Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 59
Titration Calorimetry as a Tool to Determine Thermodynamic and Kinetic Parameters of Enzymes

We have so far thoroughly discussed the use of isothermal titration calorimetry and differential scanning calorimetry in dealing with the protein folding-unfolding or protein ligand binding reactions and we have discussed in details; how recent developments in isothermal titration calorimetry. We have helped in understanding the binding interaction between a ligand and a protein within a certain affinity range. We also discussed that if the affinity is very very high then one can even use the differential scanning calorimetry to address the binding phenomena and to extract the binding parameters quantitatively.

Today, we will discuss the use of titration calorimetry as a tool to determine thermodynamic and kinetic parameters of enzymes and we will address how the enzyme catalyzed reactions can be studied by using ITC and the kinetic parameters. When we talk about the kinetic parameters, we talk about the rate and we talk about the rate constants. Let us recall one of our earlier discussions when I mentioned that thermodynamics will tell the direction of a spontaneous change, but thermodynamics cannot tell that how much time it will take that is where the kinetic comes into picture.

So, the question is; can isothermal titration calorimetry be used to address the kinetics of a process? The answer is yes and we will take the example of enzyme catalyze reactions and demonstrate that today, but before we go into derivation of certain equations, let us discuss a little about the enzymes.

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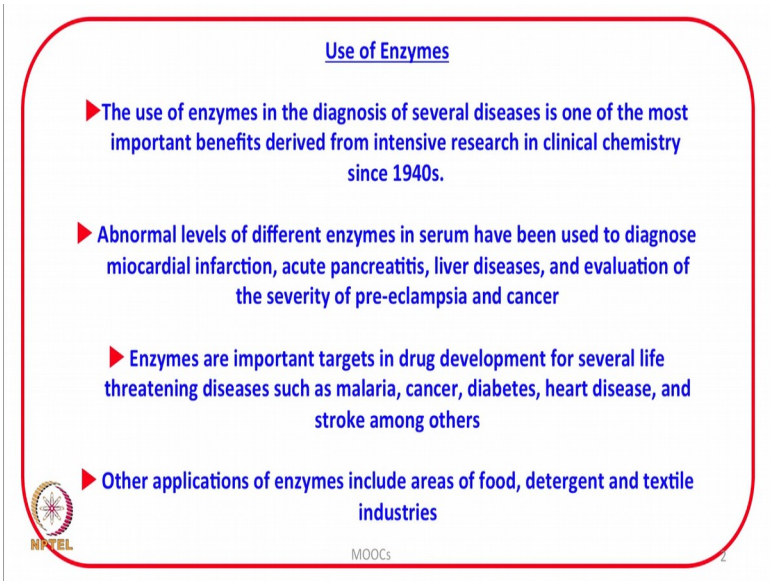

Let us take a look at the slide where are the enzymes used. Let us take a look at the first comment; the use of enzymes in the diagnosis of several diseases is one of the most important benefits which is derived from intensive research in clinical chemistry. Since 1940s, abnormal levels of different enzymes in serum have been used to diagnose miocardial infarction acute pancreatitis liver diseases and several other processes including cancer enzymes are also important targets in drug development for several life threatening diseases such as malaria cancer diabetes heart decease and strong stroke among many others.

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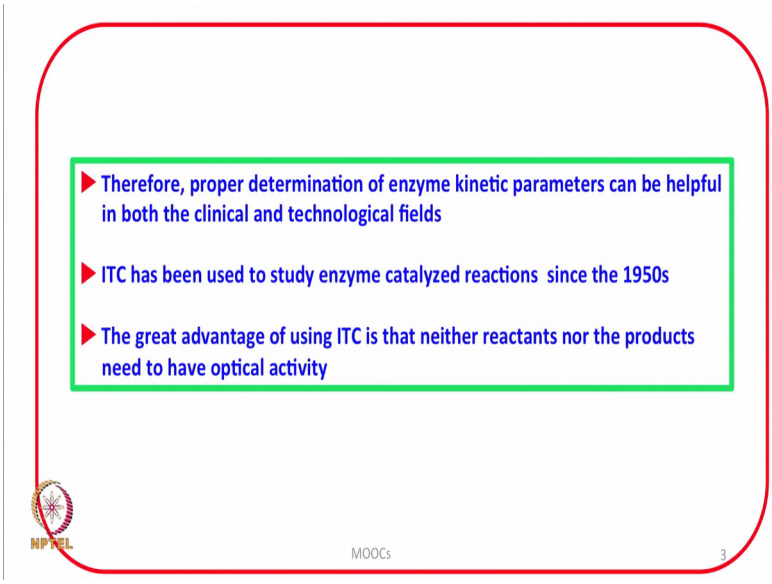

Enzymes are also very important in the areas of food detergents and textile industries, further in view of the importance of enzyme in various fields, proper determination of enzyme kinetic parameters can be helpful in both clinical and technological fields. ITC has been used to study enzyme catalyze reactions. Since 1950s, I made a comment here that ITC has been used to study the reactions pertaining to enzymes. Since 1950s, different types of titration calorimeters were available since then and with time the technological developments have happened to such a large extent, then we talk about isothermal titration calorimetry. Today we need only very very small amount of the samples.

Let us take at the look at the next comment; another great advantage of using isothermal titration calorimetry is that neither reactance nor the products need to be optically active. So, therefore, isothermal titration calorimetry offers an important platform; an important methodology even to study the rates of the reaction. Now let us derive some equations suppose that in a reaction the heat produced is Q; let us do some derivations in an ITC what we have here.

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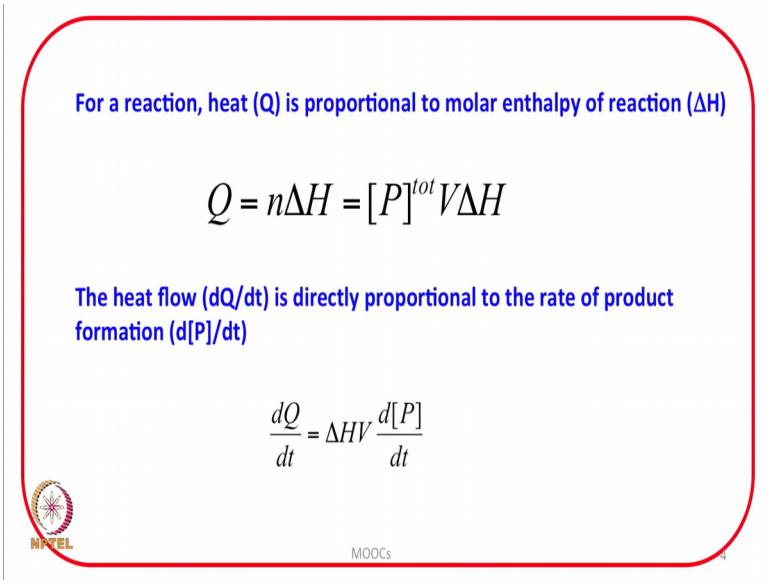

I just draw one injection peak, it is power or micro calorie per second or micro joule per second versus time initial baseline.

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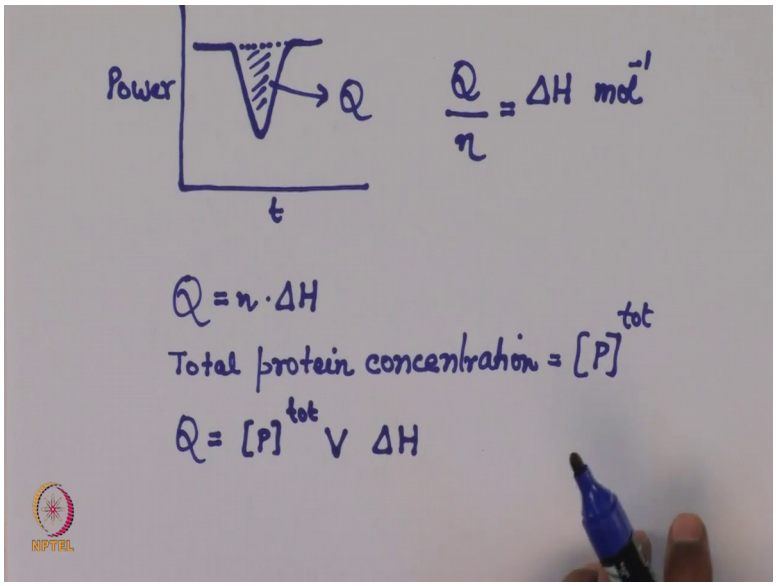

And then there is a injection and the peak and we know that this area is Q the amount of heat absorbed or amount of heat evolved. Now if I divide Q by the number of moles of the reactant added, then this becomes delta H and the units will be per mole. So, therefore, I can write Q is equal to n times delta H. Now suppose if my total protein concentration.

Let us say my total protein concentration is equal to let me write this as P total and what are the units of this P total? It is moles per liter; that means, if I want to write in place of n, it will be concentration into the volume. So, in place of n, it will be the concentration of the total product form into volume into delta H. This is the connection between the heat change or whatever is the heat absorbed or liberated. It is the total concentration of the product form which is also equal to the total concentration of the enzyme reacted; if it is 1 is to 1 binding into volume into delta H.

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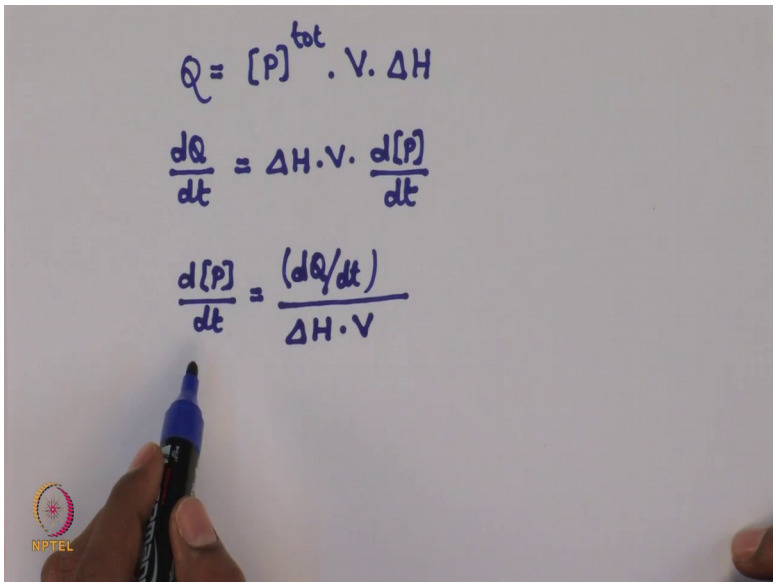

Now, let me extend this discussion; further what we have derived is Q is equal to P total into volume into delta H and now let me write a derivative d Q by d t will be equal to delta H into volume into d P. How the product is being formed with tire d P by d t and what is this d Q by d t? This is the rate of heat flow which is actually the power. Now let us take a look at the slide what I discussed is that Q is n time delta H and that can be connected to P total into volume into delta H and then if you take the derivative what I have is d Q by d t is equal to delta H into volume into d P by d t and I just discussed the derivation of this.

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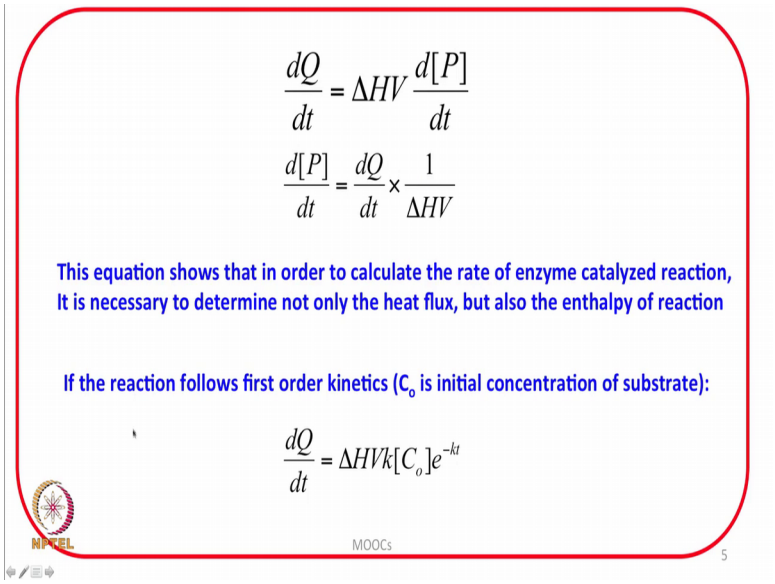

Now if I rearrange this equation I have d P by d t is equal to d Q by d t divided by delta H into V.

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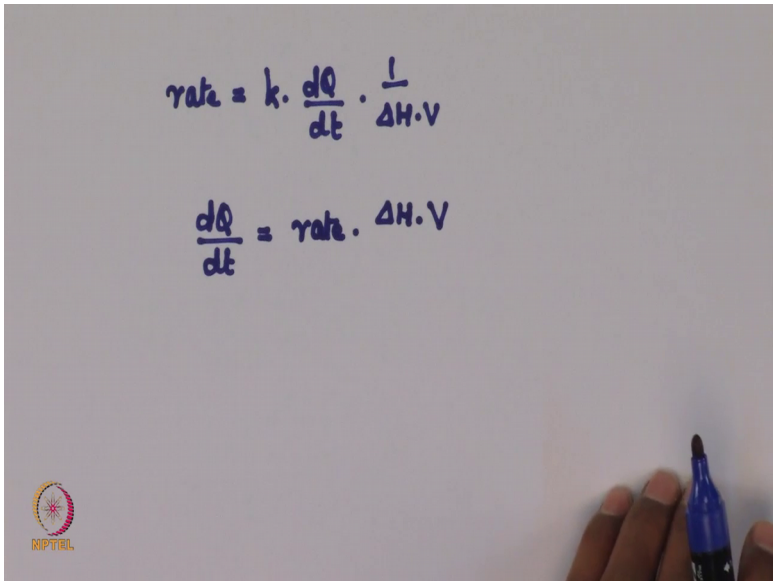

And d P by d t can be written in the form of rate so I write rate is equal to K into d Q by d t into 1 over delta H into. So, therefore, d Q by d t d Q by d t is equal to rate into delta H into V, fine.

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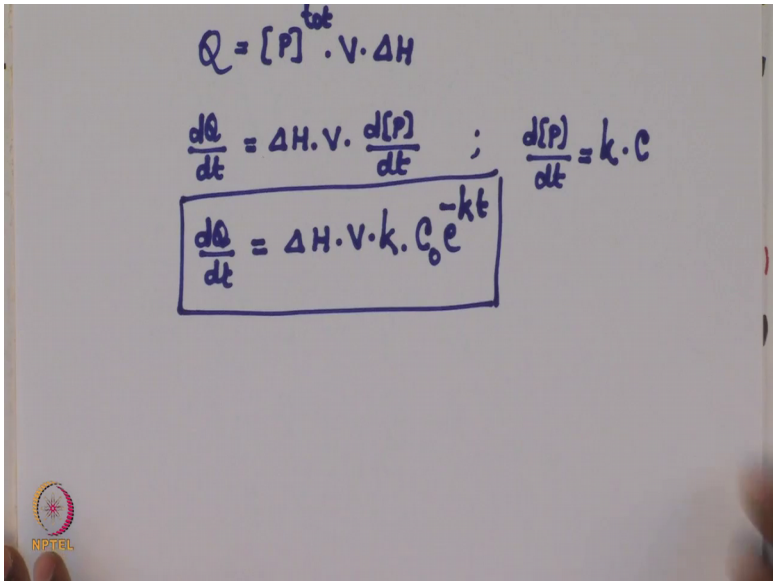

So, what I have is Q is equal to P total into volume into delta H and from this I get d Q. From d t is equal to delta H into volume into d P by d t. So, now, if I go further and

connect this d Q by d t will be equal to delta H into volume into d P by d t is equal to; what is d P by d t equal to; if I write d P by d t is equal to assuming that; this is a first order rate constant into the concentration of the protein or enzyme. So, if I write this is equal to K and for a first order reaction C is nothing, but equal to C 0 where is the initial concentration of the protein exponential minus K times t; try to understand this step where I am writing this K as K and instead of C, I am writing C 0 into exponential minus K t. This is what is expected for a first order reaction and connects the heat flow with delta H volume and the rate constant.

Now let us go back to the slide that is what I just derived that if the reaction follows first order kinetics then C 0 is the initial concentration of substrate and we can come up to this equation; that means, here if I am able to determine d Q by d t, I should be able get the rate constant for the reaction.

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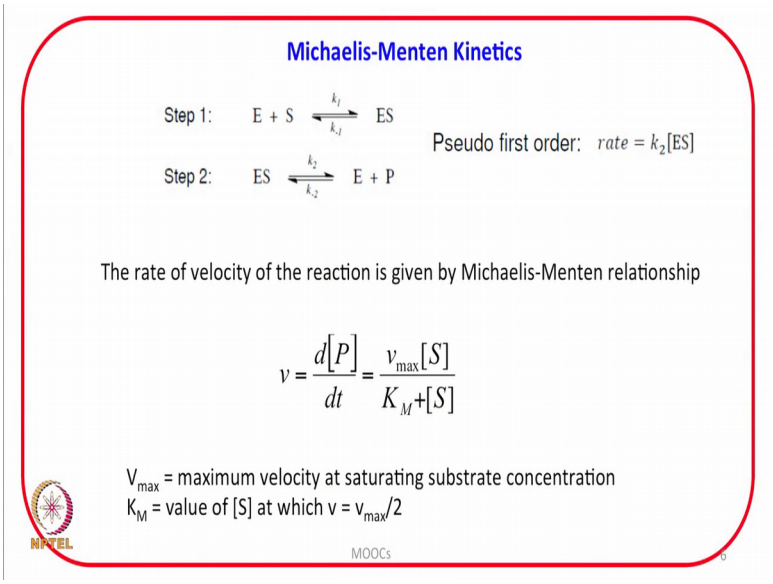

Now let us discuss a specific example of Michaelis Menten Kinetics and what is that in Michaelis Menten Kinetics, the reaction between enzyme and substrate is described by these 2 steps where step 1 where enzyme plus substrate is forming enzyme substrate complex where the rate constants are K 1 and K minus 1 and step 2 is enzyme substrate then decomposes into enzyme plus formation of products rate constants are K 2 K minus 2.

Keep in mind that K minus 2 is much smaller than K 2 and it can be; that means, the reverse reaction can essentially be ignored and only the forward reaction be considered if we consider that then our mathematical treatment will be like this.

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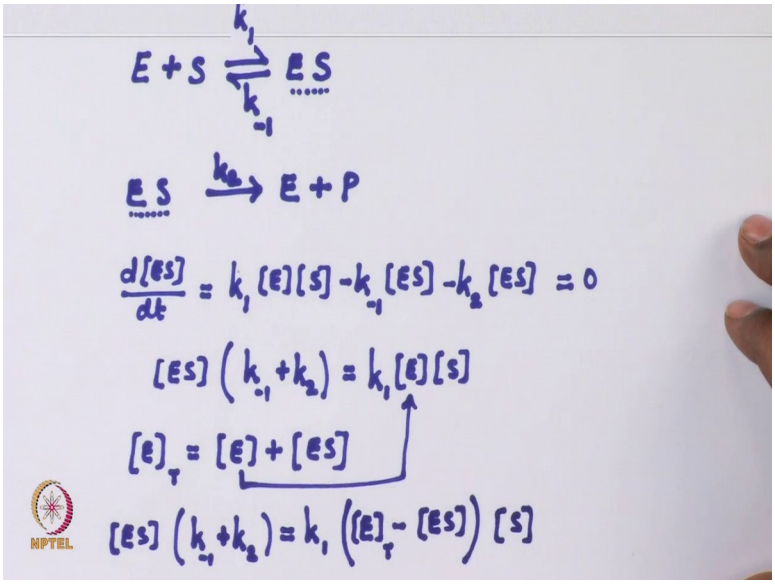

We have enzyme plus substrate this will form an enzyme substrate complex and let the rate constant be K 1 and K minus 1, then this enzyme substrate complex will dissociate into enzyme plus product and the associated rate constant is K 2. Now if we look at the intermediate in this overall reaction; the intermediate is this enzyme substrate complex and if we remember study state approximation what we use do in study state approximation is since the concentration of the intermediate remains nearly the same for most of the duration of the reaction the rate of change of concentration of the intermediate with respect to time we can set it equal to 0.

So, that is what we will do over here. We will write d E S over d t which is let us take a look at this K 1 E S is being formed. So, I will write K 1 into E into S. Now accounting for the reverse reaction with K minus 1 as the rate constant for the reverse reaction E S is consumed. So, I will write minus sign minus K minus 1 into E S and now look at this; here also E S is being consumed. So, minus K 2 into E S and this entire thing I will put equal 0 that is what I was saying that since the concentration of E S which is an intermediate here will remain nearly the same for most of the duration of the reaction its rate of change of concentration with respect to time we can set it equal to 0.

Now, form this equation what I have is if take out E S then within the bracket I have K minus 1 plus K 2 equal to K 1 into enzyme concentration into substrate concentration. Now I will make use of another fact that the total enzyme concentration has to be equal to free enzyme concentration plus that of its complex form with the substrate I can substitute for E into this and what I will get is E S into K minus one plus K 2 is equal to K one within bracket instead of E, I will use E total minus E S. This is for E and then I have left over is S.

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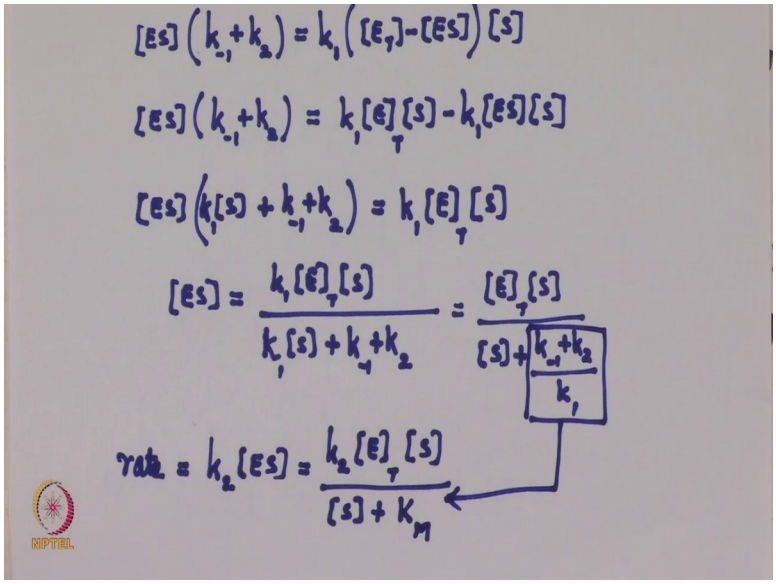

What we just derived is E S bracket K minus 1 plus K 2 is equal to K 1 E total minus E S and you have S E S into K minus 1 plus K 2 is equal to K 1 E total into substrate concentration minus K 1 into E S into substrate concentration rearrange E S, then you have if you bring over to this you have substrate concentration into K 1 plus K minus 1 plus K 2 this is equal to K 1 into total enzyme concentration into S.

So, therefore, E S is equal to K 1 into total enzyme concentration substrate concentration divided by K 1 S plus K minus 1 plus K 2 which is equal to total enzyme concentration into substrate concentration over concentration of S plus K minus 1 plus K 2 over K 1. I have divided throughout this and therefore, rate is equal to rate constant K 2 into concentration of E S that is from the definition of rate applied to equation 2. So, this is equal to K 2 into total enzyme concentration total substrate concentration over substrate concentration plus this is Michaelis Menten constant; this one.

Now let us go back to the slide and take a look at the rate which is d P by d t is V max into concentration of x over K m plus x where V max; I am writing as the product of K 2 into total enzyme concentration; why this is called V max because the value is maximum when the substrate is very very large at that time you know K M can be ignored and access will cancel and V will be equal to V max.

V max is the maximum velocity at saturating substrate concentration K m is the value of S at which V is equal to V max by 2 this you can judge by doing some little mathematics over here what we got.

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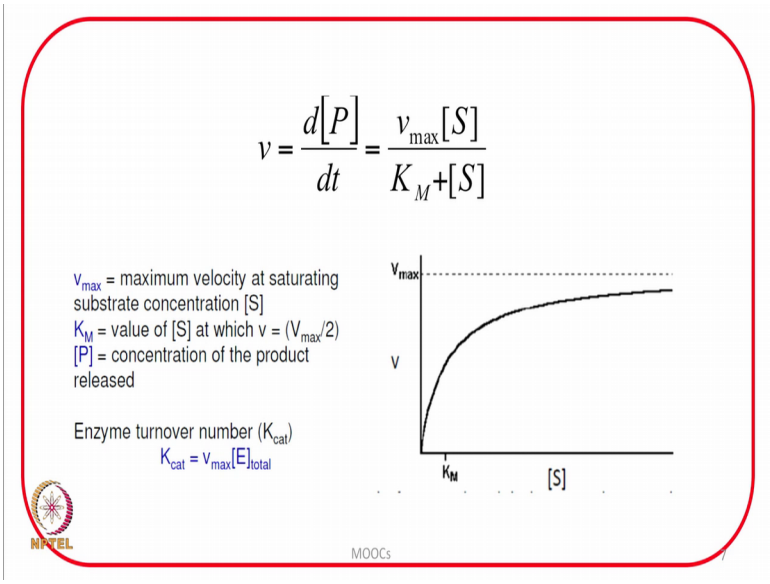

Now is the rate in terms of V max Michaelis Menten constant and the substrate concentration this is how you can pictorially present whereas, as I discussed earlier V max is actually this rate constant which is the product the V max is equal to the K cat is equal to V max into E total that is the rate constant.

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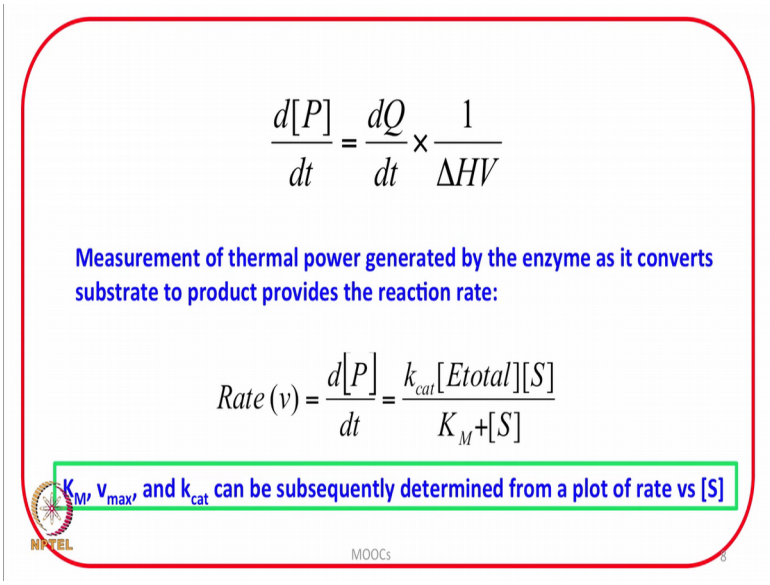

So, you remember that you just described we just derived this equation d P by d t is equal to d Q by d t into 1 over delta H into V and d P by d t. We have also just now derived is equal to K cat. K cat is the rate constant which is same as K 2 as we discussed earlier into E total into the concentration of substrate over K m plus S and now you see d P by d t can be evaluated from d Q by d t. If I know d Q by d t delta H and volume I can then get the values of the rate constant and Michaelis Menten constant.

That is what is commented over here K m V max and K cat can be subsequently determined from a plot of rate versus the concentration of S.

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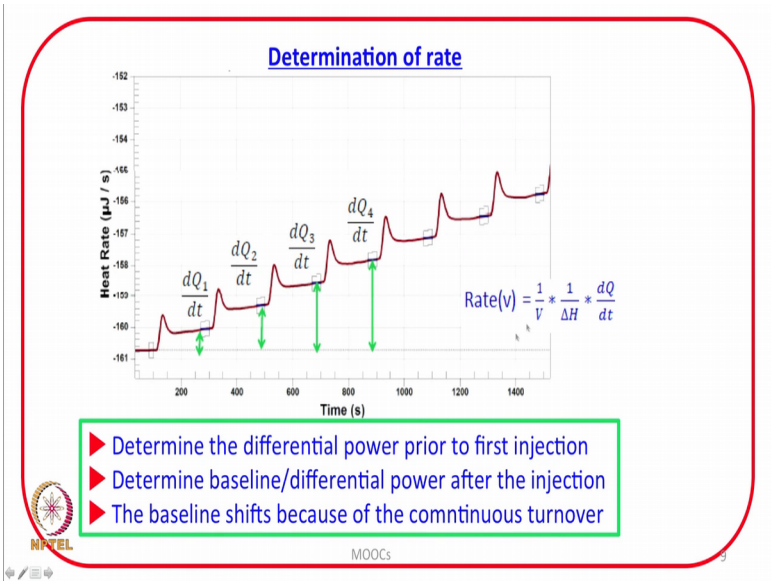

How do you determine the rate? The determination of rate requires determination of this flow of power with respect to time. So, this is how it is determined when the enzyme is added to a substrate or vice versa the initial mixing will produce some change in heat and the heat flow is given by this line d Q by d t.

After you mix the enzyme is substrate after the product is form; the new heat flow is this. After the next injection, the new heat flow is this; this is not the main event for determining the rate the main event for determining the rate is the heat rate that is the rate of flow of heat which is determined after the injection has been made you see in each case and if I know the delta H that is the area under the curve and then I can easily calculate the rate is equal to one over volume into one over delta H into d Q by d t.

And this is how to determine d Q by d t that is determine the differential power prior to first injection; determine the baseline differential power after the injection and as you notice here there is a drift in the baseline and the drift in the baseline shifts because of the continuous turnover product is being continuously form.

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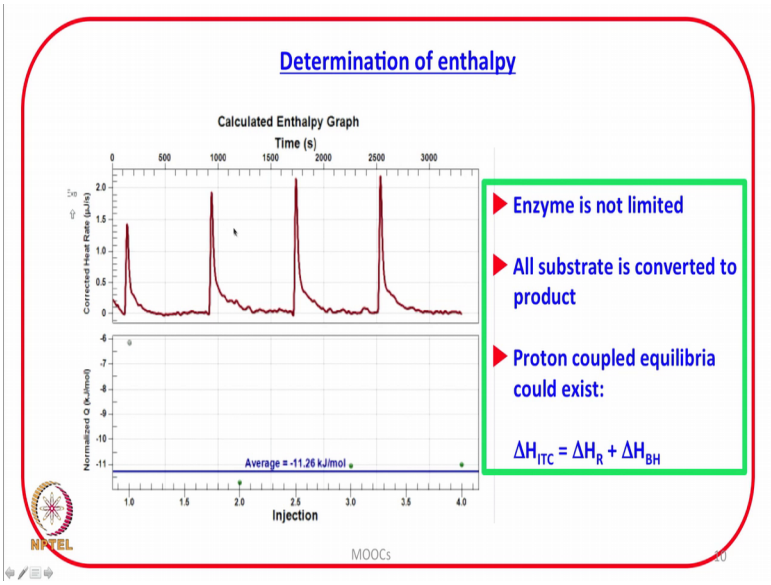

Now, how do you determine enthalpy you mix enzyme with the substrate or substrate with the enzyme the way you want to design the experiment and suppose if you are adding substrate to enzyme, you make sure that enzyme is not limited. There is excess of enzyme. So, whatever is the amount of substrate added it is converted to product then the area under the curve after proper dilution corrections can be divided by number of moles and that will give you the value of the delta H as you see on the average as long as enzyme is not in the limiting amount on the average the value is minus 11.36 kilo joules per mole in this specific rate in this specific example.

So, area under the curve divided by number of moles of the substrate added will be equal to delta H. The assumption is all substrate is converted to product and as I discussed earlier you have to always apply for the buffer ionization effect otherwise the result will be erroneous. So, delta H b; H corresponds to n times the standard enthalpy of buffer ionization S. Therefore, if I know delta H, if I know the rate of heat flow, I can derive the kinetic parameters associated with the enzyme catalyze reaction. Once I know the rate constant then the activation enthalpy and activation entropy of the reaction can be determined from Eyring equation.

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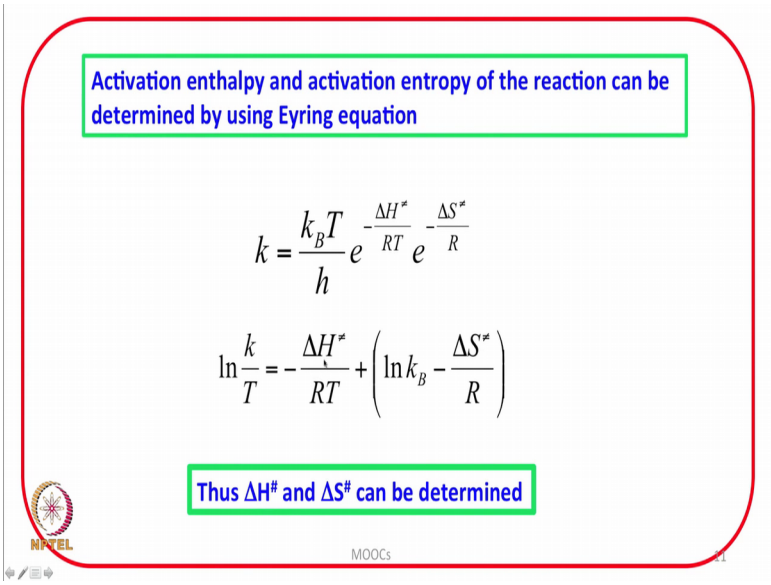

Let us take a look at what is Eyring equation. This is Eyring equation where rate constant is connected to Boltzmann constant temperature Planck's constant and the activation enthalpy of reaction and activation entropy of reaction.

If you take the logarithm on both sides and rearrange the equation; take logarithm rearrange, you will get log K by t is equal to minus delta H by R t delta H of activation plus log K b minus delta S of activation by R. So, if you plot log K by t versus 1 by t, the slope and intercept will give you the values of enthalpy of activation and entropy of activation. So, what we discussed in this lecture is that isothermal titration calorimetry is not just important in getting the thermodynamics of the process; it also very accurately very nicely helps us in addressing the kinetics of the process.

We can get the rate constant of the process. We can determine the rates of the reaction and along with that these thermod; these kinetic parameters we can also determine the activation enthalpy and activation entropy of the reaction thus demonstrating that isothermal titration calorimetry is also an important tool in addressing the kinetics of the reaction.

Thank you very much.