do not know.

(( ))

No, what is phenomenon, what is the phenomenon? that is going to come in.

Dispersion.

No dispersion yes? You are in the close to the answer, but.

(( ))

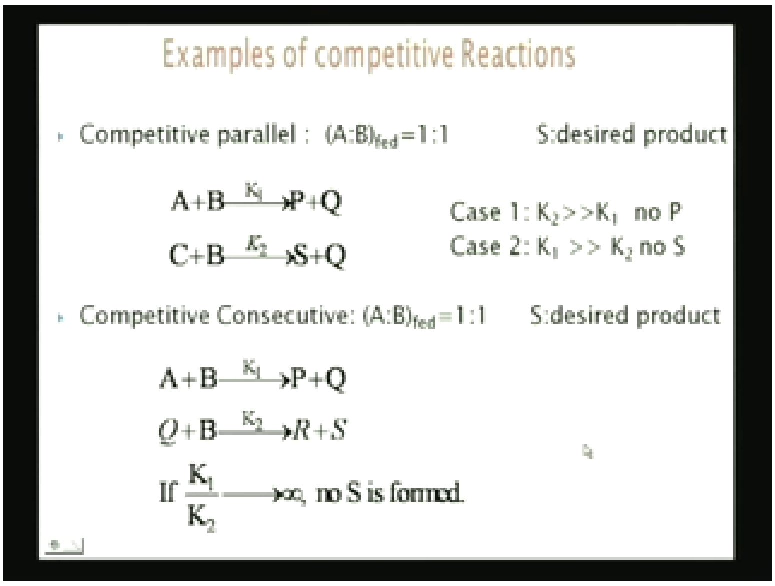
No what is the phenomenon what is going to happen I do not want coefficients and all that what is going to happen, what is the phenomenon that will kick in. So, this is simple reaction happening, and new phenomenon will kick in, once I start to do that once I start reducing the stirring rate what is that phenomenon, what is going to happen is there will be transport limitations or mixing limitations in the system. See right now, there is no mixing or transport limitations in the system. So, what is happening is that whatever the reaction rate that is going to be the final in this going to depend on K 1 and K 2, but as soon as you increase your mixing limitations so, slow down your mixing rates then you are going to introduce mass transfer resistance is another system. So, your reaction rate is no longer going to be the reaction rate that was prevalent.

Now K you have to you have to figure it out is the is very straight forward thing and deals with the basics of reaction engineering which you are interesting, you know which are something that we are interested in this class which is that when you introduce mass transfer limitations in the system and their reactions going on which reaction does it hurt?

The faster reaction, because the faster reaction is not inhibited by mass transfer and when you introduce mass transfer resistance in the system it is excuse me. If the faster reaction that it hurt so, what will happen is that, because K 1 is much faster than K 2 it is going to slow down the K 1 the first reaction, and it is going to have hardly any effect on the second reaction, because see what mass transfer does is it sets on upper limit on the rate of reaction.

So, if your rate is already much lower that lower the, below that upper limit it does not does not make any difference but, if it is above that upper limit then mass transfer will pull it down, is it clear, everybody? So, this is this is the trade to do so, introduce mixing limitations in the system, and another result of which what will happen is that the first reaction would be slowed down well the second reaction will remain as it is, and the relative yield will go up of it and these are at the reason I go went into such details about this because these are the things that we need to understand, let see.

(Refer Slide Time: 19:25)



So, the second reaction is competitive consecutive type reaction here what you do is you is A and B are feed you know one is to one feed, and again an S is a desired product as the difference as you see over here that A plus B giving P plus Q and it is a product that reacts if you compare the first one you know the second one what is difference in the first one it is some other reactant or some other substrate or some other product from some other reaction coming, and you know reacting with B whereas in this reaction it is not that it is a product of the first reaction which reacts with a second reaction with B to in the second reaction which means as this is essentially competitive consecutive or it is also known as competitive series. So, the first reaction is a parallel and the second reaction is in a series the you cannot pull of a lot of tricks in this case really but, again is you can still do the same thing.

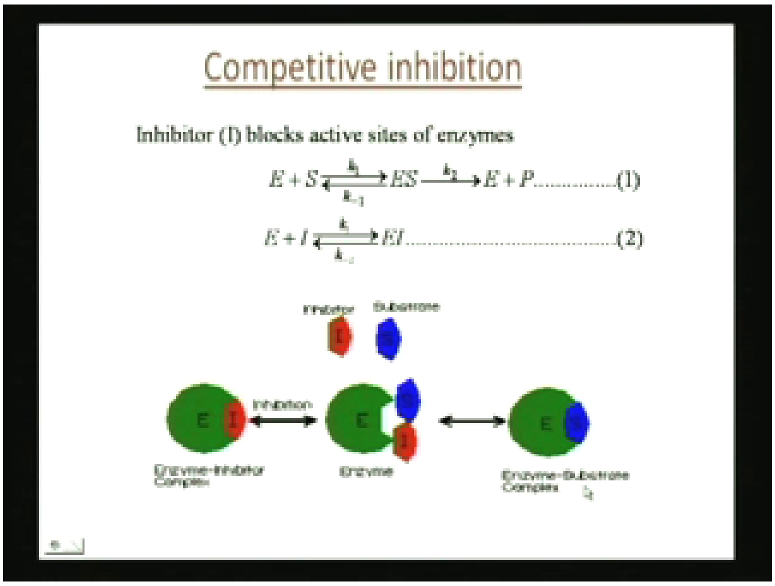
So, again you know what happens is this K 1 is much faster than K 2 as I said then what happens is all the all the what? B is all the B is consumed in reaction A, reaction one and there is no B left that can react with you to form the product and again you can pull out the same trick, you can use a you can use a stirring limitation or mixing limitation through (( )) and introduce that as a result of first one with what will happen is the first one will not be compete and the second one will be will be allowed to happen, what I want you to do is you know.

When you go back home is that for this case competitive consecutive for example, it is a little easier there is an asymptote see can you keep on increasing mixing limitations and can you can you do that? Yes, you can but, there is an asymptote why there is an asymptote? because it is no longer governed by reaction when you introduce mixing limitations in the system the yield is dictated by the mass transfer resistance in the system.

And what I want you to do is to figure out what the yield of S is going to be under mass transfer limitation, because you have been taught in the reaction engineering yield of S in the absence of mass transfer limitation you can only get in terms of K 1 and K 2, what I want you to do is in the presence of mass transfer limitation what is going to be the yield of S? figure out the question what is going to be the yield of S if you introduce mass transfer limitation such that K 1 is much greater than the K 2, another words the I want you to do this case particularly K 1 over K 2 goes to infinity so K 1 is instantaneous K 2 is reasonably slow.

So, introduce mass transfer limitation as a result of which the first reaction will be hurt the second reaction is not going to be hurt, and I want you to do figure out that what is the amount of S that is formed under those condition so, if this goes to infinity and introduce mass transfer limitation S is formed in the absence of mass transfer limitations no S is formed, in the presence of mass transfer limitation S is formed, and I want you to figure out what that amount is?

(Refer Slide Time: 22:26)



So, now what we will do is we will go on to competitive inhibition and I will try and explain what it is, because I gave you the background in terms of competitive consecutive and competitive parallel reaction this would be very easy for you to understand so, I just wanted to give you a very familiar background in reaction engineering, and then you can map that to what we are doing over here. So, this is a competitive inhibition slightly more complicated than the thing that we did last time but, still very straight forward.

So, the first reaction as you see is the same as what we have done before which is E plus S reversible giving ES which then irreversibly breaks down into the enzyme, and the generates the product as well. The second reaction is the one that makes a difference as that I told you these are competitive what kind is this competitive, competitive what, which kind of competitive, competitive inhibition of course, what kind of competitive?

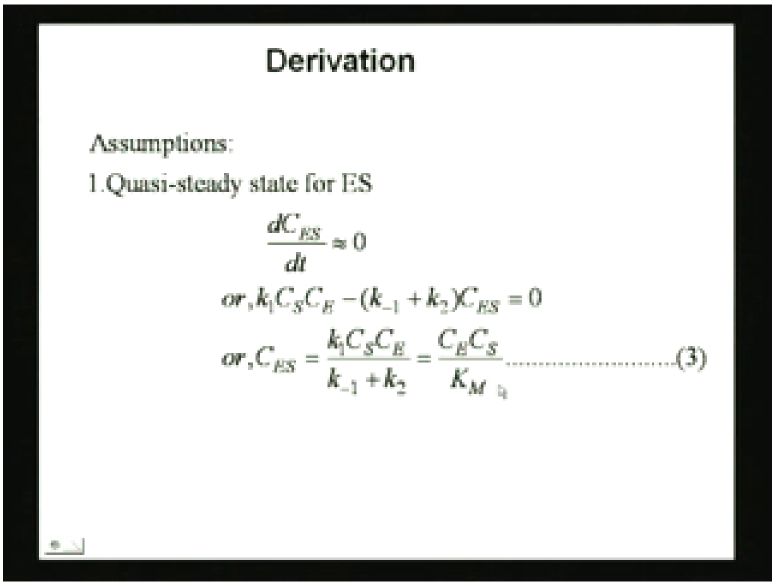
we did two kinds of competitive just now which one is this? just look at it and tell me you do not have to go back to the note, I am sorry.

So, then say first one is the E plus S getting ES which breaks down to enzyme and the product, and the second one is E plus I giving EI. So, all I am asking is this is competitive inhibition? So, which kind of competitive is, competitive parallel look a very straight forward why? because S and I are acting parallelly, parallel to each other in interacting with the enzyme. So, as I said you know in put it in more different way is that both are substrate, and the inhibitor are competing for the parallelly competing for the attention of the enzyme.

So, this is the nice picture here so, this is you know so this is the substrate and this is this is the substrate S and then this is the inhibitor what you see there, what is interesting in the in the in the structural substrate if you can see that the blue one is a substrate, the red one is the inhibitor what is interesting is that the similar stereochemistry, similar structure of that. So, this is a lock and key mechanism. The enzyme and the and the substrates are always specific to each other, and there is a lock and key mechanism. So, there is then enzyme is active site of the enzyme and this fits in the fits into the substrate.

So, the enzyme and the in substrate and the inhibitor they have similar structure as a result both of them can fit in for they both are competing to this picture shows that the both competing for the for to occupy that slot in the enzyme and either the inhibitor can occupy that spot or the substrate can occupy that spot? As a result the inhibitor enzyme complex could be formed or a substrate enzyme complex could be formed, is it clear?

(Refer Slide Time: 25:21)



So, now what we will do is like we did it in last class. we do the derivation and try to figure out what is the rate is because at the end of the day what we are interested in their rate the question that we have to ask ourselves are, or is this a Michaela Menten kinetics after inhibition it may or may not be so because sometimes kinds of inhibition are not Michaela Menten it deviates from the Michaela Menten structure. Some kinds of inhibition retain the Michaela menten form so, question A is that we have to discuss that is that the Michaela Menten form if there is a Michaela Menten form what are reduced is the maximum reaction rate reduced or the K M the Michaelis constant that is reduced.

So will you one of you tell me at this point from what we did in the last two classes, what does it mean what does a maximum reaction rate being reduced imply, what does the Michaelas constant being reduced imply you just quick if you want to go back to your notes you can look check any of the plots that we did and you see what I am trying to ask, it is very straight forward question but, we need to understand that.

What are the maximum reaction rate implied if you imply that whatever is the maximum reaction rate attainable and in that is reduced that is a sort inhibition see I now want you to see look at why I am asking raising this question at this point of time is now I now want you to look at the whole concept of maximum reaction rate, and the concept of Michaelis and the and the Michaelis Menten, Michaelis constant in the light of inhibition till now you just looked at it as constants of your reaction but, now I want you to look at it in the light of inhibition.

So, in the light of inhibition what does the maximum reaction rate being reduced means or increased or reduced means as that the maximum attainable or attainable reaction rate is reduced means it is being inhibited in a way, then what does the Michaelis constant K M imply? it gives you in the plot it gives you what the.

(( ))

But, in your plot just to answer my question simply in your plot what does the Michaelis constant correspond to? the maximum reaction rate corresponds to a line parallel to the x axis. So, which is one of the limits, saturation limits which is, which limit is the which order? kinetics.

(( )).

Which order is that the maximum reaction rate is the zero-th order asymptote, and the small C S is the first order asymptote so, what does the Michaelis constant correspond to in your plot. So, have your notes what is corresponds to is a.

(( )).

It gives you the slope in a way, once you fix your maximum reaction rate your slope is came over R max. So once you fix a maximum reaction rate it will give you the slope and therefore, it will give you the reaction rate in the case of the first order kinetic. So, how fast that is the first order rate? So, if you can reduce now again coming back to the in the light of inhibition so, if you can reduce your Michaelis constant K M what can you reduce, what, can you inhibit? My question to put in most simply you know you people are not getting it may be too early in the morning so if can you reduce can you inhibit the reaction by increasing or decreasing the Michaelis constant.

(( )).

So, we will see that yes but, the answer is that it is possible. So, what I want you to do is first so, we will go through this in the next you know half hour or whatever we have we will go through the derivation of competitive inhibition, and I want you to actually work it out on with pen and paper, because that is the only way you can learn it. So, the first assumption that we make is that you know let us go back to the equations again to jog of your memory. So, what is happening look at the first reaction this is what we did in the last class, the simple case where E the enzyme reacts with a substrate to produce a complex ES which then breaks down into the enzyme and the product, what was the major assumption that was there that ES is the complex, which is short lived another result it breaks down very quickly and therefore, we can assume a quasi-steady state or pseudo steady state approximation on here.

Which means that, the rate of accumulation of years in simple mathematical term the rate of accumulation of ES is 0. So, d dt of C ES is 0 that was which what we had assumed. Then, we had already also discussed and gone into great detail to try and quantify under what condition this assumption is valid and, we figured out that under certain ratios of K 2 and K 1 this is this is valid. So, here what we do is we assumed that S is valid so, we assume that the CS, CSS, d dt of C ES is 0 so, d dt of C ES 0 so this is the first assumption that we make and C is the D C, d dt of C ES is given by this number.

Ashwin, I want you to put your pen to paper start write writing this. So, d dt of C ES is 0 so, you d dt of C ES, C ES equals this so, which is the forward reaction minus the backward reaction, and minus the formation of the product so C ES is formed from the forward reaction again disassociates some backward reaction, and then the product is also formed from complex. So, once this is 0 what you can do is you can get in terms of the ratio of sorry, in terms of the substrate and the enzyme C E and C S why are we trying to do that? we discussed this in the last class why we are trying to do that.

(( )) parameters.

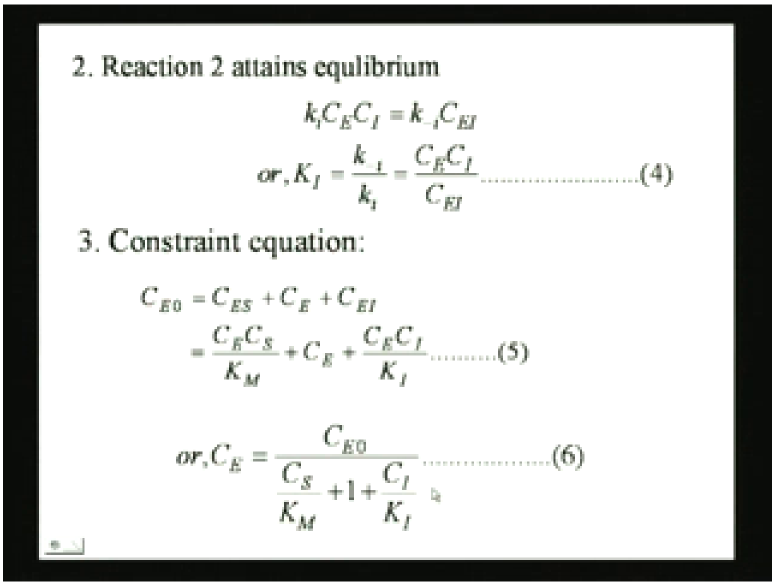
Parameters and not just that to be able to express them in terms of.

Measurable.

Measurable quantity, to be able to express them in terms of measurable quantities which are C E naught which is the initial concentration of the enzyme and C S which is the concentration of the substrate. So, that is a whole idea now once you make steady state assumption we can do this and then we can express our C E C E and C S in terms of K M which is again a function of the K 1 minus 1 and K 2 and K 1 all three and this we discussed before that we this is a precisely the answer to this is what you answer that you get first which is that to we use K M the Michaelis constant to reduce the number of parameters we use express it in terms of C E and C S so that it should be expressed in terms of measurable quantity what should be the next step what you think?

(( )).

(Refer Slide Time: 32:41)



The constraint equation, that is what called the constraint equation. We will do that but, next step that we are going to use we are using here is that say equilibrium relation. So, if you rate reaction to that was there if I need to go back I will go back here this reaction the reaction the second reaction, that is enzyme and the inhibitor reaction then equilibrium which means that you can straight away get a relationship between EI and I so this is what you do you so, can straight away get the relationship between this and k I is known as the equilibrium constant for the inhibitor which is the minus k minus I over k I, k minus I being the backward reaction rate and k I being the forward reaction rate, because see the complexity that we have over here is that what is what is the added complexity in terms of you know or solving the equation as compared to what we did in the last lecture. You have what is the added complexity in terms of actually solving this equation as compared to.

(( )) complexity

Which complex?

(( )).

Right. So, that the added complexity we have that is we had we need to eliminate one more complex last time we had just one complex here this time we have two complexes ES and EI, and we need to eliminate so the first step we use towards that process is given an equation for which is to express my EI in terms of C E and C I, and the inhibitor rate constant or the equilibrium constant. Now, with the constraint equation now I want to you the I give you the constraint equation in a few seconds but, I want you to write it down on your own and tell me what is going. So, C E naught is my initial concentration of the enzyme, and what should be equal to, yes Pallavi.

(( ))

C I then you can express your C ES and C EI in terms of other quantities, because both these reaction that they are assumed to attain equilibrium. So, you see on the screen what I intended so, C E naught equals C ES plus C EI plus C I so, those of you were not there in the last class for you so, the constraint equation is essentially the equation where you balance the enzyme present in all different forms. So initially it is a mass balance equation in a way to think off so initially if I put C E naught amount of enzyme into the system it has to be present in all the different forms of the enzyme is present which could be complexes we have two complexes here C ES and C EI are the enzyme in the free form.

So, it has to be out of these between these three forms it has to be there it cannot vanish anywhere so, and because these are as thermal reactions also. So, C ES because the you know it can assume so how do how do we get C ES equals C E, C E times C S over K M from the I just showed here before, from this the quasi-steady state assumption, and this one is equation 4 given on top and C ES it is what we have been able to achieve, we have been able to achieve eliminate both the complexes out of the systems and express everything in terms of C E and C S and C I. So, what is we still have one more step to go what is that step, what do we need to do?

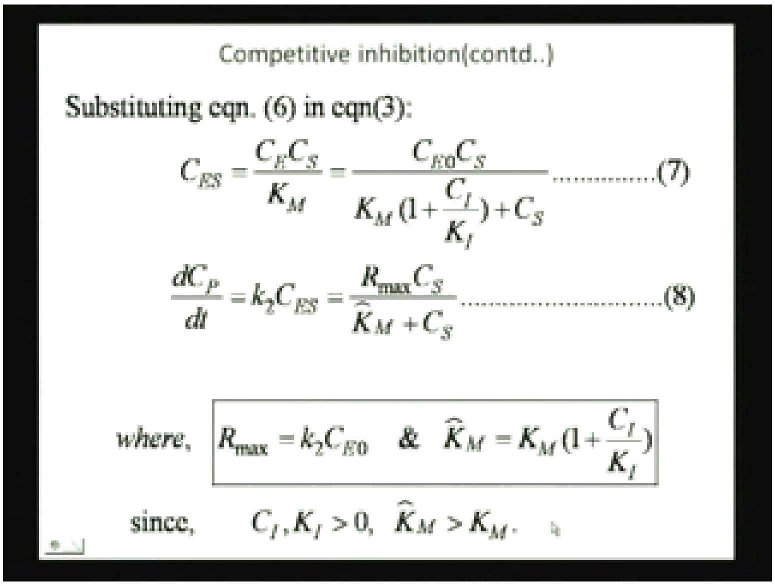
(( )).

No eliminate what one more elimination is left.

(( )).

C E C I is we already eliminated look at equation 5 on the screen C I we already eliminated what else is left to eliminate now, what all left to eliminate, we did this in the last class C E, C E we have to eliminate because C E again is a is a quantity that we cannot measure at any point of time all point of time. So, we need to measure in terms of C E naught the initial amount of the enzyme that we given and this is what we do so we take the we invert this relationship so, C E we express in terms of C E naught, C S and C I, is it right?

(Refer Slide Time: 37:20)



So, C ES is now you know if I so, I have express my C E so if we wants to write down we can write down equation 6, I will give you few more seconds to do that, done? So, C E is now expressed in terms of C E naught C S and C I so once I have done that my C ES equals C E times C S times over K M this is from the quasi-steady state I got, now I can replace my C E by C E naught and that function over here so, what I get is C E naught C S divided by K M into 1 plus C I over k I plus C S. Why am I expressing C ES in terms of other quantity because very simple, because the?

(( )).

Reaction, because of rate of product formation is given by k 2 times C ES, because if you remember the rate of product formation was the irreversible reaction that lead to P formation of P, Lisa. So, that was simply equals k 2 times C ES now we have been able to express C ES in terms of known quantities such as C E naught and C S and C I and everything, and so have been able to express this and this is the where we put it so once if we have put it then you from analogy what you can see, this retains the Michaela Menten form, because this was one of the questions that we thought was important, I said we have to answer various questions but, first one of that is a fact that whether it retains the Michaelis Menten form or not and what we figure out is that yes this type of inhibition the competitive inhibition retains the Michaelis Menten form, which is that it can be expressed in terms of the rate can be expressed in terms of R max times, the substrate concentration divided by a constant plus substrate concentration.

So, by analogy what would be your, what would be your R max and your K M and so on what is R max? R max remains the same which is?

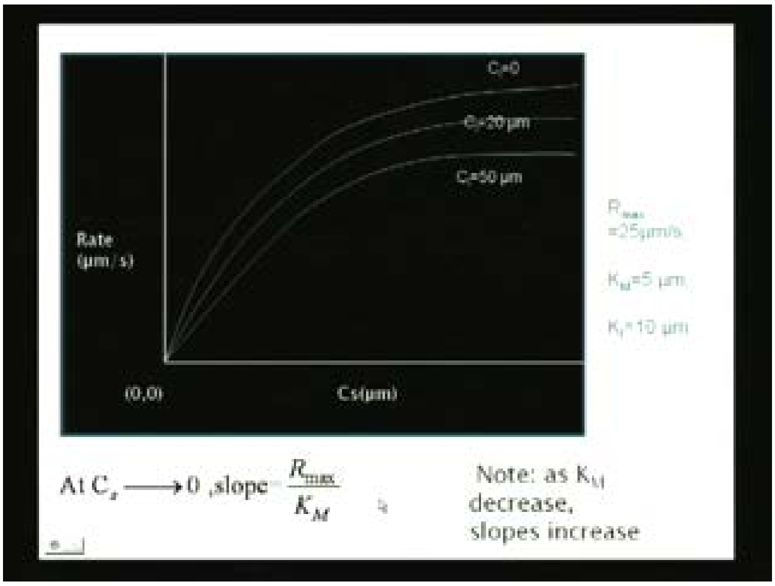
(( ))

k 2 into C E naught, R max gives k 2 into C naught and I discussed this in the last class so who will tell me that why is R max equals intuitively why is R max equals to k 2 into C E naught.

(( ))

If all the all the enzyme actually reacted, and formed product then the reaction rate would simply be k 2 into C E naught into C S so, k 2 into C E naught so your R max equals k 2 into C E naught, and K M hat is this quantity so, which means what that your R max is still the same in competitive inhibition, but your K M has changed, and I think you mention, you said this before at the end of the class that once your K M increases then you can inhibit. So, that was your prediction and in terms of that is that is correct once K M increases it you can inhibit and is K M is going to increase here or decrease it is going to increase very clearly, because C I and K I both the greater than 0 so therefore, K M hat is greater than K M. So, you will be able to inhibit.

(Refer Slide Time: 40:51)

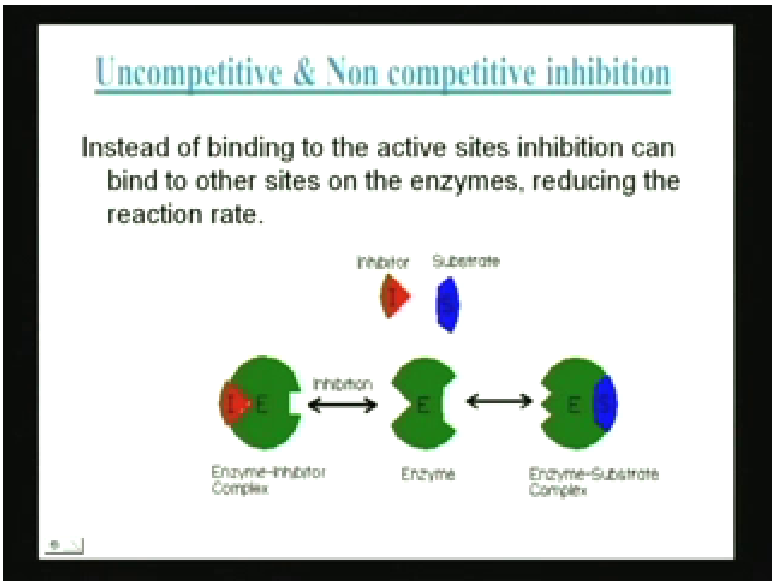


So, I will show you a plot here. So, I am not sure you can see the plot properly but, these are let me run it for you so these are the rates in micro molar per second on the left and C S on the y axis is micro molar per second, the x axis is the concentration of the substrate in micro molar, and what are these C Is on the top you see these are the inhibited concentration. So, the top graph is for no inhibitor at all, the second one is for inhibitor equals to any micro molar, the third one is for inhibitor equals 50 micro molar. So, this is without inhibition you see that it is whatever is the rate so if you increase the certain amount of inhibition it goes down, and it goes down further as you keep increasing the rate of inhibition and these are real actually numbers from experiment, and the R max that you get over here is around 25 micro molar per second this M actually big M actually, and K M Michaelis constant is around 5 micro molar and K I is an inhibitor rate constant is around 10 micro molar.

So, the first thing that we see is that as the slope goes to 0, and this we have discussed in last class as well this is the first asymptote the first order asymptote. So, as the slope goes to as the C S goes to 0, the slope goes to R max over K M. So, though your R max remains the same your K M, because this K M changes so the slope changes and what you will see. So, the R max over here remains the same and what you will see is that what is not obvious from the graph but, what will actually see that if you go on for a very long time they will all merge all these graph will merge. So, as a result the R max will actually will not change but, these are over a certain period of time but, because of slope changes at the beginning, because slope is R max over K M, because K M changes the slope changes so, they will attain that asymptote at a very different rate. Is it clear?

Those asymptote could be the same but, because your initial slope is very different from each other because your K M is very different they will attain that asymptote the dynamics of attainment of that asymptote are going to be very different, and as K M decreases the slope increases. So, this is this is understood I guess.

(Refer Slide Time: 43:20)



So, is there any question on competitive inhibition at this point of time? if there is you can ask and then otherwise I will go, and do a little bit on uncompetitive inhibition. No question? So, before you start with un-competitive inhibition let me ask you what are your thoughts on so after so had not been able to tell me what I am un-competitive inhibition is at that point of time. So, I gave you a hint and what it could be now after having the un-competitive inhibition can do you want to take another try at that what do you think would be un-competitive inhibition.

(no audio from 43:57 to 44:14)

I said that before but, if you if you want a give you the answer. So, what happens is the inhibitor in un-competitive inhibition instead of binding to the active sites in the enzyme the go the inhibitor goes and binds in the other sites of the enzyme. It goes and binds in the other sites of the enzyme it does not bind the active sites it goes and binds in other sites, and you might ask that well how does it hurt the reason it forms in altogether different complex that is how it hurts you might ask that well if it binds to the active site does not bind to the active site then how does it matter well the reason why it matters is this, because it can it will form altogether different complex which can form give rise to different sets of products altogether.

So, this substrate and as you see what is the difference now the stereochemistry of inhibitor and substrate is different so, they do not go and fit to the same slot as before earlier they were going and fitting to the same slots so, how do you if I ask you this question how do you ensure that is certain kind of inhibition is competitive, and another kind of inhibition is un-competitive by manipulating the manipulating the structure of the stereochemistry of the structure of the inhibitor.

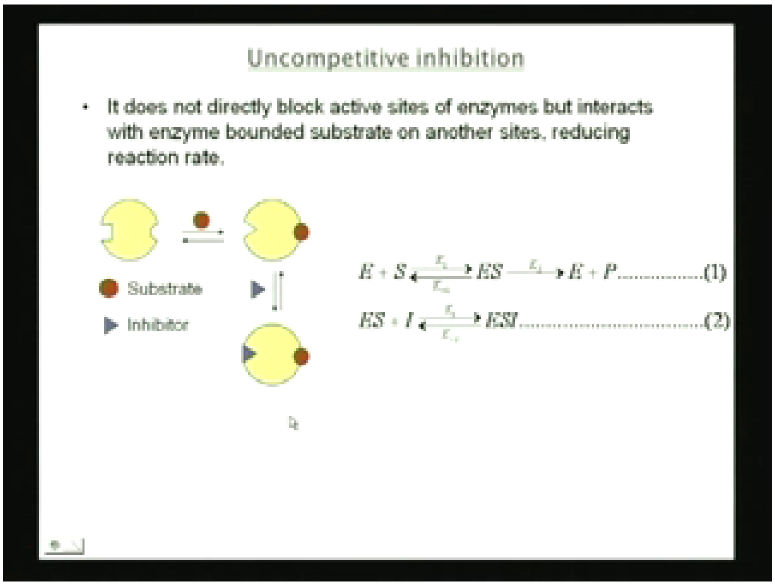
So, if you want to have a competitive inhibition then you mimic the structure of the substrate if you want to have different kind of inhibition for example, un-competitive then make sure that it goes and bind this enzyme but, it does not have the same structure as the as the complex. So, these are the some of the you know do these kinds of biochemical interactions and experiments. So these are the kind of the things that you might want to manipulate with.

So, this what happens the substrate has a certain structure the inhibitor has a different structure over here as you see, and this will go and slit sit to this slot and this will go and sit to this slot so, let us let us you know go and go and understand this so what will happen is this so, some of the enzyme inhibitor that you will have will go, and react with the substrate bound enzyme. So, this substrate will allow to go and bind to the enzyme always, why am I saying so, because the substrate typically is present in what kind of quantity?

(( ))

Very less, no abundant rather substrate is present in abundant quantity the enzyme is present in minute quantity is the substrate is typically present in a abundant quantity we know you know how much substrate you waste in the lab. So, the substrate is present in abundant quantity as a as a result of which the substrate is always say it is a enzyme is present in small quantity substrate is always going to bind to the enzyme, the inhibitor is also present in small quantity just remember so, some of the inhibitor will go and react or bind to some of the substrate (( )) bound compound that, is that clear? the mechanism.

(Refer Slide Time: 47:14)

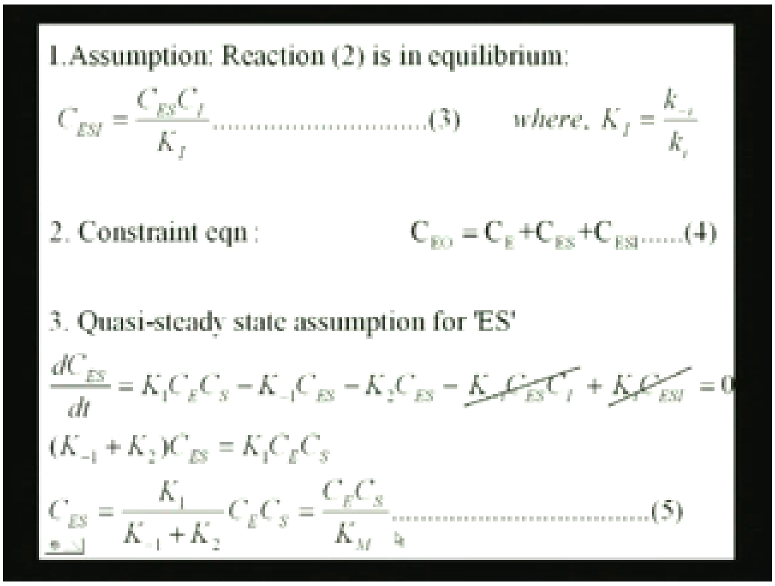


Now, we can go to the reactions, because it otherwise does not make sense to go into the reaction and unless the mechanism is physically clear to you. So, as the un-competitive inhibition and that is why it is called un-competitive so, both are not competing for that active site earlier what was happening was both the substrate and the enzyme sorry, the substrate and the inhibitor they were competing for the active site. Here both are not competing for the active site, as a result they that is why the name nomenclature, and if I tell you know this the best way to remember it.

So, that is why it is called un-competitive inhibition. It does not directly block the active sites of the enzyme but, it interacts with enzyme bounds substrate on another sites reducing the reaction rate. So, this is the equation that you get so, first equation remains as it is. Earlier what was happening the second equation so just you know just remember how this mechanisms work? So, in first equation, equation 1 is like it is always there. So, in presence of inhibitor, in absence of inhibitor it is there so, that is the E plus S giving ES which generates E plus P, P being the product. The second reaction last time in competitive inhibition it was what E plus I giving EI which means that it with competing with the active site. In this case it is not competing with the active site but, it is going and binding to the complex their enzyme substrate complex forming some other compound ESI, which is not leading to products.

So, essentially it is not blocking the active site itself, but it is generally in some other complex which does not lead to product formation. So, this is the this is the picture of the thing so, this is the you know another picture of the thing so, this the substrate is given here, and the enzyme by a triangle and this goes and binds so the substrate goes and bind so typically much of it quite some of some of it is in this form but, some of it this ES that is formed again binds with the inhibitor to form the ESI.

(Refer Slide Time: 49:07)



So, what I am going to do now we are going to start working on the derivation for the next few minutes and, I will and I will give you some hint but, I want you to work on it. So, let me ask you that first assumption is that reaction 2 that you had over here if this is going to attain equilibrium. So, if you want to go back to your calculation what I suggest is that the calculations for equation 1 remains the same so, you do not need redo those calculations what you need to redo is this calculation for the reaction 2 so, I would like you to do that so, the first assumption here is that reaction 2 is in equilibrium.

So, what would that lead to? The relationship between a, (no audio from 50:02 to 50:14) what is that lead to? if you assume that the first reaction is in equilibrium what will you get? second reaction is in equilibrium so, what will you get?

(( )).

Right. So, this is what you will get so C ESI equals C ES over C I over K I where K I is the equilibrium constant for the inhibitor, backward reaction over the forward reaction fine next what will you do the which assumption next.

(( ))

Quasi-steady state you can use then you get that will help you get the relationship between C E, C S and C E, C ES. So, it is the same thing that we had before slightly different. What will do you know do next is the constraint equation will come to the quasi-steady state a little later just after this so the next thing that I want to ask you the what is the constraint equation going to be similar as before slightly different which is C E naught equals C E plus C ES, C S, C E plus C S plus C S I as simple as that. Now the quasi-steady state assumption so, the quasi-steady state assumption is, and I want you to write it but, you know because we have do not have a lot of time today, I just you know one to make sure that you understand that it have to be written in this form. Just look at what is on the screen, and explain to me why you know these so, the difference that you have over here is that I am just explain to me what is the difference between the quasi-steady state assumption here I am showing you this, and the quasi-steady state assumption where is that, see this has three terms the quasi-steady state assumption and I want you to understand this very clearly so, this has three terms the quasi-steady state assumption. Where is the one in the next that I showed it has five termed why is that?

(( )).

Yes, that is a reason, because ES is also taking part in the reaction as a result of it so ES is taking part in the second reaction as well in the first earlier case in competitive ES was not taking part in reaction 2 so, it was only there in reaction 1 but, here ES is taking part in reaction 2 as well so, it is a part of that but, when I cancel these two why do I cancel those two?

(( ))

Because of equilibrium so, the reason I want you to understand this is that see there is a difference between steady state and equilibrium and many times I go I find the students confuses happen they assume that equilibrium equal steady state. There are times when equilibrium equal steady state but, there are many times when equilibrium does not equal the steady state. So, in this case for example, the you have the equilibrium for this reaction so, equilibrium relates to a particular what fill in the blank equilibrium relate to when I talk of equilibrium I mean equilibrium of a of a.

(( )).

No in terms of reactions if I say that if there are reactions happening in the system when I say the there is an equilibrium I speak over it in terms of equilibrium of a what? Of a particular reaction when I speak of steady state I speak of steady state of a particular?

(( )).

Species, that is the fundamental difference between a steady state and an equilibrium and you have to make this get this very clear in your head. So, when I speak of equilibrium, equilibrium is for a particular reaction whereas steady state is for a particular species. So, here if you look at it this reaction has attained equilibrium does not mean that ES is that in steady state, ES actually does not does not steady state at all we assume that it attains steady state for a simplicity over calculation, and for certain condition which we are able to justify but, it does not attain steady state in general.

So, this is what we have so E see and this is what we have so, that these two will cancel out with each other and K minus this, because the equilibrium these two terms will cancel out, and then you will have a relationship which is again similar to what we had but, make no mistake this the derivation is not the same. So, let us and go to I think we do not have a lot of time, I think probably we would not be able to finish the derivations today or I will do is will we would not do the derivations and just for the next couple of minutes we have I will just summarize quickly what we what we did.

So, in this lecture what we essentially did was with started talking about inhibition, and why we need inhibition which was to regulate the product quantity of product that is formed, and the reason we regulate the quantity of product that is that is formed is that we want to stimulate a certain biochemical pathway and not stimulate other biochemical pathways and, because the product is typically active over a small range in concentration B and third reason is that if you generate lot of product it will accumulate and lot of more energy will be formed. So, we understand why we need to in regulate and the way we regulate is by using inhibition.

So, in most cases we introduce an external inhibition, and it could be intrinsic as well so, inhibition here it says could be intrinsic as well as extrinsic. So, in most cases we introduce an external inhibition, and there four different kinds of inhibition that we study these lecture competitive, non-competitive, un-competitive, and substrate out of these four the first three are extrinsic inhibition, which means that we introduce an inhibitor and the last one is an intrinsic inhibition which means that system itself inhibit these are auto inhibitory systems, and they are very interesting systems they have their dynamics, and we would not to able to study those dynamics in this class that very interesting.

So, today we did the competitive inhibition and we started of finished of competitive inhibition. We showed that in competitive inhibition the maximum reaction rate remains the same while as the Michaelis constant changes as the result of which the slope changes and the reaction is inhibited the reaction rate is inhibited we were working on the non-competitive, and will continue that in next lecture. So, that is all for today thank you so, and I will meet you in next lecture.