Introduction to Chemical Thermodynamics and Kinetics

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Lecture – 36
Chemical Kinetics: Mechanisms – Part 5

Let us now, discuss enzyme Inhibition, or what are the Kinetics of enzyme Inhibition. So, by enzyme inhibition as we discussed already that, there should be some other molecule. Some molecule which is other than the substrate, that actually binds to the enzyme and reduces the probability of the substrate going into the product.

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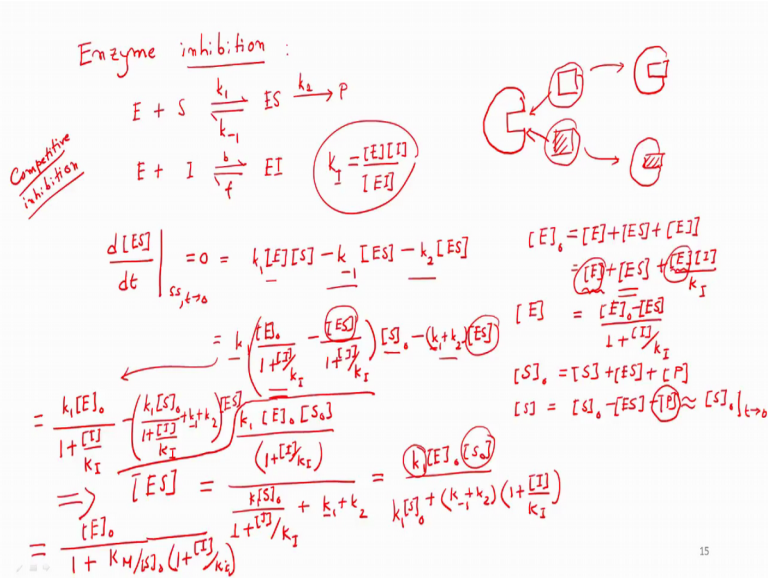

So, you can write the equation like there is of course, an equation with enzyme in substrate as before. So, you can write the rate equations like this and then I have enzyme substrate complex which forms the product. Now, the enzyme can also bind to an inhibitor and we call that complex and enzyme inhibitor complex; and it has some equilibrium instead of writing the rate constants. You can just consider the equilibrium and usually this equilibrium constant is written as K I because, its I's you means actually inhibitor.

And the equilibrium constant is written in the reverse way, in the sense it is at the equilibrium constant for the reverse reaction. If this EI to E plus I is the forward and the

E plus I is the backward or reverse reaction; then the equilibrium constant can be written as, concentration of E into concentration of I divided by concentration of EI.

Now, you see 1 interesting thing here ; so, what I am saying right now is that the enzyme has a particular pocket and a substrate can bind to it. But not only the substrate an inhibitor which also has the same size and shape can also bind to it, then if this binds then I will have the enzyme substrate complex. And if this binds the inhibitor then I will have the enzyme inhibitor complex which will look like this.

Now, this means actually the substrate and the inhibitor competes among themselves for the active site. So, this kind of inhibition is known as competitive inhibition, competitive inhibition. Now, let us try to understand what will be the rate law or the how the Michaelis Menten Equation or the Line weaver-Burk form of Michaelis Menten equation, will take the form for Initial Rate versus Initial Substrate concentration.

Now, let us consider that as before we are going to use the steady state approximation for the enzyme substrate complex and that would be 0; and that is nothing but as you know that, enzyme substrate complex appears here. So, it will be k1 E S and then will have minus k minus 1 sorry it is forming ES. So, it will be k 1 into E times S minus k minus 1 into ES, minus k 2 into E S. So, that is 0 under steady state approximation then, as before we before we proceed we can actually calculate the material equilibrium.

So, at any point the concentration of the initial concentration of the enzyme will be the amount of the enzyme present at that time, plus some amount of enzyme is forming the enzyme substrate complex; some amount is also forming the enzyme inhibitor complex. So, we can write for; and the enzyme inhibitor complex we can also write it like E plus E S plus, you can use this equilibrium. So, you can write it as E into I divided by K I. So, you can see that I can take the enzyme thing common here.

So, it will be this I am just keeping as step; so, if I move this term on the left, it will be and just switch my left to right it will be E 0 minus ES. And then it will be divided by 1 plus because the for the enzyme term have 1 plus I by K I. So, that is the solution for enzyme concentration at any time, similarly we can also write the substrate concentration in the similar way. And substrate concentration will be nothing but, the free substrate concentration plus enzyme substrate complex plus some substrate has given rise to product.

So, this will be the same as the Michaelis Menten equation; so, we can write over write it will be S 0, minus E S minus p and this E S thing we can ignore always and the p thing also will ignore eventually. So, this will be nothing, but S 0 itself, in the limit t tends to 0 because only in the limit t tends to 0 this term goes to 0; and E S we can always assume is much smaller than S 0.

Now, let us say that what will be the we can actually put the values here and try to figure out what is ES? So, this is under steady state as well as t tends to 0 I am writing both together. So, this will be k 1 instead of E, I will write E 0 divided by 1 plus I by K I and minus E S E S, I am just writing separating the terms. Because I will just collect the terms which are E S enzyme substrate complex 1 plus I by K I, this will be I concentration of I and then I have S S is nothing, but S 0 already you know that. And then I have minus k 1 plus k 2 into E S that is equal to 0.

So, I have to collect the terms for E S. So, the E S will be nothing, but this term which is left which is k 1, into E 0 into S 0 divided by 1 plus I by KI. Let me actually write it here because it is a big term and then divided by the common part which is coming with the k s. You can see here it had basically it had 1 plus.

So, E S is a multiplied by as you can see 1 divided by 1 plus I into the concentration of I divided by KI, plus k 1 plus k 2, it will be k minus 1 not k 1 and this is a concentration term.

So, see this is the expression for ES, we can actually multiply the numerator and the denominator by 1 plus I by K I and simplify this form let us do it. So, it will be nothing, but k 1 0 into S 0, and divided by of if I do that. So, the first term will be 1 and then have k minus 1 plus k 2 times, this entire part which is 1 plus I divided by K I. And then we can further divide everything by k 1 into 0.

As we did earlier, so in the numerator and the denominator and then we will have or we can actually keep that at this stage and you can do it later also. Because the I mean just for material balancing you see that k 1 it will be k 1 k minus 1 plus k 2 divided by k 1. And that we can just keep it for the for the time being we can get back to it later also, or the Michaels Menten part the way we did it if we go back.

So, we actually divided by k 1 into S 0 so that we can do. So this k 1 and this S 0 that is remaining here so that k 1 into S 0 combination we can divide the numerator and the denominator in this case, but we are missing 1 k, 1 S 0 here. So, let us see what is the k 1 into S 0, so this constant the way will have written this is this plus when we collected the terms there will be a k 1 into S 0. As you can see that these down for E S maybe we can write it clearly in a one more step.

So, this is nothing, but first to write the term which is the constant term it is k 1 into E 0 divided by 1 plus I divided by K I and then I will have all the E S terms, but then the first term of the E S also has given an S 0. So, it is k 1 into S 0 divided by 1 plus I by K I and then there is k 1 plus k 2 plus k 1 plus k 2, this k minus 1 actually and that multiplied by E S and this is equal to now the rearranged form.

So, you can just draw line just to have at confusion so that keeps this is the E S concentration. So, you can divide the numerator and denominator by k 1 into S 0. So, this is at this stage is k 1 into S 0 and if you do that. So, then I will have here k minus 1 k 2 by k 1 maybe we can go to the next page and write or maybe you can write it here, so I am writing the next step.

So, we will write it as where dividing the numerator denominator by k 1 into S 0. So, we will have nothing, but E 0 divided by 1 plus. Now, k 1 k minus 1 k 2 plus k 2 divided by k 1 that we called as Michealis constant K M, and then will have divided by S 0 multiplied by this factor which is 1 plus inhibitor concentration divided by K I.

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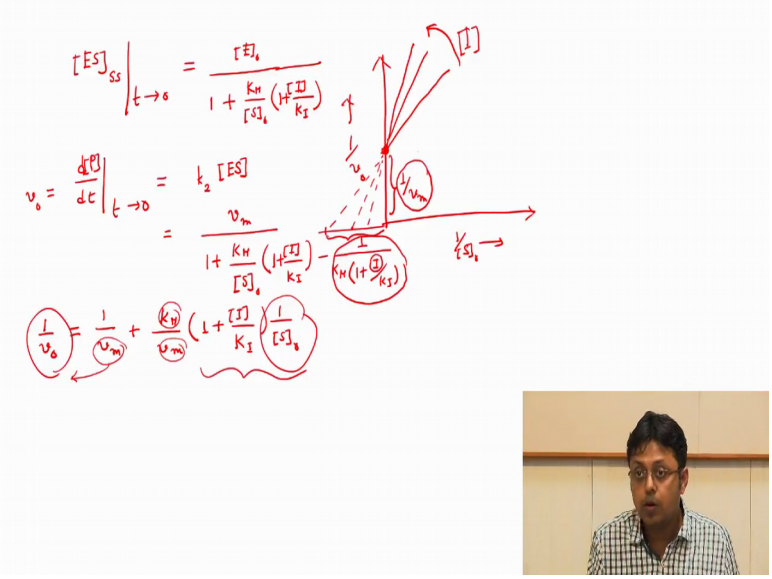

Now let us write it very clearly. So, what we got is the enzyme substrate complex concentration under steady state at t tends to 0 is nothing, but k 1 k 1 into E 0 already removed it is E 0, divided by 1 plus K M by S 0 into 1 plus I divided by K I.

So, the rate of the reaction are d P dt at t tends to 0 is k 2 into E S which is nothing, but so the k 2 will be multiplied and we know that k 2 is nothing, but k 2 into E 0 is nothing, but v max divided by 1 plus K M divided by S 0 into 1 plus I by K I. Now let us just plot the line weaver Burk plot for this competitive inhibition pathways.

So, as before let us just draw so of course, if I plot here this is actually v 0 still not written in the line weaver Burk form. The line weaver Burk from if I write it will be 1 over v 0 and it will be 1 over v m plus it will be K M divided by v m into 1 plus inhibitor concentration divided by KI, into 1 of over S 0, I am writing the 1 over S 0 slightly differently. And then we are plotting 1 versus v 1 by v 0 versus 1 by S 0 and then let us describe the try to understand what is the nature of the plot.

Now, suppose again we will do the y intercept and x intercepts y intercept means actually x is 0. So, if I put that S 0 this enter thing goes to 0, and then the value of 1 buffer v 0 is the same it is 1 over v m. So, this intercept is nothing, but 1 over v m always.

Now if you ask what is the value of the other if we just extrapolated it at what point it crosses the x axis for that we have to take 1 over v 0 is equal to 0 because that is the x

intercept and at that point what is the value of 1 over S 0 that we can easily figure out we take the to the other side it will be minus 1 over v m.

So, that v m and this v m cancels and now this K M will be brought down. So, it will be 1 over K M with a minus sign it will be minus 1 over K M into will have also this other factor because where is the writing the 1 over S 0 term and it will be exactly 1 plus I divided by K I. So, it is not just 1 over K M as in the I mean enzyme kinetics without any inhibition.

Now, you see a very interesting situation here that if I now vary the inhibitor concentration what will happen? So, if I go to a higher inhibitor concentration now you see that this is the intercept I have seen I have drawn here, but if I go to a higher concentration of the inhibitor then you can see this formula of this x intercepts will reduce because actually the inhibitor concentration appears in the denominator.

So, this intercept will be smaller suppose it comes here at a higher inhibitor concentration; however, 1 by v m this is unchanged because this does not depend on inhibitor concentration. So, the new curve for an increased inhibitor concentration will be something like this and if you keep on increasing the inhibitor concentration it will look like this. So, by varying the or increasing the concentration of the inhibitor we will see a family of curves, but they will all cut the y axis at the same point.

So, the y axis intercept will be the same if the if we vary the inhibitor concentration and if we see it then we conclude that it is a competitive inhibition pathway. Now there could be another situation which we call as non competitive inhibition.

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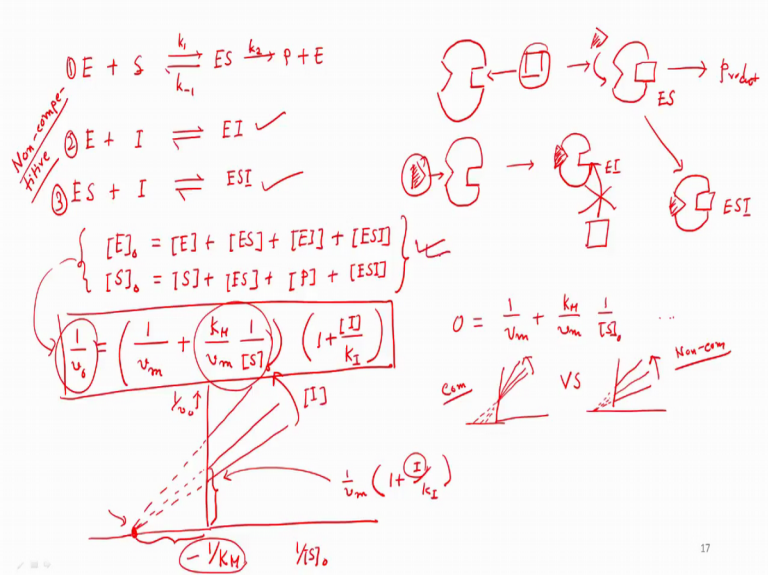

And there another interesting thing happens you have enzyme plus inhibitor that as before have an equilibrium to form enzyme substrate complex that forms the product plus the enzyme back. So, here also just remember the enzyme will be back and then you also have the sorry this will be substrate not inhibitor enzyme plus inhibitor that is in equilibrium with enzyme inhibitor complex and plus you can have enzyme substrate adding with the inhibitor to give you the enzyme substrate inhibitor. Now what is the mean actual?

So, here the enzyme can also have multiple binding site and suppose I have shape of the enzyme pockets what the substrate or the inhibitor can bind in something like this. Now what can happen a substrate can bind to it which is fine and so this is this will give my enzyme substrate complex which is something like this, and the inhibitor can actually bind here from this other problem which means if I draw it again. Suppose this is the shape of the inhibitor which can now bind here and to give the enzyme inhibitor complex.

So, this is the EI this is the E S the inhibitor can also bind to the already formed enzyme substrate complex. So, meaning the inhibitor can further come here and then form something like this so this is E S I complex, because both the substrate and the inhibitor are bound to the enzyme.

So, this type of enzyme inhibition is known as non competitive inhibition and the nomenclature is pretty straightforward. Because you can see that the enzyme are the substrate and the inhibitor do not compete for the same site on the enzyme because they have very different shape. But now the enzyme can be attacked by the inhibitor, or the substrate.

And the assumption here is that once the substrate binds to it there in the binding of the substrate cause some conformational change in a Enzyme, so that the binding of the inhibitor causes some conformational change in the Enzyme, so that this substrate now cannot bind. So, there is no such pathway that EI enzyme inhibitor complex is going to the enzyme substrate inhibitor complex. So, it is inhibiting whenever the enzyme is whenever the inhibitor is binding. So, the only pathway that the product can form is this pathway.

So, the first step so we have an interesting third step here or you can actually use the similar way you can actually write an equilibrium constant for this equation, you can write a equilibrium constant also for this equation and do the material balance very carefully. Because you remember that for this case E 0 will be the free enzyme concentration, plus some enzyme substrate concentration, plus some enzyme inhibitor concentration, plus some enzyme substrate inhibitor complex concentration.

And similarly your S 0 will be free substrate at any time plus some enzyme substrate complex some of them some of the substrate has been converted to product and plus will have also the ESI complex and using these two you can actually figure out what is the value of the steady state concentration of E S enzyme substrate complex. And now you can put it back into your rate equation and you can get the write it in terms of the line weaver Burk way and then you will figure out a very very interesting equation. This will be like this let me write it and then I will explain what it is all about times 1 plus I divided by KI.

Now let us try to plot it in the line weaver Burk plot which is a plot of 1 over v 0 versus 1 over S 0. And as before you will have say for a typical enzyme inhibitor concentration I have a intercept. So, now the question is what is this intercept the or the x y axis intercept for y axis intercept the x axis value has to be 0.

So, 1 over S 0 is 0 so this term basically vanishes. So, what I will have is that 1 over v 0 will be 1 over v m so let me just write it somewhere else. So, this will be 1 over v m times 1 plus I divided by K I and then you can also ask this question what is this x intercept. So, for x intercept I will make this S 0. and ask this question what is the value of 1 over S 0.

So, if I make that S 0 now you can see that the x intercept it will be something like minus 1 over v m and then I will have K M by v m, but that gets cancelled. And so if that S 0 first of all you can actually write it 0 is 1 over v m plus K M by v m into 1 over S 0 into something, but that is something also gets 0 because it is a 0 on your left. So, I will have this one. So, if I just arrange this will be nothing, but minus 1 over K M.

Now have a very very interesting situation here I see that the x intercept now is independent of the inhibitor concentration because it does not appear. However, the y intercept depends on the inhibitor concentration if I increase the y in the inhibitor concentration. So, I will have a value which is more because it is proportional to the inhibitor concentration. So, what will happen I will have a, but the intercept x intercept does not change.

So, what I will have is that if I keep on increasing the inhibitor concentration. So, I will have something like this, so the curve will be like this, the inhibitor concentration is changing and you can see that how it is changing. So, look at the striking difference between the competitive Inhibitor where we actually did not have does this step we just had two steps and the non competitive inhibition where actually had a third step. And look at the curves very carefully in the case of competitive inhibition as we are increasing the enzyme inhibitor concentration we have a common point on y axis the intercept is fixed which is 1 over v m; however, the x axis I mean intercept it changes and the family of curves looks like this.

However now the other situation is when we have the non competitive inhibition where the x intercept is kept fixed and; however, the y intercept keeps on changing you will have more and more y intercept. So, by looking at the curve further it is if we just plot it the enzyme the line weaver Burk plot. And if we just ask this question like how it looks like as we vary the enzyme inhibitor concentration if it looks like this versus if it looks like this, then we can readily figure out what is the nature of the competition.

So, this will be the competitive inhibition pathway and this will be the non competitive inhibition pathway. So, you can actually use these equations and try to get the equation of the line weaver Burk form and see whether you are getting this form. So, that you can try and you just follow the same way like we followed to arrive at this expression. You can look at look at these equations I mean like how we derived it is just simply based on the material balance which is I have already written here.

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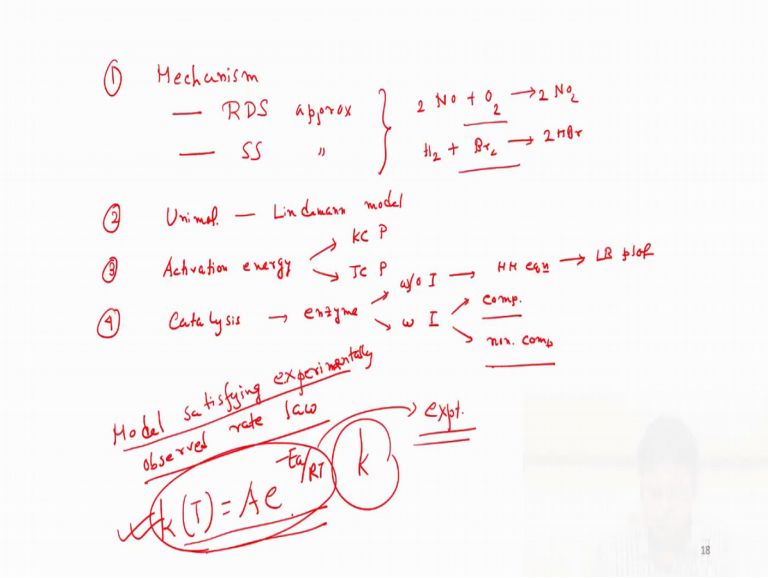

So, what we what we studied in this entire lecture is that we first started with a mechanism of a what is a what is meant by a mechanism? And we talked about two approaches or two approximation one is known as the rate determining step approximation, and the other one is basically steady state approximation.

And we solve very interesting equations or interesting reactions for that one was this nitric oxide going to no two which is apparently simple, but and the rate laws are also simple, but we showed that it can have very different mechanism both of which can like to the same observed or predict the same observed rate law.

The other one was basically H 2 plus B r 2 all in gas phase reaction 2 H B r which looks very simple. But the rate law is fairly complicated, but we derived we proposed the mechanism and that successfully explains the rate law that we discussed. And then will also discussed the little bit discussion on the unimolecular reaction.

How unimolecular reactions happen? And we particularly talked about the Lindeman model for the kinetics of unimolecular reaction. Then you talk little bit about the activation energy or energy of activation to be more precise and then you showed how we can think about the kinetically controlled product or the thermo dynamically controlled product, and how basically you can control the branching of the product ratios by controlling the temperature or waiting for long time.

And then we switch to catalysis we give an example of acid base catalysis of ester hydrolysis, but most of the time we discussed about enzyme catalysis and we also discussed what will be the enzyme without any inhibitor and also with any inhibitor. And with inhibitor we saw showed that there can be competitive and non competitive pathways and under the enzyme we actually discussed the Michaelis Menten equation. And how one can get the line weaver Burk plot from Michaelis Menten equation.

So, that will be more useful particularly when you describe the competitive and non competitive nature of the inhibition. So, this concludes our discussion of this lecture on reaction kinetics and mechanism of reaction. So, by mechanism again it is a it is a model that actually satisfies the observed or we can more specifically say it is experimentally observed rate law, so that is known as a mechanism and you can have actually multiple mechanism for the same reaction.

And once you are done so you actually then propose the mechanism, but then actually you also have to verify the that any this mechanism is correct by trapping the intermediates that we briefly discussed. And then in the next lecture will be discussing how one can calculate k from the very fundamental principle. So, far we have used k as it is only thing we know about k is that k has a temperature dependence and expression of k is something like A into e to the power minus E a by RT.

Now, that expression of the Arrhenius law is actually experimentally determined relation it is an empirical relation. Because Arrhenius did measured some rate constant at did for an temperatures and he arrived at this equation. Now the question is can we actually develop a model based on collisions between molecules and our atoms or our atoms and molecules and then get an equation where we can actually get a form of the rate constant something like this in the Arrhenius equation. And that is covered under reaction dynamics that will be discussing in the next lecture.

Thank you.