Chemical and Biological Thermodynamics: Principles to Applications

Prof. Nand Kishore
Department of Chemistry and Biochemistry
Indian Institute of Technology, Bombay

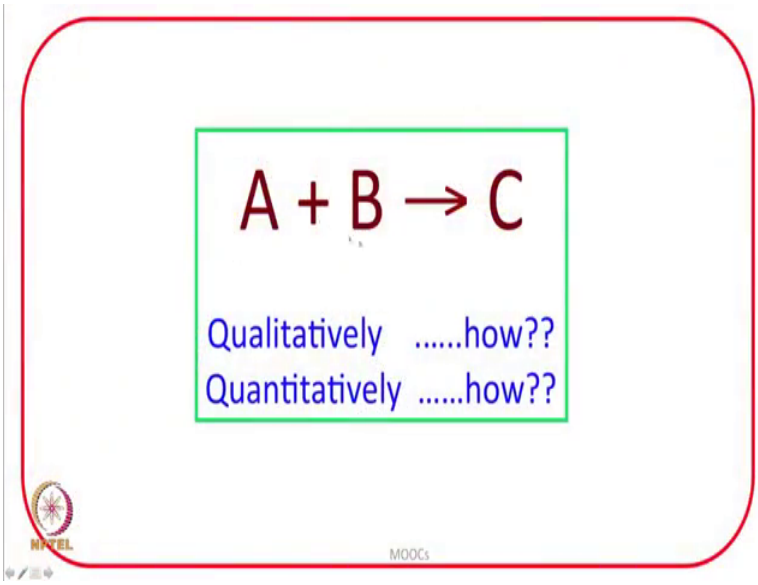
Lecture – 45
Isothermal Titration Calorimetry (ITC)

In this lecture we are going to discuss another very important technique which is used to determine the heats of reactions in which the amount of heat absorbed or evolved is very very small and that technique is called isothermal titration calorimetry as we earlier discussed that calorimeters have been there in the market since 18th century. However, with the time the technology developed and it was always felt that we should have calorimeters available which are capable of measuring the heats of reaction of the order of micro joules.

Most of the biological interactions taking place in the living systems are weak the heats of interactions can be very very small and therefore, if you want to address those phenomena those interactions we need very sensitive micro calorimeters earlier we discussed differential scanning calorimetry. And today we have differential scanning calorimeters which are very sensitive which require only a few micro grams of e of a sample to give the values of the transition temperature enthalpy of unfolding entropy of unfolding another thermodynamic quantities to a very good degree of accuracy emphasis.

And now let us discuss a technique which can give us how much heat is released or absorbed when 2 interactions 2 specious are or more specious are interacting.

(Refer Slide Time: 02:45)

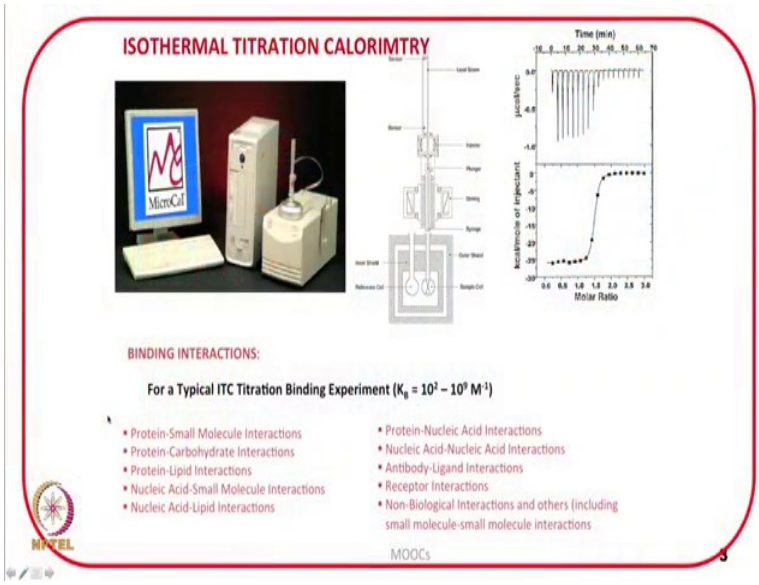


Let us take a look at this slide and we just start with an example of A plus B forming C and whenever we talk about this kind of reaction the questions can be can we answer qualitatively how this happening qualitatively means A is mixed with B and it forms to C and qualitatively we establish that there is A, there is B, there is C in the mixture and when we talk about quantitatively. Quantitatively means we must assign the numbers here particularly if I talk about this reaction in order to make quantitative I must be able to tell how many moles of A are interacting with how many moles of B and how moles of C are been informed and. Secondly, how much heat is liberated or how much heat is absorbed when this reaction takes place.

And the third thing is what is the equilibrium constant of this process and if I know the equilibrium constant I can immediately get the change in gives free energy standard gives free energy and if I know now the heat if I know delta g naught I can calculate delta s naught; that means, by making it quantitative I am talking about establishing stoichiometry of the reaction establishing the value of equilibrium constant of this reaction determining or measuring the values of heats of interactions and entropy change once I have all these thermodynamic quantities I can describe the process both qualitatively and quantitatively and then when I talk about the interactions when 2 specious are interacting there is some amount of heat is either released or absorbed.

And there are certain interactions which release heat and certain types of interactions absorbiate and therefore, if we are able to measure the heat changes very very precisely; that means, we can connect the heats liberated or heat absorbed with nature of intermolecular interactions and these things we will be discussing in details which are of tremendous academic and industrial importance. And we will take up in the future lectures isothermal titration calorimetry today has become a popular choice amongst the biologist biochemists and chemists who are interested in understanding various types of interactions in which the amount of heat changes or the heat liberated or absorbed is medium to very very low why isothermal titration calorimetry is important.

(Refer Slide Time: 06:43)



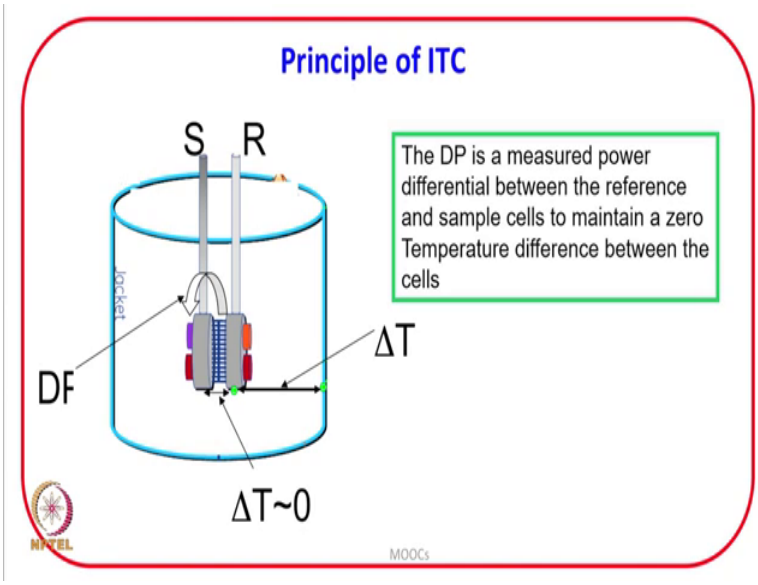
Let me just read out some of the areas where the isothermal titration calorimetry has been applied very very successfully.

Typically the binding constant whether you write K B or you write K A; K A is use for association constant K B in literature many times K B is used as binding constant physical chemist would like to express in terms of K A association constant or K B or binding constant or you can simply say K biochemist prefer to express in terms of dissociation constant K D and we know that K A and K D are reciprocal of each other. So, therefore, we should not be confused when we are talking about K A or we are talking about K D whether we talk about K or K A or K B or K D the meaning should be very clear to us now let us take a look at isothermal titration calorimetry has been very

successfully used to study protein small molecule interactions protein carbohydrate interactions protein liquid interactions nucleic acid small molecules interactions nucleic acid lipid interactions protein nucleic acid interactions nucleic acid nucleic acid interactions antibody Ligand interactions receptor interactions as well as non biological interactions and others including small molecules small molecule interaction.

In short if I say when anything is mixed with another thing there is going to be some heat change and if we are able measure to able that heat change we can connect that heat change with the mechanism of the process and that is what our purpose here in these discussions will be to understand the process is both qualitatively and quantitatively in terms of the thermodynamics involved in it.

(Refer Slide Time: 09:10)

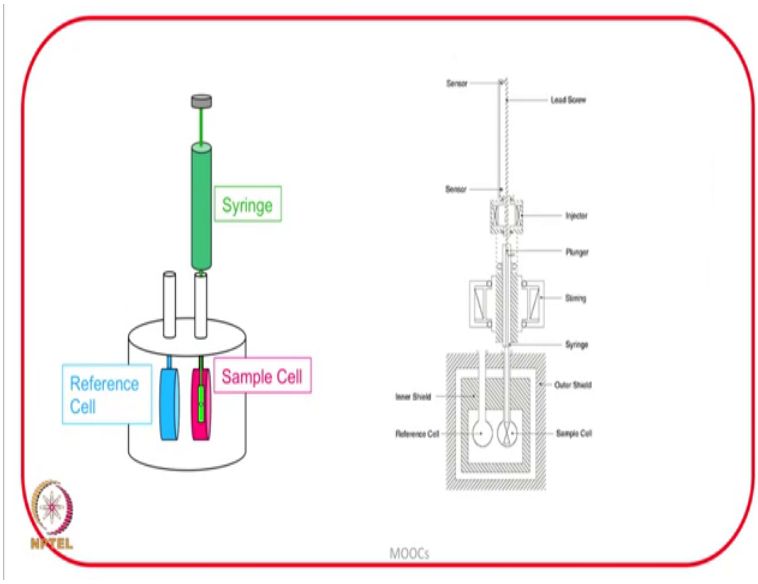


So, therefore, let us first now discuss the principle of ITC principle of isothermal titration calorimetry in an isothermal titration calorimeter there is a sample cell and there is a reference cell constant power is supplied to both the cells to maintain the temperature difference between the cell 0 and everything is to be kept isothermal because look at the name title isothermal titration calorimetry.

Unlike in differential scanning calorimetry remember in differential scanning calorimetry the temperature was changed the sample was heated at a constant scan rate in isothermal titration calorimeter temperature is not changed temperature is kept constant. So, delta T is maintained 0 and then suppose if any reaction takes place in the sample cell that

reaction can be exothermic or endothermic if the reaction is exothermic temperature will tend to increase if the reaction is endothermic temperature will tend to decrease. Therefore, additional power is supplied to one of the cells to maintain delta T equal to 0 and that differential power is measured that is what is commented over here the differential power DP is measured power between the reference and sample cells to maintain A 0 temperature different between the cells this is the basic principle of isothermal titration calorimetry.

(Refer Slide Time: 11:27)



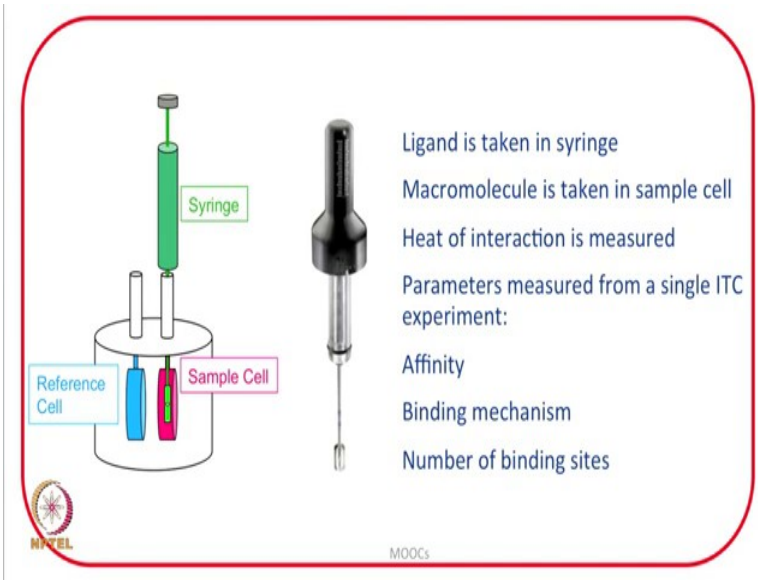
And everything is protected by an adiabatic shield let me discuss in a little bit more details what you have in an ITC is a sample cell a reference cell and in the sample cell an injection assembly can be inserted as we can see here there is an injection assembly over here this is a syringe in which the Ligand can be filled you are filing the Ligand in this syringe. And at the end of this syringe you see there is a small needle and there is small pedal over here at the end with a hole this pedal will act as a stirrer because this entire thing this can be rotated and the solution when the plunger is pushed the solution can come out of here and get mixed with the content of the cell same thing we can see in the figure in a little more clearer way that within these adiabatic shields you have a sample cell you have a reference cell.

And in the sample cell you have this injection assembly inserted through which the Ligand can be injected and the end of this injection assembly is in the form of a pedal we

can see here and this entire assembly that is that syringe can be rotated. So, that as soon as a small injection is made and the solution is delivered outside it is immediately mix with the surroundings.

So, this is how the thing look inside the calorimetric cells the reference will contain usually the buffer and the sample cell will contain the sample of interest or the macro molecules of interest and the syringe will have the Ligand.

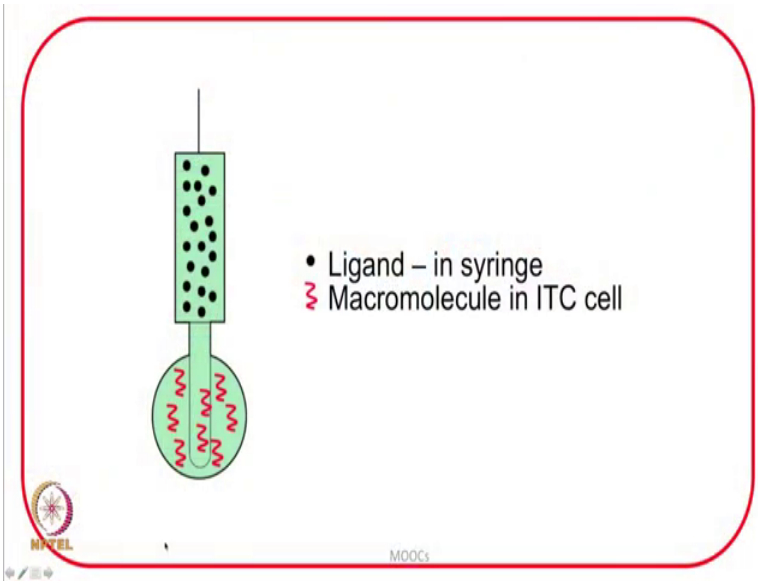
(Refer Slide Time: 13:47)



let me show another picture the syringe can either be affects kind of syringe that the whole assembly come out of the cell where the syringe cannot be dismantled or the syringe can be like this and you can clearly see here; here this assembly from this assembly the syringe can be taken out that we can show in other lecture. But you see here the needle which is extending out of the syringe has the sought of pedal at the end and there will be a hole in the pedal through which the solution can be delivered and that is what is commented in here Ligand is taken in the syringe macromolecule is taken in the sample cell heat of interaction is measured and the parameters which we eventually get from a single ITC experiment are affinity; affinity means the binding constant or equilibrium constant binding mechanism and number of binding sites.

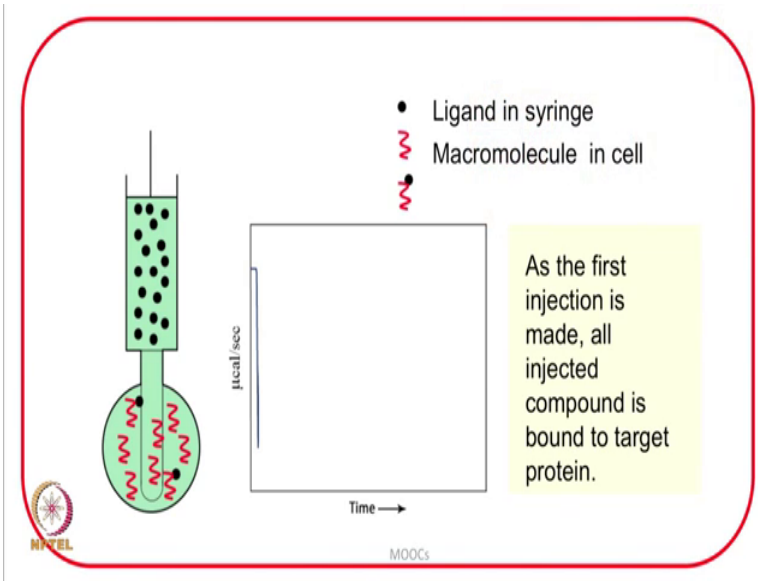
We will discuss the principle in a little bit more details now let us concentrate on the sample cell.

(Refer Slide Time: 15:24)



And consider this as sample cell in which you the red this moieties take this as macromolecules in the ITC cell and this is the injection assembly and this dark circles the black circles let us consider these as Ligand molecules in the syringe and what we want to study is how these molecules when injected inside will interact with the protein bind to the protein and lead to changes in the heat signals.

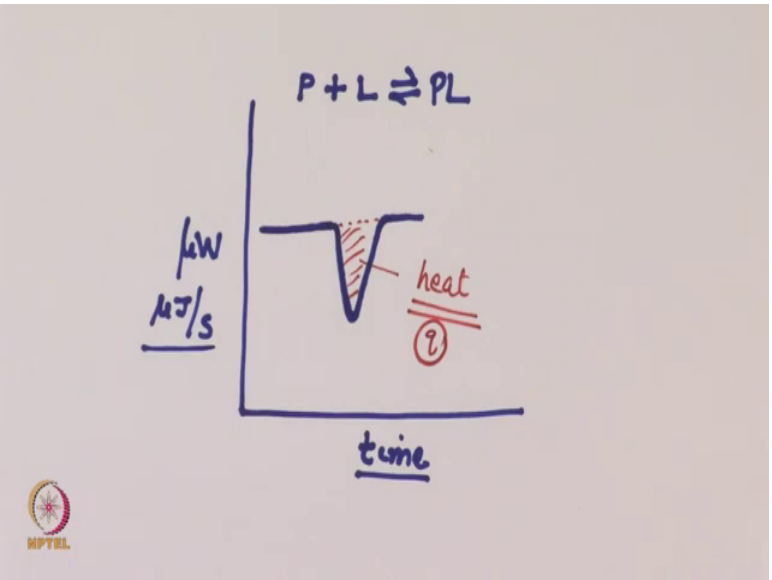
(Refer Slide Time: 16:13)



let us take a look at this as we just discussed micro calorie per second or micro joules per second suggests that we should talk in terms of joules, but many journals will except

even in calories, but whatever units we used micro calorie per second is power. So, you see initially when no injection was made what you have is a horizontal line let me explain this a bit more details.

(Refer Slide Time: 16:55)

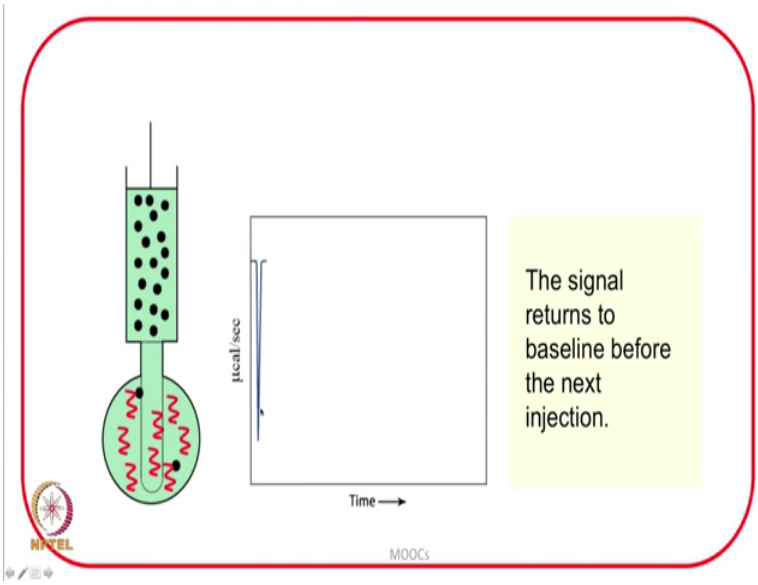


What we have either micro calorie per second or micro joule per second let me just represent microwatts or here I will have time let us say I also write this as micro joules per second initially when no a no Ligand has been added to the sample cell no additional power is supplied. So, the output is 0 means there is no differential power no additional power needs to be given either to the sample cell or to the reference cell. So, you get a straight line when you make the first injection that when you when you add a little bit of Ligand into the protein solution this reaction will start P plus L this reaction will start and depending upon whether the reaction is exothermic or endothermic additional power will be supplied to either the sample cell or the reference cell or vice versa means you can supply less power to the sample cell the reaction is exothermic the idea is to maintain the temperature difference equal to 0 and entire things should kept isothermal.

So, that differential power is monitored and after the reaction is over it goes back now you see y x is micro calories per second or micro joules per second x x is time therefore, the area this area within the line and the curve is nothing, but it is heat q now let us go back to the figure and that is what I was saying after some of the Ligand is added and it

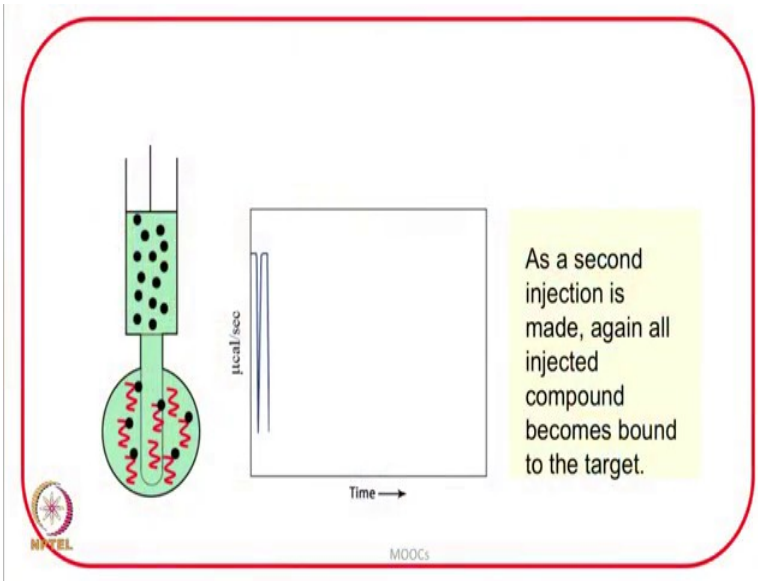
attracts with the protein additional power or less power needs to be supply to the sample cell and it is observed.

(Refer Slide Time: 19:37)



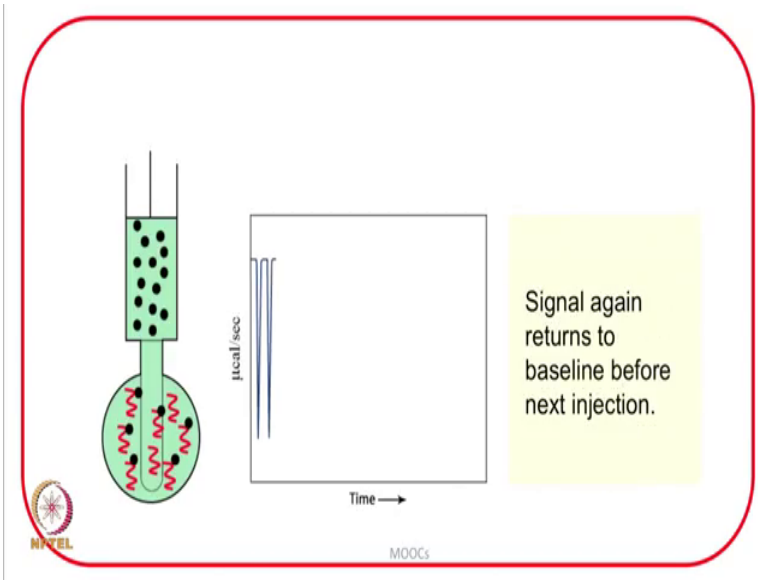
In the form of a deviation and after the reaction is over it comes back to the original now you see each protein will haves a binding site or different proteins will have binding sites for different Ligands and if we take a large amount of protein in the cell and add only a little bit of Ligand; the Ligand will go and bind to the protein at the binding site, but since there are still so many other protein molecules available with the binding sites available.

(Refer Slide Time: 20:30)



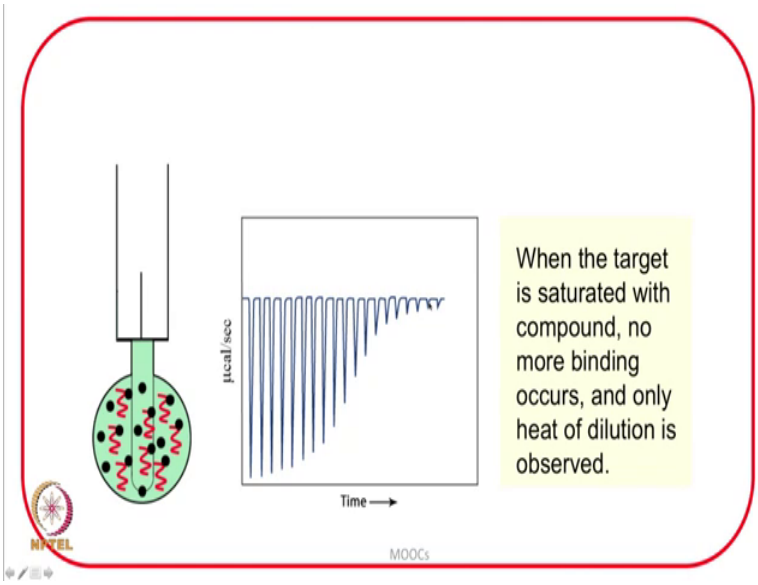
When the next injection is made or when some more Ligand is added it will again go and bind and saturate eventually it will saturate all the binding sites.

(Refer Slide Time: 20:23)



So, now let us take a look at when next injection is made again there is a deviation and you can see in the figure more and more this dark circles are interacting with the protein, but there are still proteins with free binding sites available.

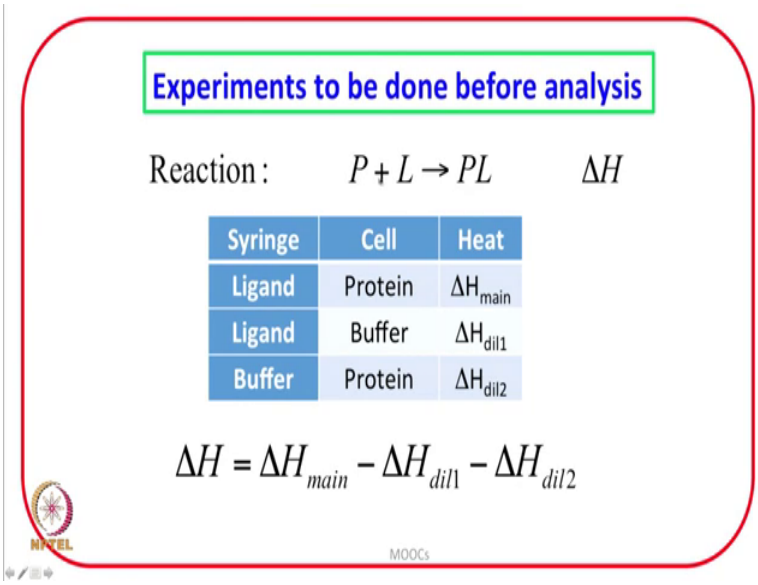
(Refer Slide Time: 20:45)



And you continue the titration and eventually after all the binding sites are saturated the amount of heat change towards the end is nearly 0 and you see the entire thing represents the binding profile area under each area between the line and this whatever the peak if we calculate all the areas and just plot the areas versus injection number. We will get binding profile and area is equal to heat and I will discuss this a bit later that is how this areas can further be utilized to calculate the values of delta H.

But let us first try to understand the principle very very clearly the principle is that when a small amount of Ligand is added to a protein solution either an exothermic reaction or endothermic reaction will take place accordingly more power or less power will be supplied to the sample cell and the differential power is noted at each injection and that is what seen in this figure that at each injection the differential power is monitored and when all the binding sites are saturated you see overall the change is turning towards 0. So, this is a one complete experiment for the reaction of a Ligand with a protein now the question is what experiments to be done before the analysis before we actually go for analysis how many experiments needs to be done.

(Refer Slide Time: 22:55)



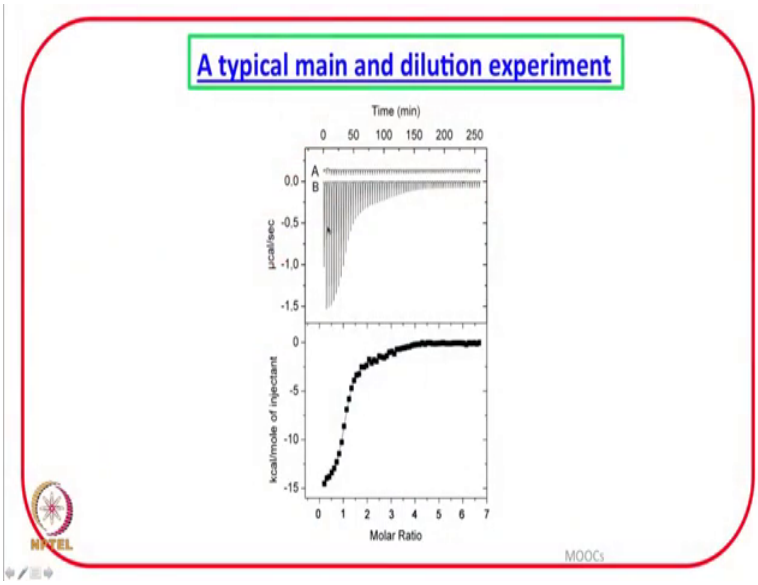
We are interested in understanding the interaction with a Ligand of a protein to form a complex P plus L is forming P L and the associated change in enthalpy is delta H at is what we want to experimentally determine, but let us examine carefully that the protein is prepared in buffer Ligand is prepared in buffer. So, therefore, along with this main reaction that is Ligand binding with the protein the protein also interacts with the buffer that is a small amount of dilution and the Ligand also interacts with the buffer when this is injected that is a dilution.

Therefore, heats of dilutions must be determined and corrected for a complete experiment before the analysis of the data is done the following should be done the first experiment is you take Ligand in the syringe protein in the cell and let us say the associated value that we measure is delta H I will explain how do how to get this delta H the second experiment should be Ligand in the syringe and buffer in the protein and what we will monitor what we will measure is the enthalpy of dilution of the Ligand delta H dilution 1.

The next experiment should be buffer taken in the syringe and protein in the cell this is the dilution of the protein and the corresponding change is delta H dilution 2 so; obviously, these enthalpies these heat changes delta H dilution one and delta H dilution 2 should be subtracted from the main delta H to get the accurate value of the heat of reaction or accurate value of the enthalpy change associated with the prospects.

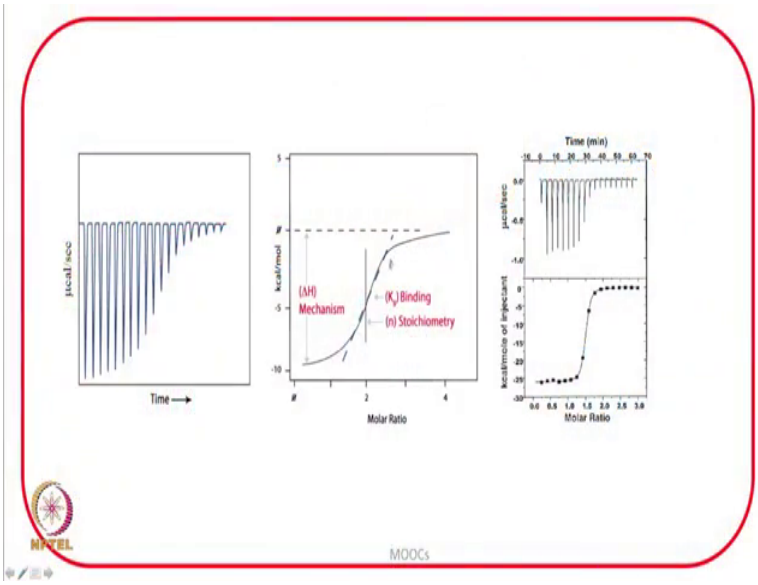
Therefore, delta H this delta H which is associated with protein plus Ligand formic P L will be delta H main minus delta H dilution one minus delta H dilution 2 that is we need to do these three one 2 three experiments in order to get value of delta H and let me show in this figure that area under each peak is represented by this dot see it is kilo calorie per mole or you can plot kilo joules per mole.

(Refer Slide Time: 26:10)



Area under each peak is represented by this dot and this is how the heat liberated varies this is the main experiment heat changes associated with the main experiment and a with the dilution experiments and let us appreciate the heats associated with the dilutions are very very small compare to the main heats and that is how it should be because the blank effect should be minimum. So, what do we get overall if this is the binding the profile.

(Refer Slide Time: 27:02)



Which is an integrated you know if we plot heat evolved at each step against molar ratio molar ratio is number of moles of Ligand divided by number of mole of protein we can get delta H delta H is the total heat liberated and we can get the binding constant the as I will explain in details later on the slop is associated with the value of binding constant and how this binding constant is extracted that also I will explain in the next lecture and we can get the stoichiometry of a reaction you see the stoichiometry here is shown in the same way as we discuss during the acid base titration.

The stoichiometry how many number of moles of Ligand are enough to saturate the protein and another example the conversion of this area into the heat integrated heat profile upper one is row data following is the integrated heat profile and the solid line represent the best fit to the experimental data. So, what we have discussed in this lecture is the principle of isothermal titration calorimetry that is how the isothermal titration calorimeter measures the amount of heat associated with the interaction of a Ligand with a protein. And also that it is the area under the each peak which represents the amount of heat liberated or absorbed and when this amount of heat liberated or absorbed is plotted against times or against injection number or against molar ration lot of thermodynamic quantities is can be determined which are those thermodynamic quantities the enthalpy of reaction the binding constant stoichiometry change in standard gift free energy and change in entropy that to from a single experiment all these things we will discuss in detail in the next lecture.

Thank you very much.