Aspects of Biochemical Engineering
Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture - 25
Kinetics of Enzyme Catalyzed Reactions Using Free Enzymes – V

Welcome back to my course Aspects of Biochemical Engineering. Last couple of classes I mostly I discuss the theory part of enzymatic reaction kinetics. We try to explain what do you mean by enzymes, half the enzymes substrate interaction take place what are the different inhibitions all this things and also we tried to analyze the batch process CSTR and plug flow reactor, and try to find out that what is the time required for batch reactor, what is the time space time required for the CSTR, what is the space time required for plug flow reactor.

Now, today actually we are going to discuss some problem because some now coming 2 lectures I will be concentrating on different problems that we have involved in different type of enzymatic reactions. Now first problem that I want to discuss that is very common problem that we have in the enzymatic reaction, that suppose we carry out any kind of reaction in a Bessel, now question come that at different time of reaction what is the percentage of substrate converting, now how can find out.

Now, in my previous lectures we try to develop the correlation between the substrate concentration and time of reaction, that correlation of enzymatic reaction we try to develop. Now in this particular problem we will we see the application of that, how we can apply in the enzymatic reaction.

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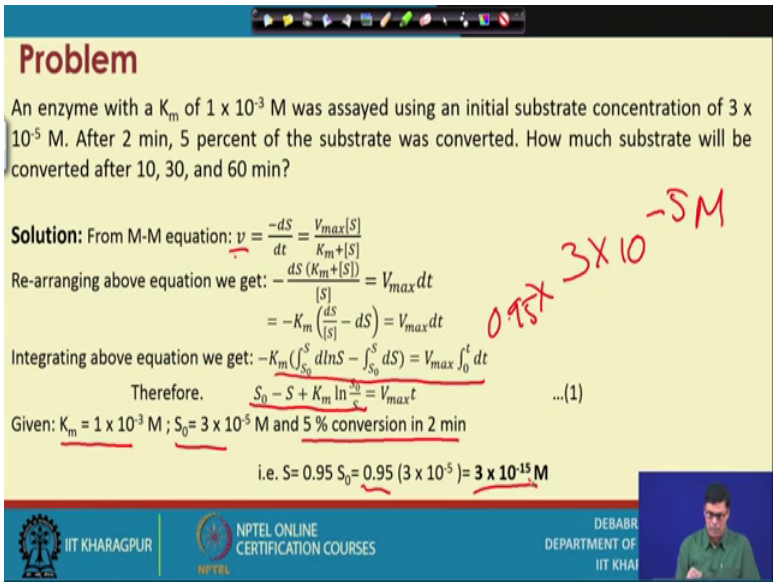

The first problem that I am going to discuss that is that an enzyme with km 1 into 10 to the power minus 3 moles were assayed using the initial substrate concentration 3 into 10 to the power 5 moles substrate, after 2 minute 5 minutes 5 percent of the substrate was converted, how much substrate will be converted after 10 minutes 30 minutes and 60 minutes.

So, in this problem if you look at what is happening that we know that this is the Michaelis-Menten equation we already discussed v equal to V max S km plus S am I right now this is equal to velocity of reaction equal to minus dS by d t. Now from this we can develop this correlation this is. So, this correlation final correlation will be coming this km that t you know d l n S and dS S 0 to S and V max 0 to t d t. And if you solve it, it will be S 0 minus S km l n S 0 by S and V max. Now in the problem what is happening that km is given and say S 0 initial substrate concentration and 5 percent substrate converted in 2 minutes.

So, time of reaction is 2 minutes and S value is the 5 percent. 5 percent means initial substrate concentration was 3 into 10 to the power minus 5 moles am I right if you multiplied. So, how many substrate will be remain after the reaction? Reaction will remain is the above 0.95, 5 percent substrate converted. So, we can multiplied by nine 5 will get the substrate conversion after the reaction is over.

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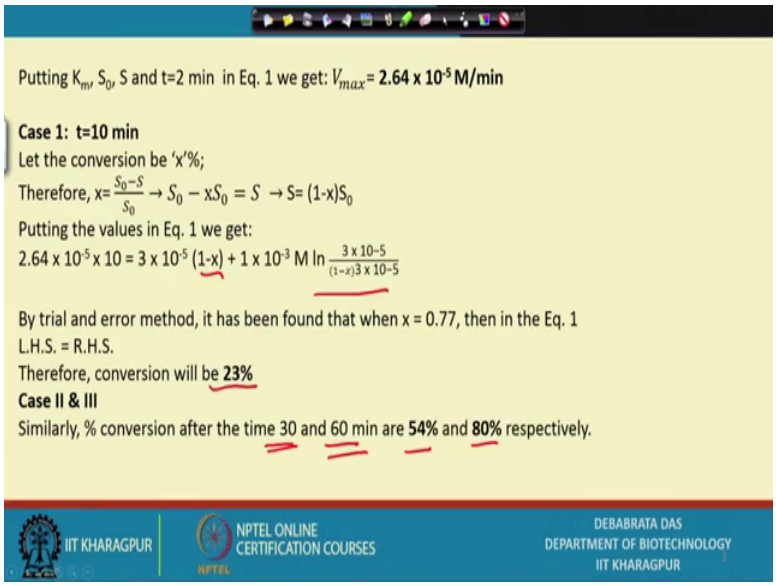

Now, next is that. So, here we from this we can find out the value of V max, we can find out this value of V max were next problem that we have that is, that we shall have to find out how much substrate converted how in 10 30 and 60 minutes.

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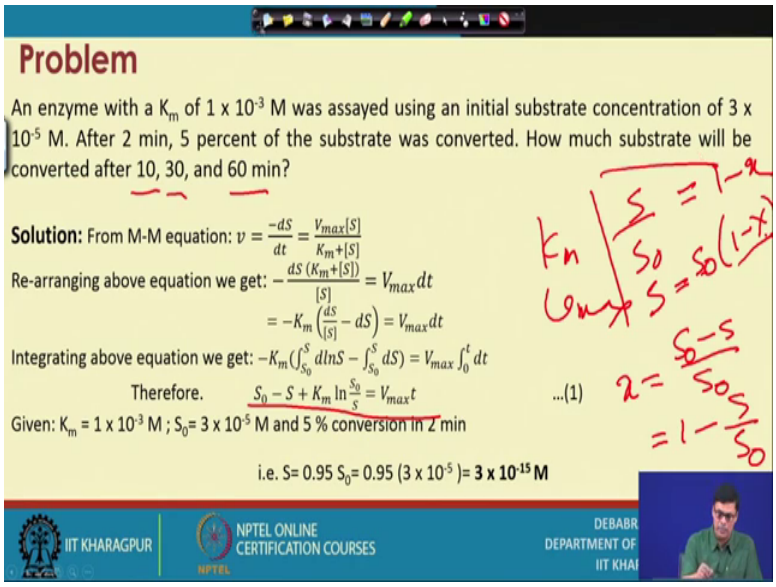

So, now, we know the km value, we know the V max value and we have this correlation am I right.

So, what we can do we can here, we can this x, x is equal to what? S 0 minus S by S 0; so we can write in term of x this is 1 minus S by S 0 am I right. So, S by S 0, I can write

S 0 equal to 1 minus x, so S equal to S 0, 1 minus x. So, we can convert this term in the form of S 0 and x. So, you know if you can convert then it what is the x? X is the nothing, but that how much substrate that is converted S 0 minus.

So, that is the exactly we shall have to find out how much substrate is converted. Now here we have a now we can put the put the values in this equation, and we can find out through the tri-level method, that we shall have to find out what value of x, left hand side is required to right hand side. And we find that that after 10 minutes, the 23 percent substrate converted and similarly if we calculate after 30 and 60 minutes we will find 54 and the 80 percent substrate will be converted.

So, this is a very common enzymatic reaction that we have, and we can any kind of enzymatic reaction we can analyze and find out how much substrate will be converted and different time of reactions.

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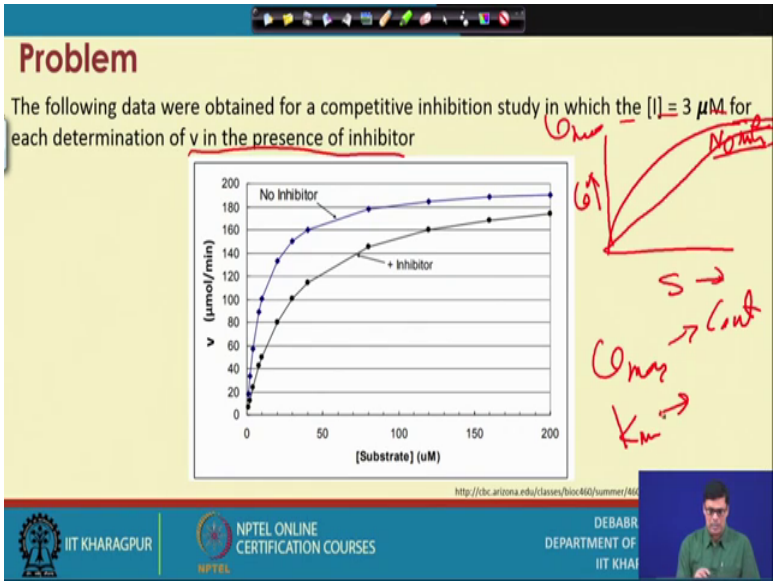

Now next problem that I am going to discuss the following data's were given, to obtained for a competitive inhibitor inhibition study in which I; I is the concentration of inhibitor and equal to 3 micromolar for the determination of value of v in the presence of the inhibitor. Now here I want to tell you very interesting thing that we already discuss, the in case of competitive inhibitor what I told you? That this is v and this is substrate am I right.

And this is this is with the no inhibition, when there is no inhibition then we have this plot, now in case of in competitive inhibition this will be like this. So, at infinite substrate concentration it will be becoming V max this is equal to V max. So, we know in case of competitive reaction V max will remain constant and what km will increases and if km is increases the velocity of reaction will decrease. So, this is. So, in this problem though we have this is no inhibition and this is inhibitor.

So, you can see the nature of plot is given here this is inhibitor and this is no inhibition that we have.

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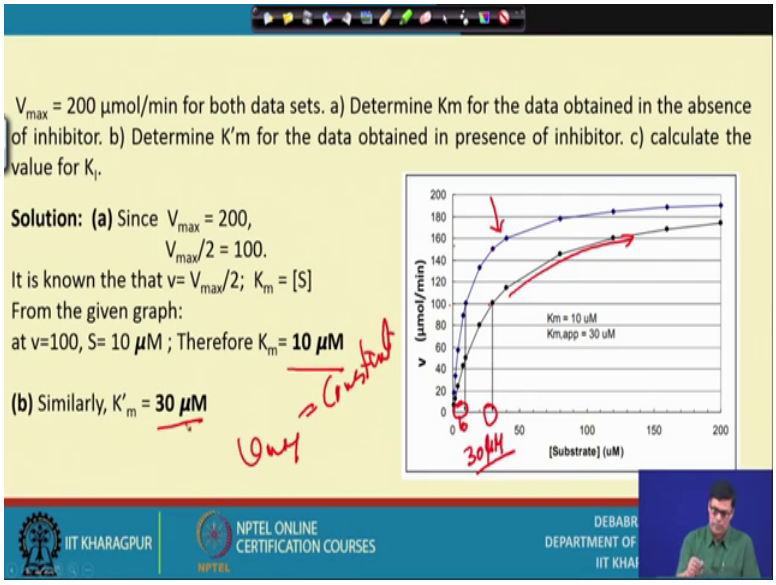

now V max is given here V max value is given 200 micromoles per minute for both data set and determined what I shall we shall have to determined value of km for the data obtained in absence of the inhibitor, and km dash for the data obtained in presence of the inhibitor and calculate the value of K I. So, 3 different things we shall have to find out we shall have to find out that km value without any inhibition and k km dash value with inhibition and K I value that what you call inhibition constant, that we shall have to find out. And this is the common feature of any kind of enzymatic reaction and let us see how we can find out this is the difference values.

Now, when you talk about the km value, we know what you know that when v equal to V max by 2 then your S the substrate concentration is equal to km value.

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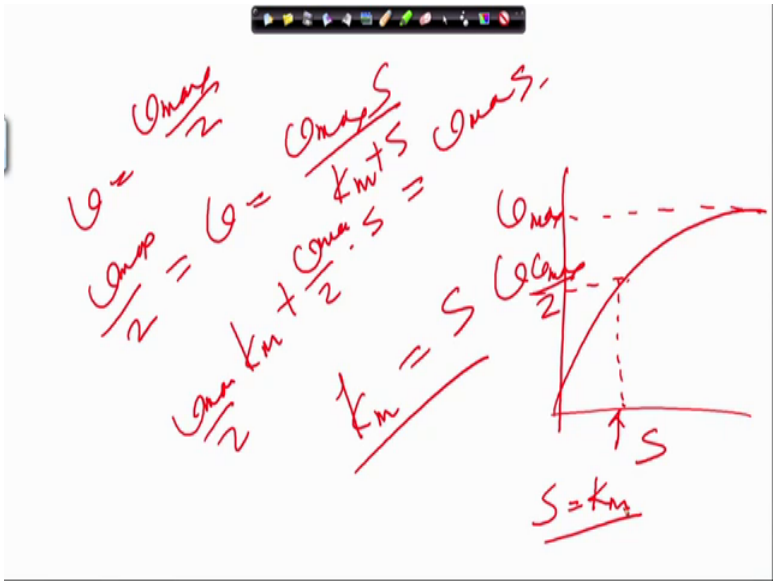

Because I can show you that we have v equal to V max S km plus S. Now when if we assume v equal to V max by 2 then what will happen? This is v max by 2 am I right. So, this is v max by 2 into k m plus v max by 2 into s equal to v max s. So, I can bring it that side then we will find the k m is equal to S. So, now, what is happening that in case of enzymatic reaction that the v equal to v versus S plot is like this without inhibition.

Now, we know this is v max value now this is half v max v max by 2 am I right. So, this is this value at this, this substrate concentration this is equal to km. So, we can easily find out the km value from this particular plot. This is exactly what we have done here that you know that this is no inhibition, this is no inhibition, now in case of no and this is v max and there is 200 the 200 micromoles per minute is the V max value and. So, half of this is a hundred am I right.

So, this is this values what is this values? This is equal to 10. So, this is exactly this is the 10 micromoles the; what will be the km value? That in this competitive without inhibition that is the 10 micromoles now let us see in case of the inhibition. When inhibition that we have the plot is like this, this is the inhibition plot. Now here that half of the inhibition because we know V max is constant because in case of competitive inhibition V max is constant, am I right.

Now, if this is constant then this corresponding value here how much is coming this is 30 micromoles micromole, this is exactly what we have written here the. So, with inhibition the km value will be 30 micromoles without inhibition it should be 10 micromoles.

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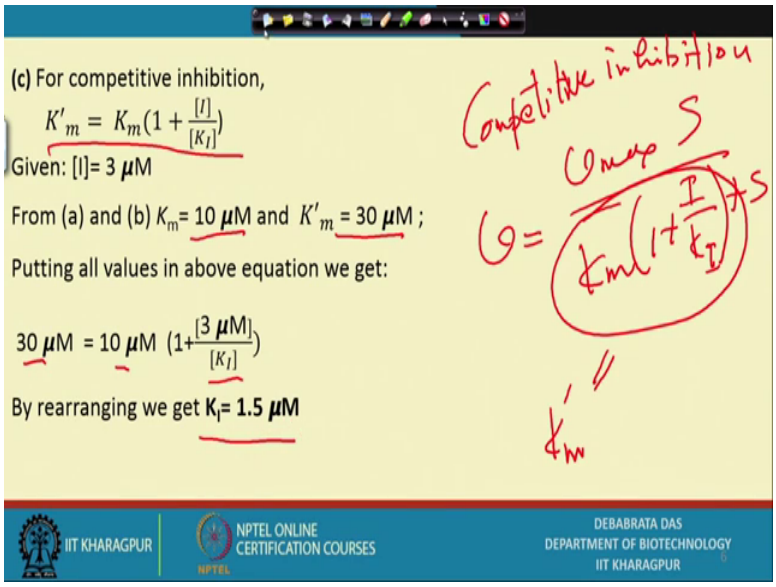

Now, next is that we shall have to find out the value of km. So, in case of competitive inhibition, we know the km dash equal to km into this.

Because we can remember that we have shown this equation in case of competitive inhibition, V max S km 1 plus I by K I plus S am I right I told you when I equal to 0 it will be becoming the Michaelis-Menten equation. So, this is the actually the km dash value this is equal to km dash value. So, we have already calculated the km dash value is the 30 micromoles and km value how much we have calculate 10 micromole. So, this we can write 30 micromoles 10 micromoles in this equation.

I that inhibitor concentration is 3 micromoles that is given. So, we can easily calculate the K I value. K I value is nothing, but that equilibrium constant between the enzyme and the inhibitor complex that you know that is the equilibrium constant, this is K I value we can easily calculate. So, in this particular problem what we try to find out we try to find out that in case of competitive inhibition, how we can determine the value of km how we can find out the new km value or due to inhibition, and from this km value we can also determine the value of K I that is the equilibrium constant between the enzyme and the inhibitors.

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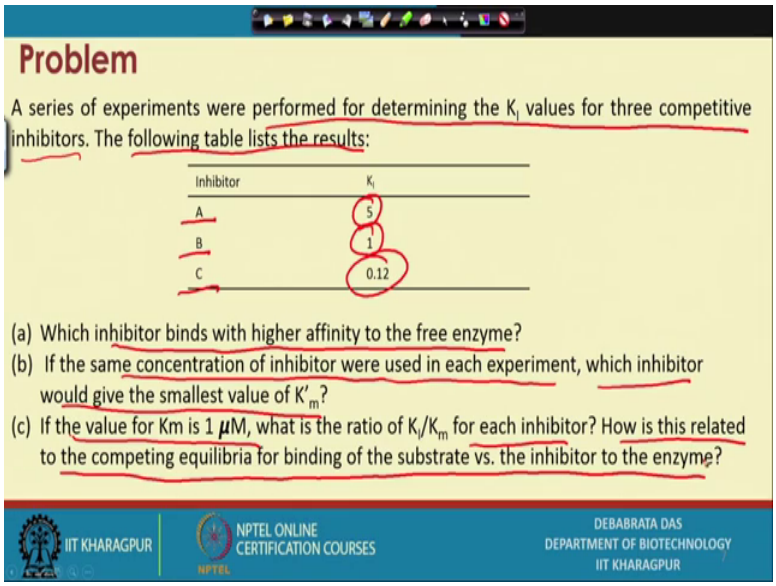

Now, next problem is also very interesting. If you look at a series of experiments where perform to determine the K I values for 3 competitive inhibitors. So, the following tables listed the results are like this, this A inhibitor B inhibitor let us assume the 3 different inhibitors and their competitive in nature, and their K I value is different one is 1 one is 5 one is 1 another is 0.12.

Now, what we shall have to find out which inhibitor binds with higher affinity with a free enzyme, this we shall have to determine. If the same concentration of inhibitor were used for each experiment which inhibitor would give the smallest value of km dash, that is the that we shall have to find out and if the value of km is one micromole, what is the ratio of K I by km the each inhibitor and how this related when the competing equilibrium for binding substrate versus the inhibitor to the enzymes.

So, how low what is the; we know that during discussion this discussing this competitive inhibitor, we come across different type of competitive inhibitor you might be knowing that one is completely competitive inhibition, another is partially competitive inhibition. Now in case of completely competitive inhibition both inhibitor and substrate they compete for the same active sites of the enzyme, but in case of partially competitive inhibition, the inhibitor compete other than the active sites of the inhibitor. So, that it makes some kind of conformational change of the active sites. So, the substrate cannot interact with the enzyme.

So, this is this with the that kind of things we want to find out how they compete with the active sites, same active sites that we are going to shot out here in this problem.

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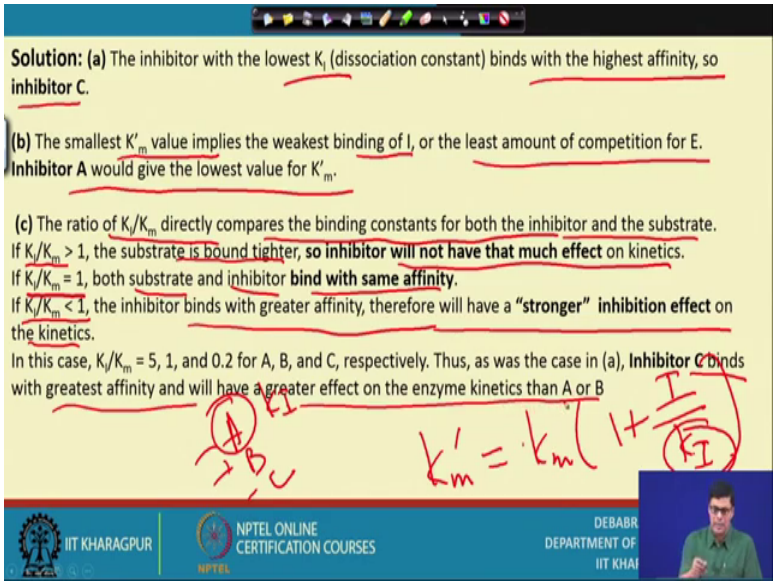

So, the first solution that if you look at that the first question that was like this, first question of the; which inhibitor binds higher affinity with the free energy. That you know we have 3 inhibitors that different K I value; where one is K I value is high and the low and this.

So, we know since it is competitive inhibition again let me write km equal to what km dash equal to what km plus I plus K I am I right. So, this is the equation that we have. Now that the question is that if I value is the inhibitor with lowest value of I binds the highest affinity. So, the inhibitor c that is the why if the I value is very low then what will happen this value will be high, and if this value is high your inhibition will be more. So, since K I value in case of thought inhibitor is K I value is lowest. So, we can always say inhibitor with the lowest K I value binds with higher affinity with the active sites of the enzyme and.

So, if you look at the second the smallest value of k km value implies the weakest binding of inhibitor the least amount of e the inhibitor a would give the lowest value of km dash so; obviously, if this value is high, then this value will be lowest. If you look at A B C 3 inhibitor we have given in this problem we have find the A has higher value of k i. So, if the higher value of K I is there then in this case the km dash value will be low.

So, I can how let say that the smallest value of K I km dash value implies the weakest binding of inhibitor or least amount of competition with enzyme and inhibitor a which will give the lowest value of km.

Now, third part is very interesting that ratio of K I and directly compares the binding constant both inhibitor and substrate. Let us say let us take the example now K I if K I by k m ratio is very high. Now K I is very high as compared to then what will and the substrate binds tighter because K I, K I is very high then this will be very low am I right then substrate will bind with the enzyme. So, inhibition will not have much effect on the kinetics. So, substrate binding with enzyme should be more.

So, this is very clear and when K I by K m is equal to 1 then this is the situation where the substrate and inhibitor binds with the same affinity because then there competing in the same way that when K I by km equal to 1, but when K I and km is less than one then what will happen that then km will be higher as compared to K I. So, so then what will happen the inhibitor binds greater affinity and so, the stronger inhibition occurs in the kinetics.

So, this is very clear because the clear cut idea that we have now how K I and k m contribute in the inhibition of the enzymatic reactions. Then in this case the k K I by k m 5 1 and minus 2 for A B C respectively, thus as was in case of inhibitor c binds with greater affinity and will have the greater effect kinetics then A B. So, you know that. So, this has this is clear from this that the inhibitor C had the greater greatest affinity that and will have the greater effect on the enzymatic kinetics.

So, I hope this problem gives you how the inhibitor takes part in the enzymatic reaction and effects the enzyme inhibitor interaction enzyme substrate interaction how is the effect.

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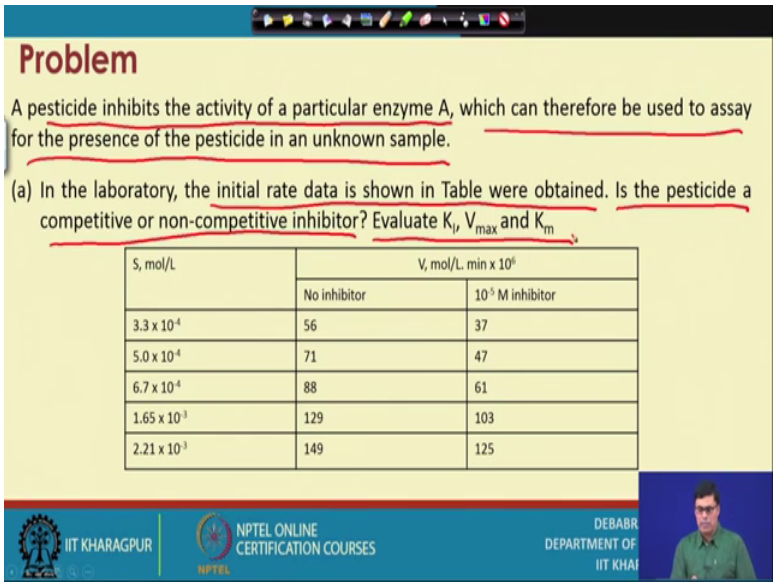

Now next problem I told you when I during my presentation that one of the application that we have in this inhibition of the enzymatic reactions, this is what is the application that we have I told you; that for the determination of complex organic molecules which is very difficult to estimate in the normal analytical techniques. I have given the example of pesticides. Pesticides the very complex chemicals and this pesticides usually we estimated with the help of h p l c h p l c we know that this is a very sophisticated instrument.

Now, by using this enzymatic reaction kinetics, it is possible to determine the concentration of pesticide. Now this particular problem deals with that, that how one pesticides and inhibit some kind of enzymes let us assume a, and how this the inhibition helps you to find out the pesticides concentration in the in your sample. So, let us go to this problem, now here the pesticide the problem is given a pesticide inhibits the activity of a particular enzyme A which can therefore, be used to assay for the presence of the pesticide in unknown sample this is the because I told you besides that I told you that inhibition has also greater role to played in the medicinal sector.

Because I told you that for the production of sulfa drug also this enzyme can be logically used and also some other purpose also it can be used. Now this is pesticide this is this is this problem deals with how the pesticides concentration can be determined. Now in the laboratory the initial rate data is shown in the table were obtained, and is the pesticide a

competitive non competitive inhibitor and evaluate the value of K I V max and k m. So, you know that there I want to tell you that we have already discussed that competitive inhibition and competitive inhibition.

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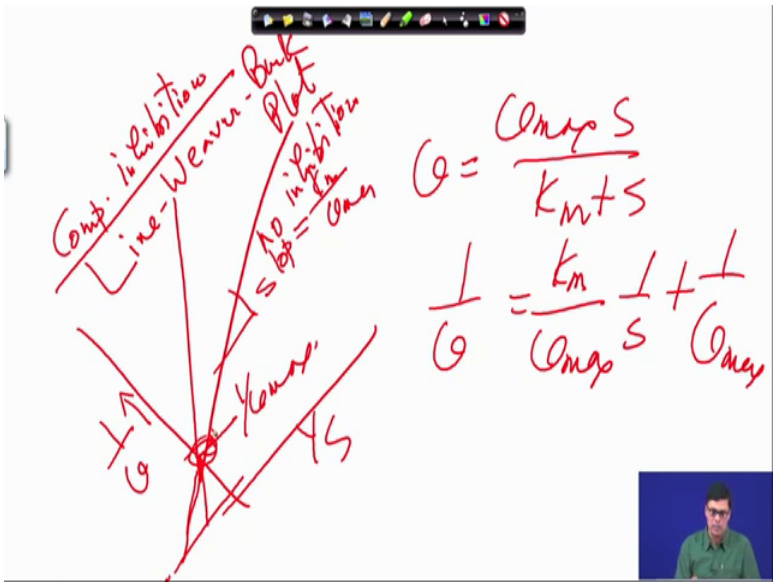

How we can find out the competitive inhibition nature with the help of Line Weaver Burk plot.

What is the Line Weaver Burk plot? This is nothing, but 1 by v versus 1 by S am I right. So, if you if you have v is the this is the Michaelis-Menten equation S k m plus S now if you write 1 by v, then you can write k m by V max equal to 1 by S plus the 1 by V max now here. So, in case of no inhibition this is no inhibition in case of no inhibition you have this plot am I right, but we know the from this equation we know the slope is nothing, but equal to k m by V max am I right and this intercept is 1 by v max.

Now, in case of in case of competitive inhibition this will be what this will be like this when 1 by V max will be intercept the point of intersection will be same, but slope will be different and this V max is constant. So, what we shall have to find out we shall have to in this in this particular problem we can in this particular problem we can we can find out that we can find out that what is the nature of plot and from the nature of plot we can find out whether the nature is coming the line weaver Burk plot.

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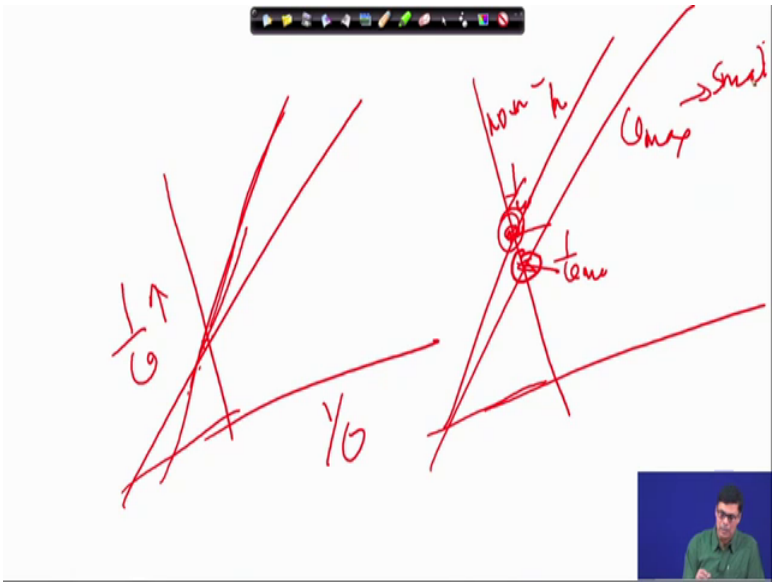

If it comes like this, the sorry this not this should go in point of intersection is like this.

Then we call it competitive inhibition, but in case of non competitive inhibition situation is different, this here it will be like this. So, this point of intersection that this point intersection that will be different this v max. So, this V max and this v max. So, this V max in case of inhibition competitive non competitive inhibition this will be higher. Since 1 by V max is higher than V max will be smaller. So, will be smaller the in case of non competitive inhibition that we can find out.

So, this that is the exactly we shall have to do from this problem that we shall have to find out that, what is the nature of the slope and from this slope we can find out whether it is competitive inhibition what is non competitive inhibition. Now in case of competitive inhibition we know the equation rate equation, and we know in case of non competitive inhibition we know the rate inhibition. So, if you use that rate equation then we can find out the value of K I V max and K m this value we can easily calculate.

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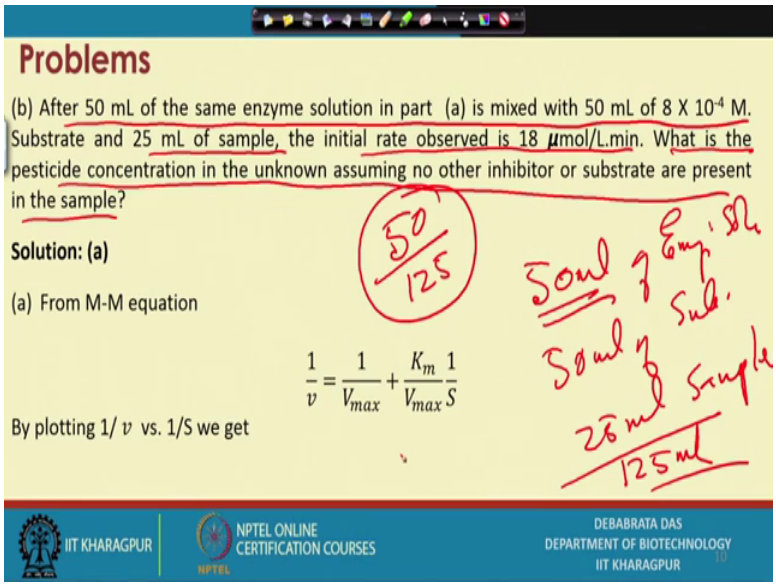

Now, second problem is very interesting. The second part what is saying that 50millilitre of the same enzyme solution of part a mixed with 50 millilitre of the substrate and 25 millilitre of the sample. The initial rate observed that is the 18 millimoles per litre per minutes what is the pesticide concentration of the unknown sample assuming no other inhibitor or substrate present in the sample, now what does that mean? Because let us try to understand what is saying that 50 millilitre of the enzyme solution, am I right enzyme solution, same enzyme solution is mixed with 50 millilitre of substrate, am I right and 25 millilitre of sample mean pesticide sample.

So, what is the total volume is coming 125 millilitre am I right. So, when we use this enzyme solution what will be dilution we have? The dilution is 50 by 125. So, whatever activity we have that is to be that concentration we have substrate concentration we have that is to be multiplied by the factor, then only then you will get the exact substract concentration. And then we can we can we can find out the exactly what is the inhibitor concentration let us see how we have solve that. Now in this Michaelis-Menten equation in the Lineweaver-Burk plot we have 1 by v equal to 1 by V max k m V max with this like this.

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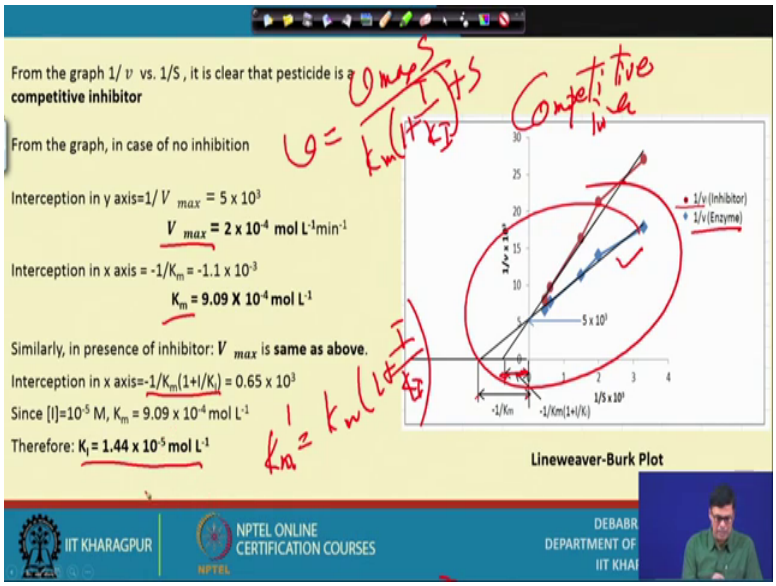

Now, when we plotted in this graph paper, we have come across this kind of a plots now this is with inhibition this is colour indicate this is inhibition and this is without inhibition. Now it is clear from this graph that this is an example of competitive inhibition am I right this is a example of competitive inhibition. So, in case of competitive inhibition what is the equation we have? V equal to V max S k m 1 plus I by K I plus S, am I right.

So, now our situation is very simple from this interaction we can easily find out the this no inhibition we can find out the value of V max and k m and from this interaction we can find out the new k m value, and once we k m value we find out we can easily find out because this is the k m dash value this is k m dash value equal to what k m plus 1 plus by K I am I right. Now here this we can directly find out this is 1 by this is with inhibition we can find out this is 1 by k m value this is and with inhibition this is without inhibition. So, we do not have to calculate from this directly from the graph we can have this value and put it and find out the value of K I, K I is not very difficult to estimate.

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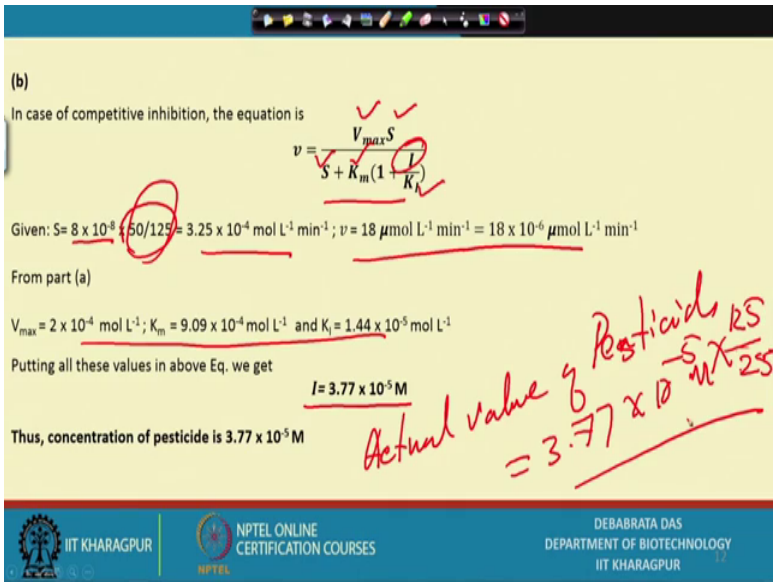

Now, then the next part as I told you the last part is very interesting, that you have to find out what is the pesticide concentration am I right now in the substrate concentration is given here the only the thing is this is the substrate concentration and this multiplied by the dilution factor 50 by 125 then we will get this. And then velocity of reaction is this then we put this in this equation we find, this equation we have we know the value of S we know K m V max value K m value K I value you know.

So, S value you know. So, I value we can easily find out and we find the I value is this. So, and this from this equation, but actual pesticide concentration will be what? Actual pesticide if we find out from this I value is this, actual pesticide actual value of pesticide cide will be what? This will be 3.77 into 10 to the power minus 5 m into 125 by 50.

Because why we have multiplied by this factor because initially we have because your pesticide solution also diluted nah, am I right. So, what a but know the I sorry I did the mistake in case of pesticide, the dilution factor will be little bit higher because we have used the 25 millilitre am I right the 25 millilitre means. So, this is to be multiplied by 25, then you will get the exact pesticide concentration that we have in the sample.

So, this is how we can easily find out that the concentration of pesticide in an unknown sample. So, today in this particular lecture we try to cover that how we can find out that the substrate concentration at different time of reaction, then we have done some kind of detailing on that how if how the interaction between inhibitor and substrate can be

determined with the help of K I values different type of K I value. And finally, we try to find out how the concentration of pesticide can be determined with the help of enzymatic reaction.

Thank you very much.