Enzyme Science and Technology
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Module - VII
Enzyme Substrate Interactions
Lecture - 33
Enzyme-Substrate Interactions (Part-III: ITC)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course, enzyme science and technology. And so, far what we have discussed? We have discussed about the chromatographic method; we have discussed about the spectroscopic method and we have also discussed about the electrophoretic method in the previous module.

And when the enzyme and substrates are interacting with each other, there are several parameters which need to be full-filled, right? One of the critical parameter is the geometric constraints or geometric complementarity. So, the three-dimensional structure of a substrate should match with the three-dimensional cavity what is present within the enzyme structures.

Apart from that, it also should be able to you know make the robust interactions with the residues present within the protein structure. And then at the end, the enzyme is always been recognizing or making a preferring a particular stereo specificity of ligand versus other stereo specificity.

So, in this context, we have discussed about the different types of techniques, what you can actually be able to use. And what we have discussed so, far, when the enzyme is interacting with the substrate, it is making the enzyme substrate complex. And in in this process, it is actually inducing the different types of conformations or different types of modulations into the enzyme structures.

And all these modulations can be studied with the different types of techniques. For example, if it is modulating the size, it can be studied with the help of the electrophoresis and as well as the gel-filtration chromatography. Whereas, if it is inducing additional charge or it is masking or giving the hydrophobic nature to the enzyme, that can be

mapped into the ion exchange chromatography or the hydrophobic interaction chromatography.

So, apart from these modifications, when the enzyme interact with the substrates, it also you know results into the either the generation of heat or the absorption of heat. You know that the enzyme is actually interacting with the substrate and after it is forming the enzyme-substrate complex, from the enzyme-substrate complex, it is actually converting into the product.

And when the substrates bind into the enzyme cavity, it is making few bonds and it is actually you know breaking some bonds. And you know that the formation of bond or the breaking of bond is always being associated with the energy or the exchange of energy right.

So, this exchange of energy can be studied in a technique which is called as the isothermal titration calorimetry and which actually going to allow you to study when you are adding a enzyme or when you are adding a substrate into the enzyme, whether there will be a generation of heat or where there will be a absorption of heat. So, let us start discussing about the isothermal titration calorimetry.

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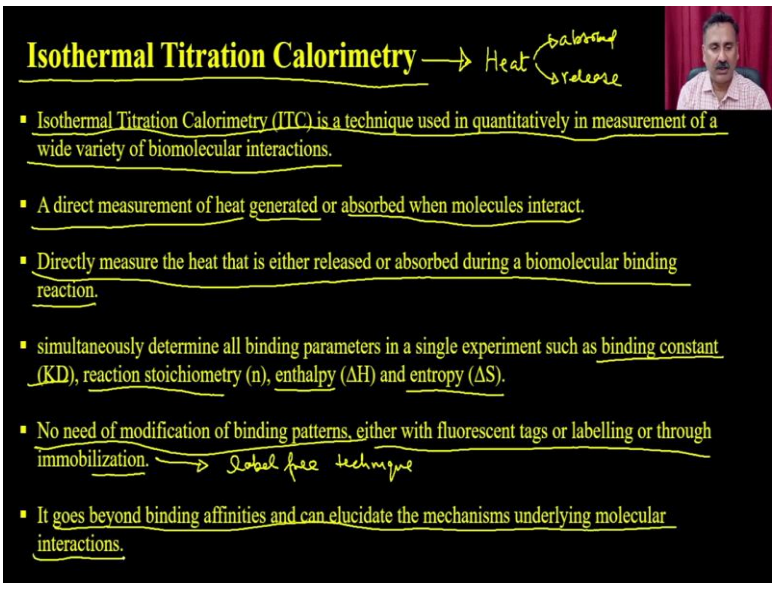

So, isothermal titration calorimetry as the name suggest, it is actually a technique which is going to which is going to measure the heat, whether it is being absorbed by the

system or it is actually going to be released from the system ok. So, the isothermal titration calorimetry is a technique used in a quantitatively in a measure of a wide variety of biomolecular interactions.

A direct measurement of heat either generated or absorbed when the molecule interacts right. And directly measure the heat that is either being released or absorbed during a biomolecular binding reactions and that is how these amount of heat what is going to be released or absorbed can be correlated to the binding studies.

So, all binding parasol, when the you can actually be able to measure the heat simultaneously, you can also be able to measure the other binding parameters such as you can be able to calculate the binding constant or KD, you can actually be able to calculate the reaction stoichiometry, you can be able to calculate the enthalpy and the entropy of that particular reaction.

Since this is a label free technique, there is no need to of modification of binding pattern, binding pattern either with fluorescent tag or labelling or to the immobilization. This means, this is a label free technique ok and this is a major of the major advantage why you have to use the ITC for measuring the interaction between the two biomolecules. It goes beyond the affinity and can elucidate even the mechanism underlying the molecular interactions.

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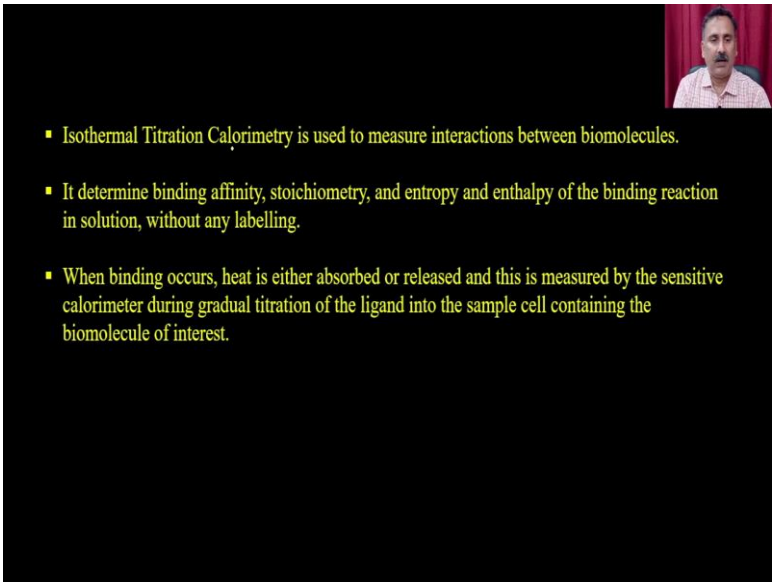

Apart from that, the isothermal titration calorimetry is used to measure the interaction between the biomolecules. It determine the binding affinity, stoichiometry, enthalpy, entropy, and the binding reaction in solution without any labelling. When the binding occurs, heat is either absorbed or released and this is measured by a sensitive calorimeter during the gradual titration of the ligand into the sample cell containing the biomolecule of the interest.

So, what you require is, you require a reaction vessels where you are actually going to perform the reactions and in this process in that particular vessel, you are actually going to measure the amount of heat what is going to be absorbed or what is going to be released.

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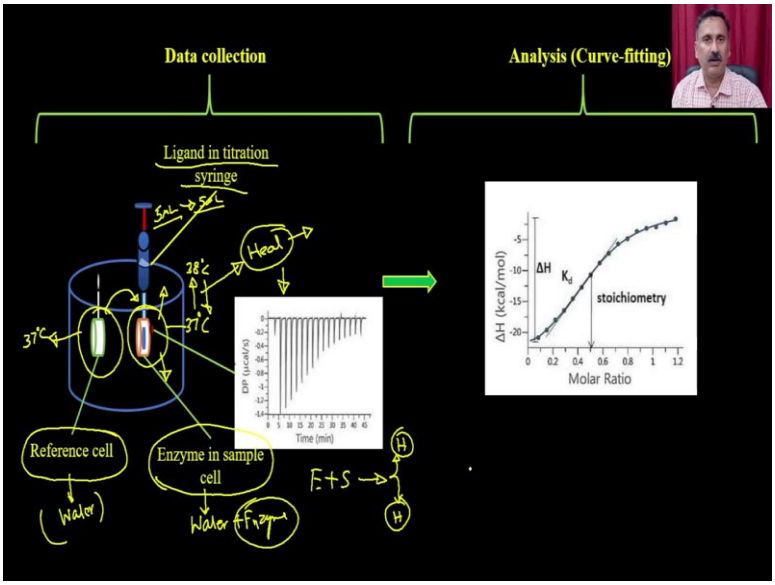

So, what you require is you required a reaction vessel where you are going to have a reference cell. This reference cell is actually going to be used to you know to know whether the heat is being released or heat is being absorbed.

And then you are going to have the sample cell where you are actually going to keep the enzyme in the sample cell and then with the help of a syringe, you are actually going to add the ligand and that is how the you can set up the reactions and that is how the ligand is actually going to be added to this enzyme in a regular fashion.

And what will happen at the end is that, when the ligand is going to be injected, it will either going to absorb the energy or it is actually going to release the energy right because of the intramolecular interaction with the enzyme molecules. And the reference cell is actually going to tell you whether this particular cell is on a higher heat or its on a lower heat. And that is how you can be able to measure whether there will be a heat absorption or the release of heat.

And then you can actually be able to have a curve, right? So, you can be able to plot the delta H versus the molar ratio and that is how these curves, analysis of this particular curve can be allow you to measure the stoichiometry. Now, let us see the description of this particular instrumentation part, right?

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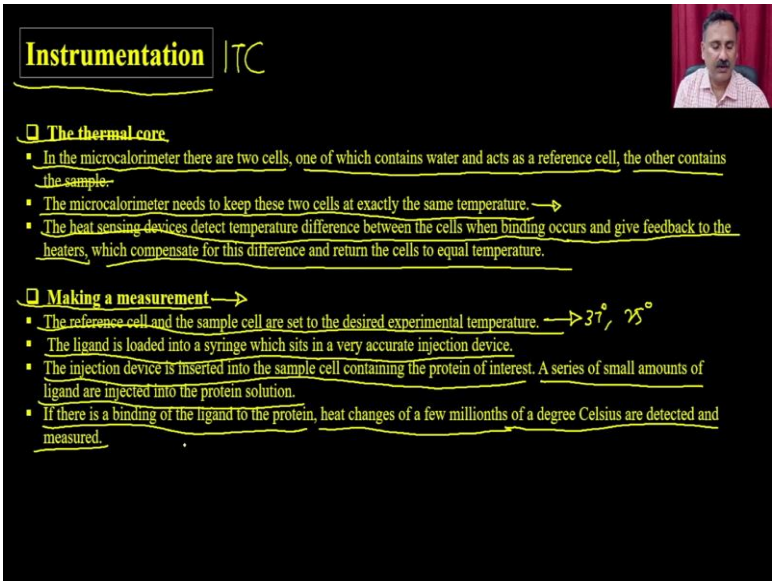

So, in the instrumentation of the ITC, what you have is you have a thermal core and you also have a measurement, you have to make a measurement mechanisms. So, in the thermal core, in the thermal core you have a microcalorimeter, there are two cell, one of which is containing water and act as a reference cell, the other contains the sample.

The microcalorimeter need to be keep these two cells at exactly the same temperature. This means, basically what is happening is that you are actually going to have a reference cell and this reference cell is actually going to have some amount of water, right? Or you can say buffer, right?

And here what you have is, you have the water plus enzyme. So, as soon as you are actually going to make any kind of addition into this water, the temperature of this particular cell is actually going to either go up or it will actually going to go down. If it goes up the heat is the temperature difference is going to be present between this cell and this cell. So, for example, imagine that the both of these cells are at 37 degree Celsius. So, this one is also at 37 degree Celsius, ok?

Now, imagine that there will be a release of some heat. So, when there will be a release of some heat, the temperature is also will go up. So, for example, if the temperature is gone up to 38 degree, although in the ITC reaction you would (Refer Time: 09:49) see a change in 1 degree or 5 degree or something, right? It is actually going to be very, very small. But just for a, just to explain you the mechanism, how the ITC is actually measuring the heat evolved or something, right?

Then what will happen is that. So, it is become 38, this become 37, right? So, reference is 37. So, what will happen is that you are actually going to spend some energy, right? So, you are actually going to withdraw some amount of heat from this particular cell so, that both of them so, that it will return back to the 37, right? This amount of heat what you are actually going to use or what is going to be utilized to bring the 38 to 37 that is going to be given here, right?

And that is how this heat can be plotted against the each injection. So, in each injection you can have one injection, you can have, you can, you can inject 5 microlitre of sample, in the second injection you can have another 5 microlitre of your ligand and so, on. So, it each injection, the amount of temperature in raising temperature is going to be keep reducing.

Because it is raised is happening because the enzyme is interacting with the substrate, right? So, if the enzyme is interacting with the substrate, it is actually making lot of rearrangement of within the enzyme and because of this rearrangement, lot of interactions are getting broken or you are actually forming the new interactions and because of that there will be either a release of heat or there will be a absorption of heat.

Either of these cases you are actually going to see a pattern like this and then this pattern can be analyzed in a subsequent steps and that is how you can be able to calculate the stoichiometry, you can be able to calculate the enthalpy, entropy and all those kind of

thermodynamic parameters. So, the heat sensing device detect the temperature difference between the two cell, between the cell when binding occur and give the feedback to the heater, which compensate for this difference and return the cell to the equal temperature.

And this is what I have explained already that reference cell is only being used to say whether there will be a heat released or whether there will be a heat absorption. If there will be an heat absorption, the temperature will come down from 37 to 35 and then there will be there will be a heater which is going to turn on and that is how it is actually going to bring the reaction or bring the temperature back to 37.

So, that is the way you are actually going to know what is the amount of heat, what is going to be released. Now, the how you are going to make the measurements? The reference cell and the sample cell are set to be at a desired temperature. So, you can actually be able to set, you can set 37 degree Celsius, you can set it as room temperature like 25 degree Celsius and so, on.

The ligand is loaded into the syringe which sits in a very accurate injection for device. The injection device is inserted into the sample cell containing the enzyme of interest. A series of small amount of ligands are injected into the protein solution. If there is a binding of the ligand to the protein, heat exchange of the few milli degree Celsius are detected and measured ok.

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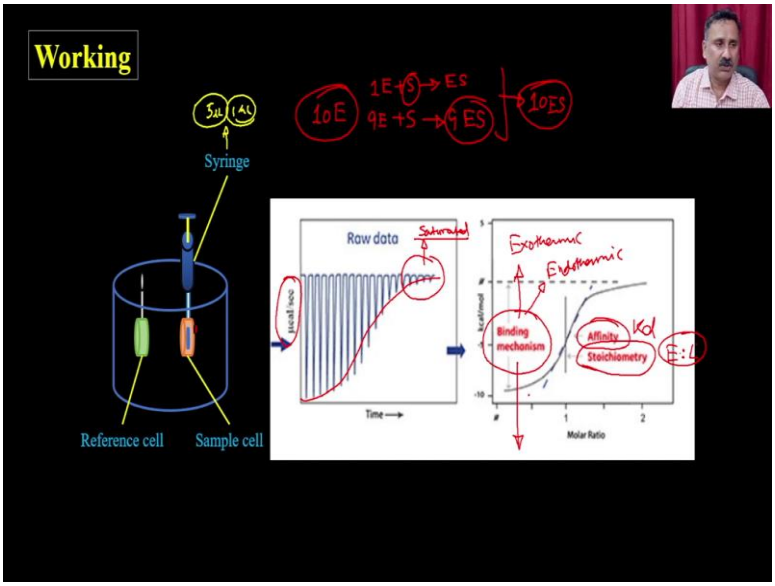

So, this is that exactly going to happen. So, this is your reference cell, you are going to have a sample cell and with the help of the syringe you can be able to inject the sample like in the 5 microlitre, 1 microlitre, whatever the increment you want right and you can make the many types of modification. And this is what you are going to have. You are going to have a raw data and that raw data is actually going to be looked like this right where it will say that how much the heat is being involved right.

So, this raw data could be of this way. So, you will see this is actually getting saturated because the binding site, for example, you have 10 molecules of enzyme right. So, what will happen is when you do the first reaction, the first molecule is going to be you know completely occupied by the substrate and it is actually going to make the substrate complex right. And you are actually going to have one molecule of enzyme substrate complex.

But the 9 enzyme molecules are still left right. So, then you when you add some more amount of substrate that also is going to bind and that is how it is actually going to form the 9 molecules of enzyme substrate complex which means together, they are actually going to be get, all the enzyme molecules are now going to have the substrate molecule and that is how this heat is actually going to be on a lower and lower and lower right.

So, heat what is being evolved or what is going to be absorbed, that phenomena is actually going to be on a lower side and that is how at this stage the system is actually going to be get saturated. So, you have to use, you have to do this titration with the ligand molecule until you will not see a saturation.

Once you see the saturation then you are saying that ok everything is fine. And using this data you can be able to calculate the affinity parameters which means you can be able to calculate the k d values, you can be able to calculate the stoichiometry which means how many enzyme how many ligands are binding to the enzyme molecules. So, enzyme or ligand ratio.

And then you also can say whether the binding mechanism, whether the binding mechanism is exothermic whether it is the endothermic right, all these information's you can actually be able to get and whether it is sequential binding or it is actually a non-sequential binding.

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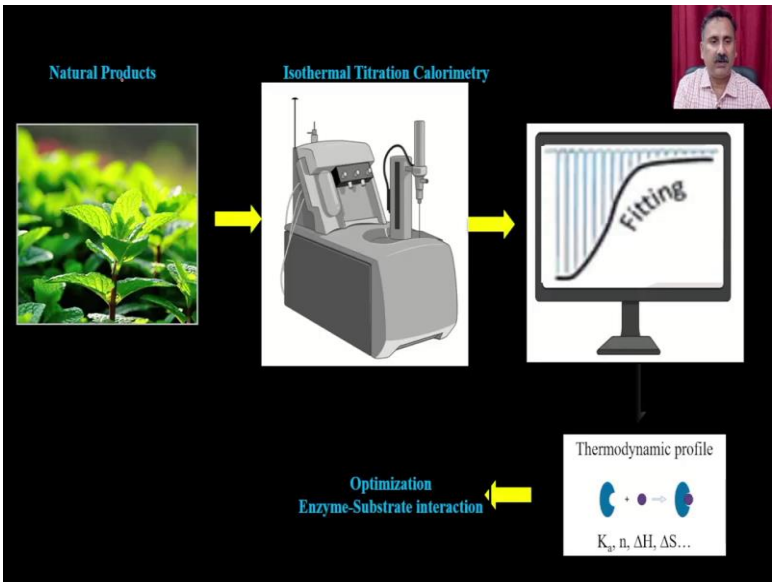

So, these are the some of the components and I have taken an example right. So, you if you can have the like natural products like for example, you can have a natural product from Mentha or you can have from tea and you want to know whether these phytochemicals are binding to some protein molecules or no.

So, this is the isothermal titration calorimetry instruments and ultimately you are going to get this particular type of curve and that curve is actually going to give you the all the parameters like kd values, affinity parameters, stoichiometry, change in enthalpy and change in entropy. And when you can actually be and using this data for multiple round you can be able to do the optimization to calculate the enzyme substrate interactions.

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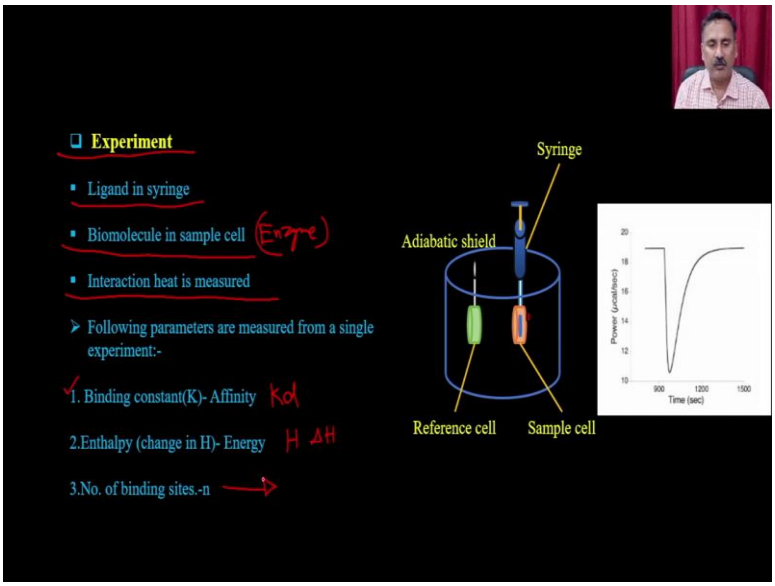

So, now let us see how you can be able to perform this experiment and what are the things you require actually. So, for the experiment what you require is, you require a ligand which is going to be present in the syringe, you require a biomolecule like the enzyme in the syringe in the vessel right.

Then you require interaction heat which is going to be measured and these are the parameters what are going to be measured. You can actually be able to calculate the kd values, you can be able to calculate the enthalpy. So, you can actually be able to calculate the delta H and then you can also be able to calculate the number of binding sites and so, on.

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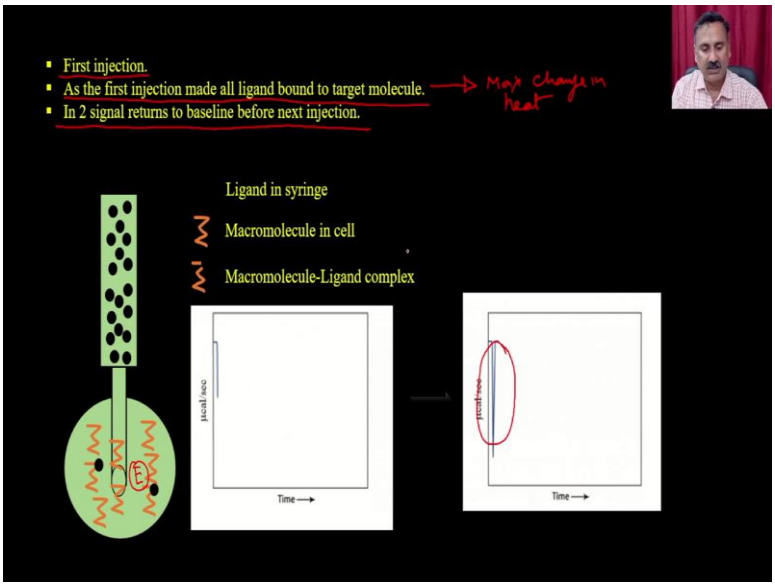

So, this is the way you are going to do. So, this is the reaction vessel where you have the enzyme right and this is your ligands. So, what you are going to do is you are going to do multiple injections.

So, when you do the first injection, as the first injection made all ligand bound to the target molecule it is actually going to be result into the maximum change in heat, maximum change in heat because all the subset molecule will go and bind to the enzyme and that is how it is actually going to make the maximum change in the heat and that is why you will see that the depth of this particular some peak is very high right.

In a 2 signal in return to the baseline before next injection.

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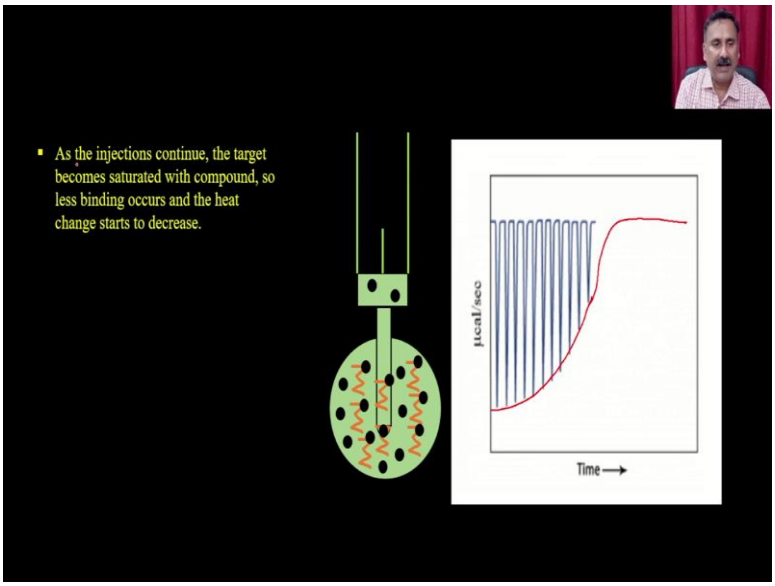

So, when you do the next injection right what you will see is that this height or this depth is actually going to be keep reducing because the number of substrate molecule which are going to be bind is not going to be 100 percent right and ultimately what you will see is that it is actually going to get saturated like this.

So, as the injection continues the target becomes saturated with a compound. So, less binding occurs and the heat change starts to decrease. So, when the ligand is not binding it is not making any kind of modifications, its not going to make any kind of alterations and as a result it is there will be a lesser amount of heat exchange.

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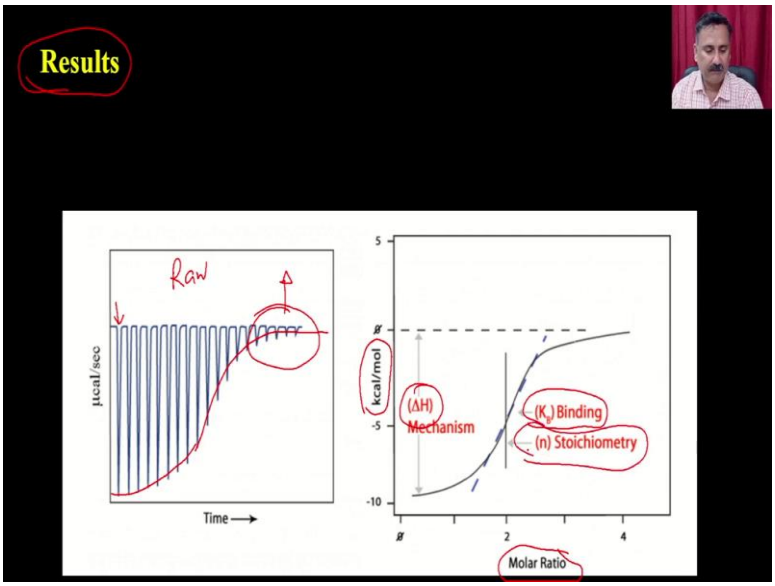

And as a result, at the end what you are going to get you are going to get this result you are going to get a change in heat right for every injection. So, every injection you are going to see a change in the heat and that heat what you see is a pattern right and that is the ideal pattern that you are going to see a saturation.

So, if you do not see a saturation; that means, there are something problematic either you are you are having a background reactions or you are there is something you know artifacts going on and because of this you can be able to do lot of calculation and you can be able to do the determination or different types of thermodynamics parameters.

So, from this data this is the raw data you can be able to plot the derived data, you can actually be able to calculate the kilo calorie per mole change in heat energy and across the molar ratio and that is how you can be able to calculate the delta H, you can be able to calculate the binding constant you can be able to calculate the stoichiometry.

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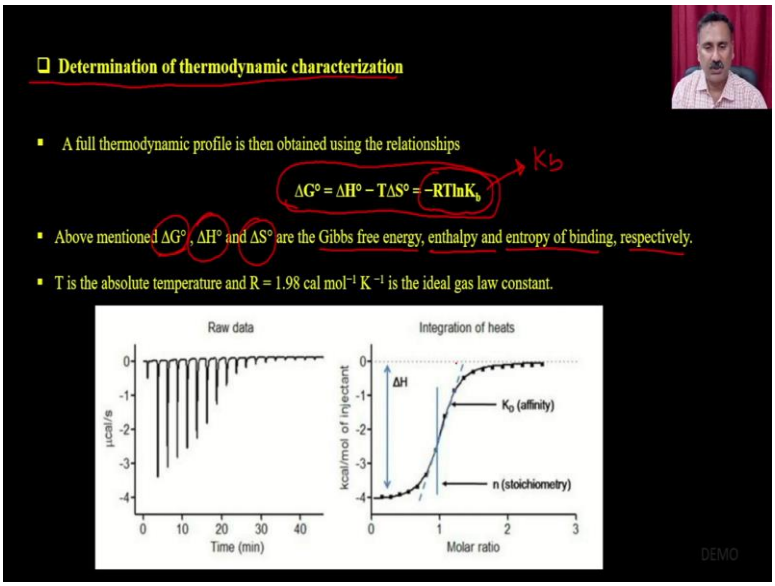

How you can be able to do the thermodynamics parameters? So, you can actually be able to use the following reactions right. So, this is the Gibbs reactions and you can be able to use the Gibbs energy and the relationship between the delta. So, delta G is equal to delta H minus T delta S or it is actually equivalent to the RT ln Kb and using this you can be able to calculate the Kb and you can be able to calculate the delta G delta H delta S and so, you can be able to calculate a Gibbs free energy enthalpy entropy and so on.

And so, putting these values you can be able to do lot of calculations and that is how you can be able to do you know all these measurements. So, to explain this all these processes and how you can be able to perform the react how you can be able to perform the experiments and how you can be able to get the raw data, how you can be able to process the raw data.

So, that you can be able to calculate the all these affinity parameters we have prepared a small demo clip where the students are actually explaining. So, first they are actually going to show you a description about the instrument and then they are actually going to show you what are the different requirements different types of things.

What you require and how to perform the experiment and once post experiments they are going to get the raw data and then they are actually going to show you how you can be able to analyze the data to calculate the affinity parameters.

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Hello everyone, I am Alok kumar Pandey a PhD scholar under Professor Vishal Trivedi in the Department of Bioscience and Bioengineering IIT Guwahati. Today in this video I will I am presenting a demo on isothermal calorimetry instrument that is ITC instrument. So, ITC is generally used to study the interaction between two molecules or between two bio molecules for example, we can study interactions between a protein and a ligand between two proteins also and many other.

So, first we will start with the what are the parts of this instrument. So, the instrument looks like this, they are two compartments this is the cleaning compartment and this is the titration compartment or experimental compartment. So, we come to the cleaning compartment first.

The cleaning compartment it contains these four bottles this one the white cap bottle is the bottle containing the detergent which is used for cleaning, the various parts like the cell and the syringe of syringe and this is the red cap bottle which contains methanol which is used for drying after cleaning.

This is blue cap bottle which contains water filtered and autoclave water which is used also used for cleaning. The methanol here should be a HPLC grade not normal methanol and this is the fourth bigger bottle it is for waste. So, all the waste after cleaning goes into this discard bottle. So, now we will come to the second compartment which is the experimental compartment or titration compartment.

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So, this is the this is called as the cell. In in the cells, we generally put the whatever entity with which is having lower concentration for example, in protein ligand we generally put protein into the cells.

You can see inside a hole is there that is the sample cell and besides this cell one more cell which is not visible is there that is called as the reference cell that is constantly filled with 280 micro liter of water and it needs not to be changed every time, but around once in two week or in a month. Now, this is the syringe in which we generally load the entity of higher concentration or in a protein ligand interaction we can say we put ligand into this syringe.

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This is the fill port adapter which is required to during cleaning or during loading of the ligand into the syringe and this is called as the cell cleaning tool which is used to clean use during the cleaning of the cell. So, now, I will explain I will start with how to clean this instrument. So, for cleaning this instrument first what we need to do is first we need to put this put this cell cleaning tool into the cell and ensure that it is nicely fitted into this there should not be any leakage.

And then for the syringe first we need to put this fill port adapter into the socket provided here we have to see it and then we have to put it into this firmly it should be firm and after that we should move this syringe into the here it is written clean into the clean location we will move it slowly and we will ensure that this clamp is nicely engaged here.

So, after doing this now we need to use the control software to set the wash parameter the way we want to wash and the method of washing generally we have to set through the software. So, we will go to the software now.

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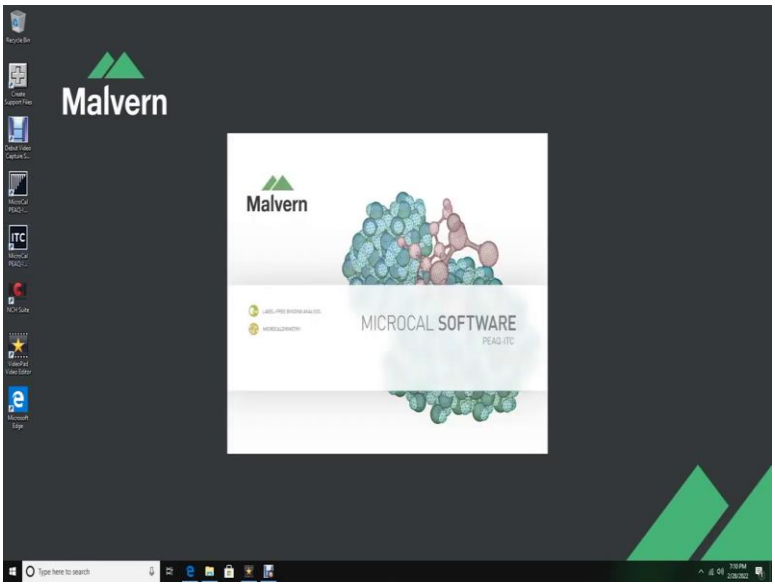

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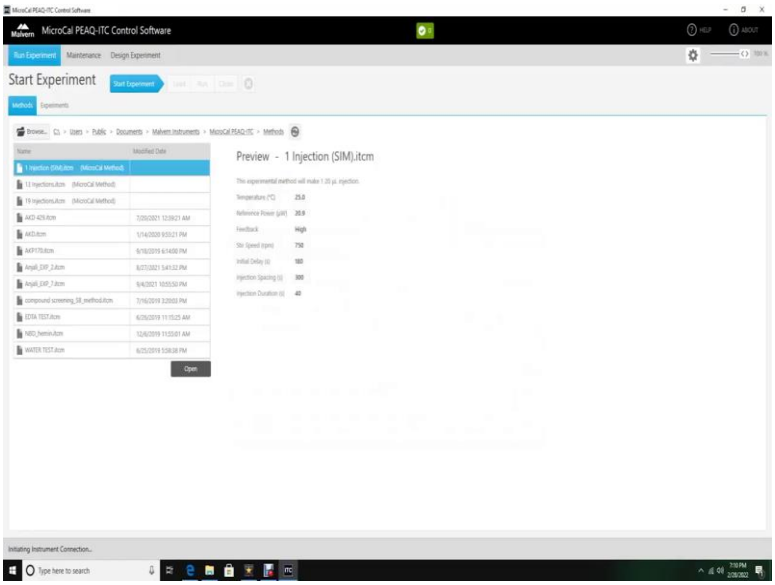

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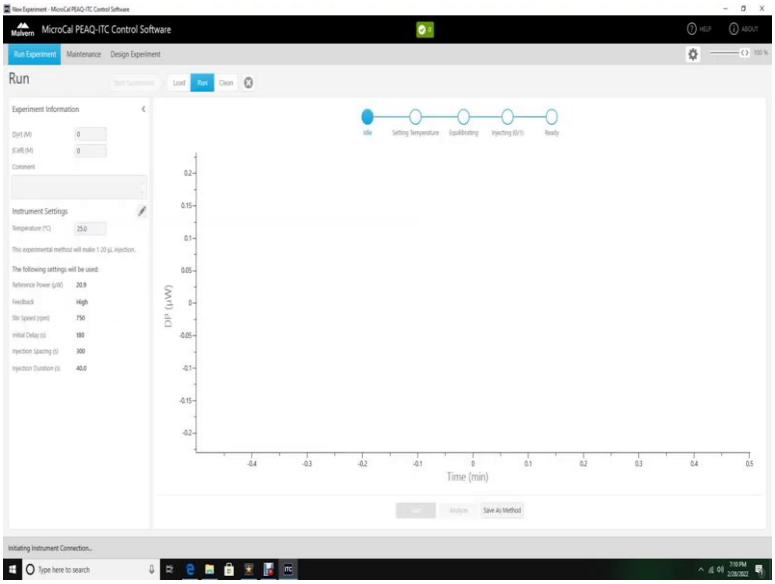

So, now we will open up the control software for ITC this is the control software this is the first window now we will click on open which will open up window with three options load, run and clean. So, we are going to clean. So, we will click on clean.

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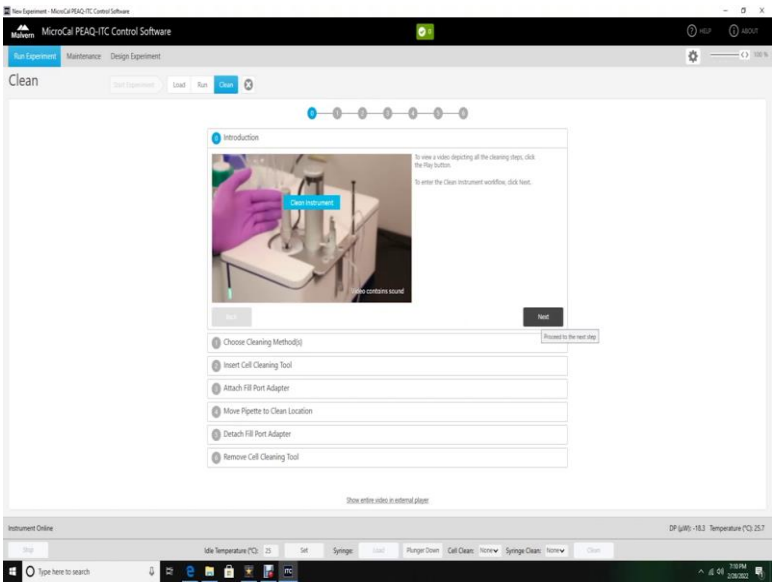

After clicking on clean it will show a video which will depict all the steps which we just to perform in the demo. So, after doing each steps we have just have to click next we have to ensure that the step is done and we have to click next.

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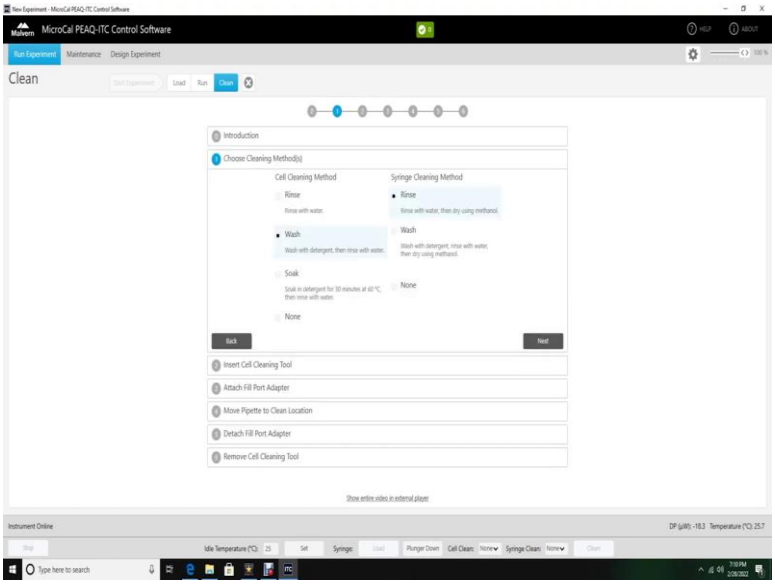

So, I will click next here it is giving the which method you want to clean the cell and the syringe in this three options are there rinse in which it will only rinse with water with in wash it will wash the cell with detergent and then rinse with water in soap it will soak the cell in detergent for 30 minutes at 60 degree centigrade and then rinse with water.

And in the syringe cleaning method similarly rinse wash and none option is there. So, we have already I have selected this wash option for cell cleaning and the wash option for syringe cleaning.

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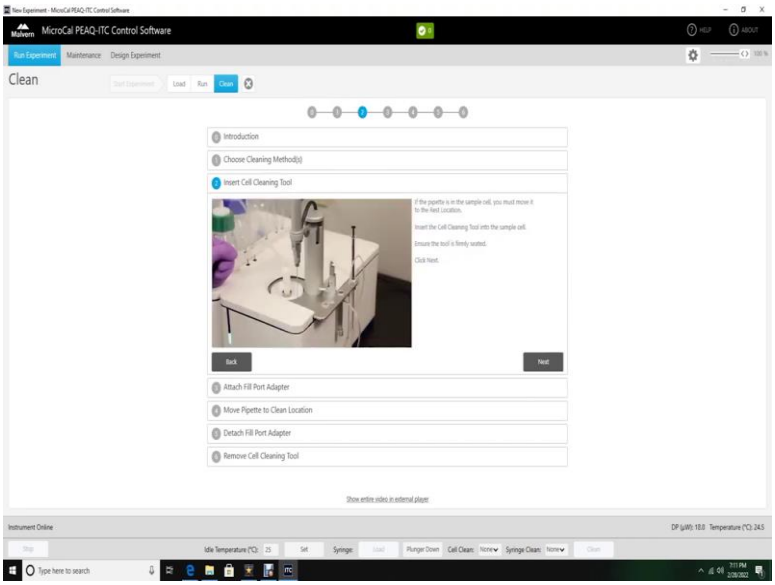

I will click next. Next again it is showing the steps which I have already discussed in the demo it to put the pipette in the sample cell how to put the pipette into the sample cell.

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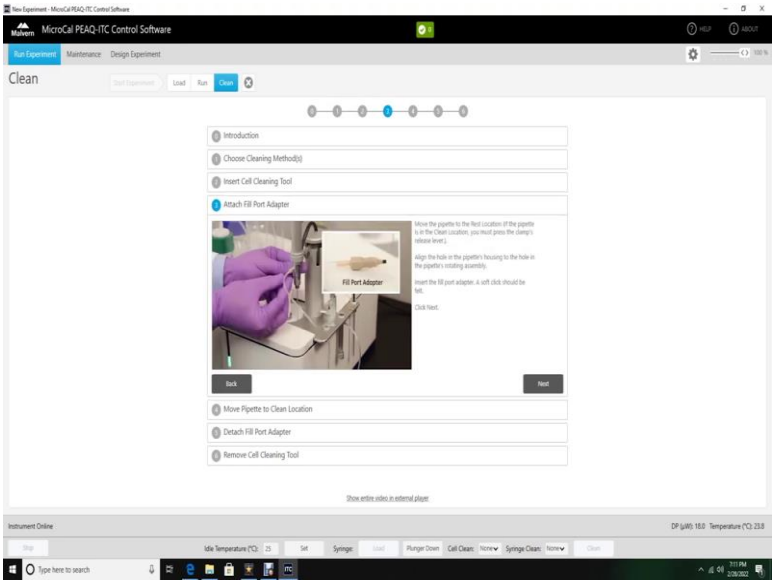

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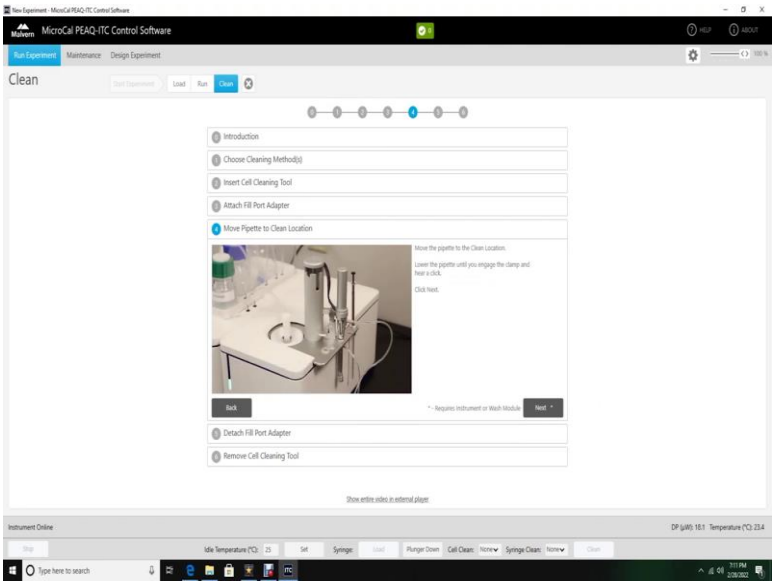

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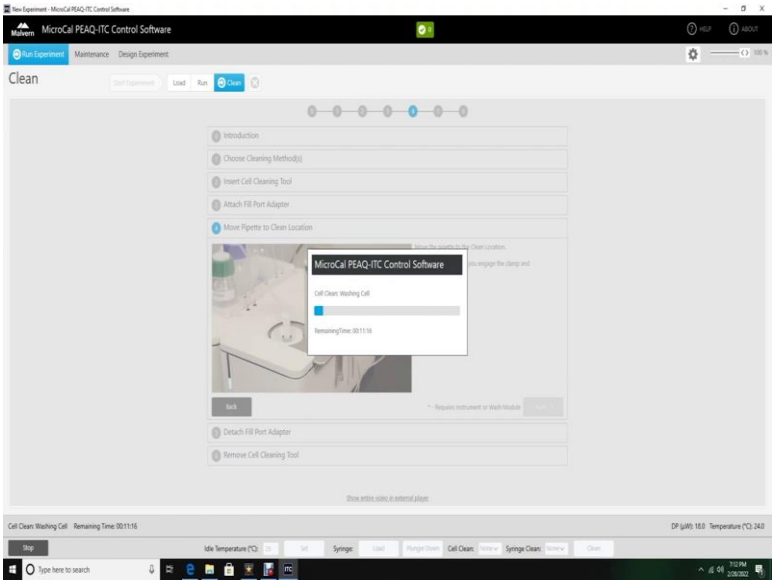

So, this step is already done now the how to put the fill port adapter and into the syringe that is step is also done now we have to move the pipette to the cleaning location this step is also done. So, we will click next now the cleaning is start and it is showing here starting vacuum.

First it will create vacuum into the instrument and if there is any leakage it may show an error. So, we have to while putting up all the cell cleaning tool and syringe fill port adapter we have to ensure that there should not be any leakage everything could be tightly and firmly seated.

So, the cleaning it has started we can see now it is testing the vacuum and now after testing the vacuum it will start cleaning the cell see emptying cell. So, now the cleaning will run for it is showing time remaining 11 minutes around 11 minutes 20 seconds. So, now, it will go for that much time and the cleaning will be done. So, now we are done with the cleaning then we will go to the loading of the sample of the protein and the ligand.

So, before loading I will like to tell two points which are very important in sample preparation. First one is that the concentration of both the protein and the ligand should be very accurately measured and the second point is that the buffer in which the protein is dissolved and the ligand is done should be exactly same or closest similar possible. So, because buffer mismatch may interfere with your results.

So, now we will come to loading. So, after cleaning we need to remove the cell cleaning tool from the cell and after that using this loading syringe we need to first take out or clean out whatever is left inside the cell after cleaning.

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So, we will discard these things. So, now we have removed everything from the cell. Now, we will wash the syringe first with water and then three times first we will wash it with water and then after washing with water we will wash this with the buffer in which our protein is dissolved.

So, that the environment of the protein does not change as much and there is no buffer mismatch. So, now the syringe is washed with buffer. Now, we can take our protein into the syringe. So, we will take exactly 300 microliter of this into the syringe and we will take it very slowly so, that no air bubble enters the syringe. Exactly three micro 300 microliter we will take ok. So, it is 300 microliter. Now, we will load this into the cell.

So, while loading we to ensure that no air bubbles is formed, but we will do first? We have to push the plunger very slowly for around 180 sorry 150 microliter and after that after that 150 microliter we have to move it little fast so, that no so, that if bubble is there it will come up and the last part again, we have to move it very slowly so, that no air bubble enters ok.

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And now after that we can move the syringe like this to remove any air bubble if any air bubble is there it will come up and then we can remove it using the syringe manually. So, now we will see the our protein sample is in the cell. So, now if any thing is extra, we will can remove it using this syringe so, that the protein is exactly filled into the cell. I will put the protein back.

Now, we will come we will put this syringe into its storage our cell is loaded. Now, we will load the syringe with the ligand solution. So, we will disengage this clamp and we can see the fill port adapter is already attached to it. So, we need not to worry about that and now we will take in a 200 microliter MCT tube we will take around 100 microliter of 100 microliter of our ligand.

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This is the ligand; it is dissolving the same buffer as the protein. Now, we will take 100 microliter of this ligand into this 200 microliter MCT or PCR tube.

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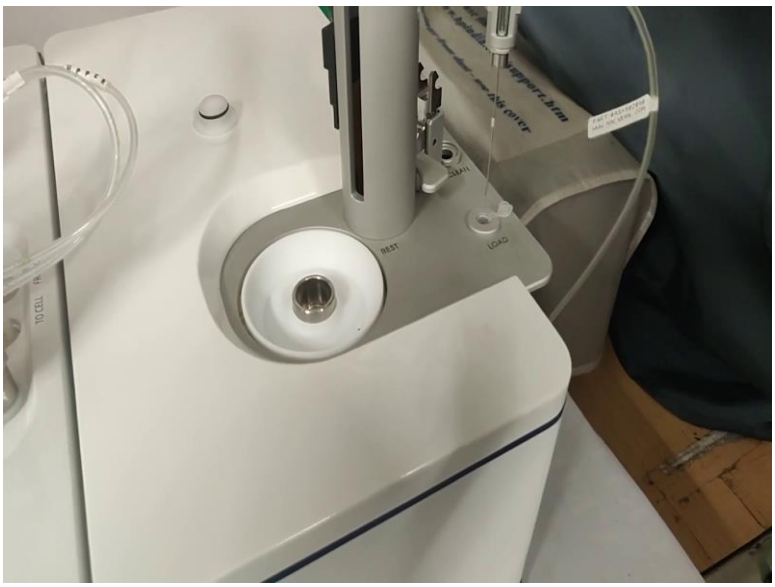

So, now we will put this PCR tube into this position where it is written as load and now, we can we will move this syringe into this tube so, that it goes till the bottom. Now, again for loading of the syringe we have to go to the control software of our ITC instrument.

So, when we give command from the software this will start loading the ligand into the syringe. So, it will be in three step. In the first step it will open up the fill port and after that it will remove whatever is the dead volume and after that it will do purging and refilling. The purging is done by the instrument to remove any air bubble into the ligand solution.

So, here we have to ensure that while refilling there should there should not be any gap between the plunger of this syringe and our ligand solution because that gap may introduce air bubbles later. So, here now we can see there is no gap between that white plunger and our ligand solution. So, the ligand is now. So, the ligand is now loaded into the syringe.

And now what we have to do? We have to bring it to the rest position we have to remove the fill port adapter and then we have to keep it back and then we have to put this syringe into the cell and firmly. Now, we can start our experiment we can set the parameters in all and start the experiment for that we need the control we need to move to the control software.

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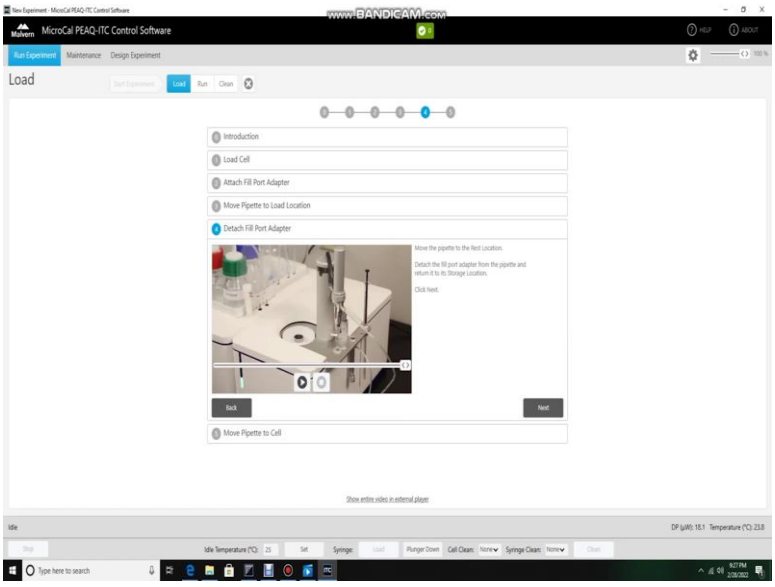

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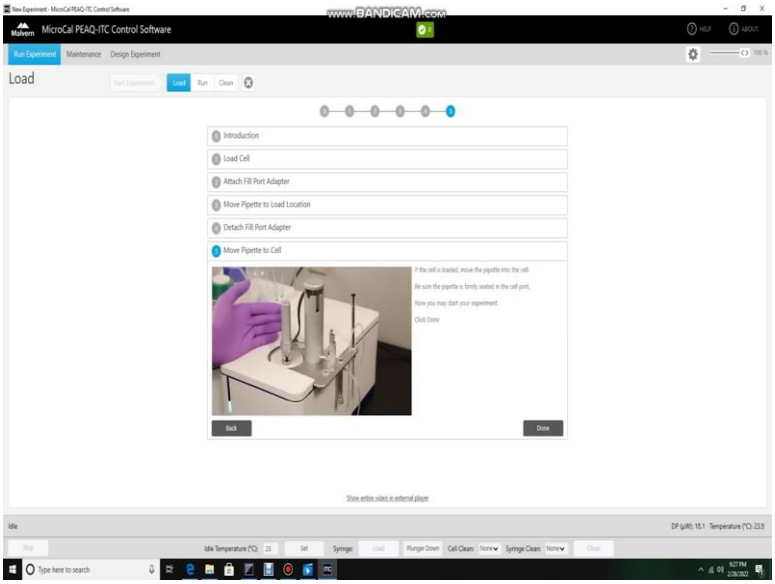

Now, after the syringe when the syringe is firmly seated into the cell, we will again come to the software and we will keep next and now the this the loading is completely done.

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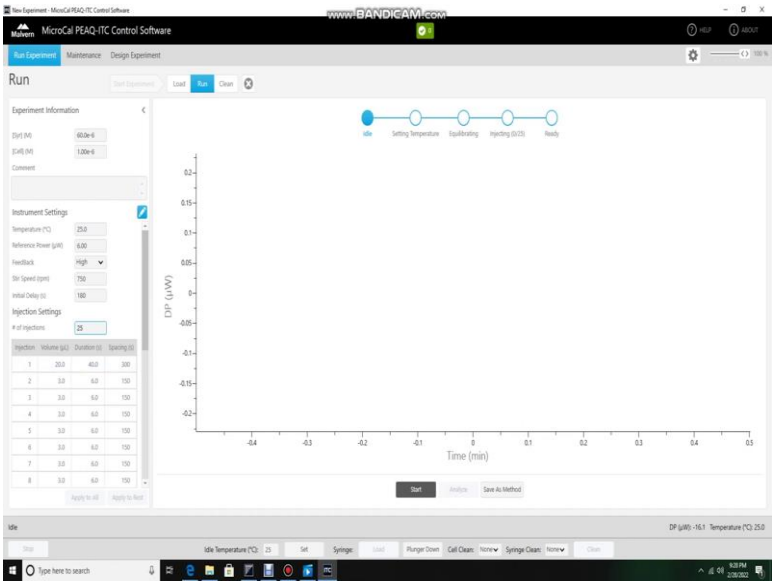

Now, this page will come where we have to set the parameters for our experiment. The first parameter is syringe concentration syringe molar concentration. So, which is 60 micromolars. So, we will put 60 micromolar here and the next is the cell concentration which is 1 micromolar which we will put to 1 micromolar here and the other parameters we can set by clicking here the temperature 25 degree centigrade which is fine.

Reference power for protein ligand instruction we are using 6 micro watts, stirring speed 750 and initial delay is the delay between the equilibration and the first injection this is 180 seconds and now the number of injections which I am setting to 25.

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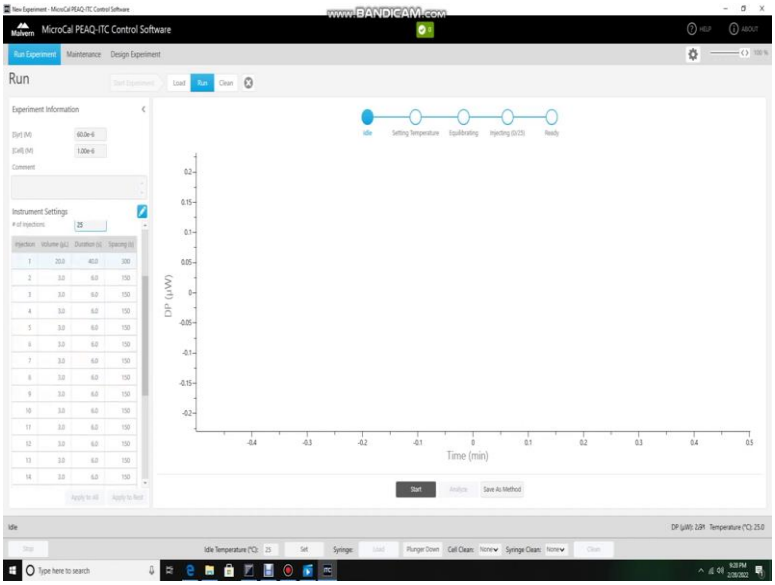

Now, after setting the injections this table will come where we can set the volume of each injection.

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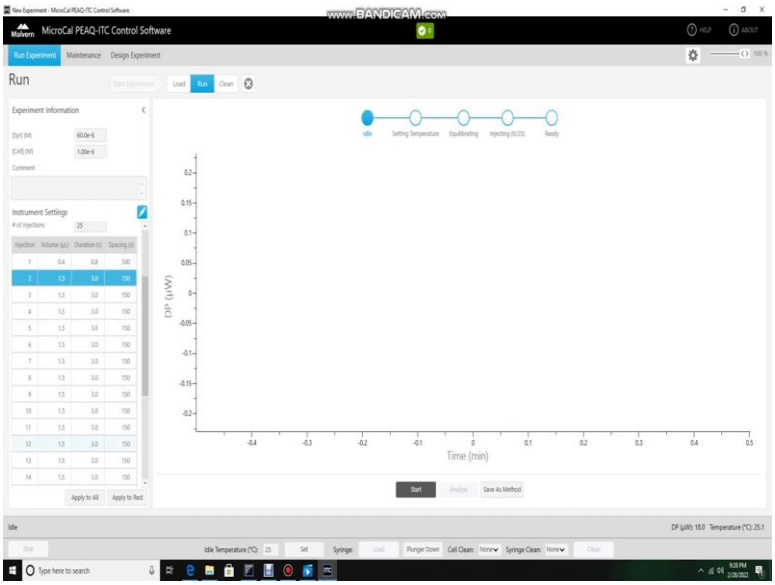

First injection should be very small. So, I am putting it 0.4 micro liters and after that the other consequent injection I am putting as 1.5 micro liters and then I will click apply to

rest then all the injection will be all the other injection will be of 1.5 micro liters. Now, here we have to keep in mind that the total volume of all these injection should not exceed 40 micro liter because the volume of ligand loaded into the syringe is 40 micro liter by default.

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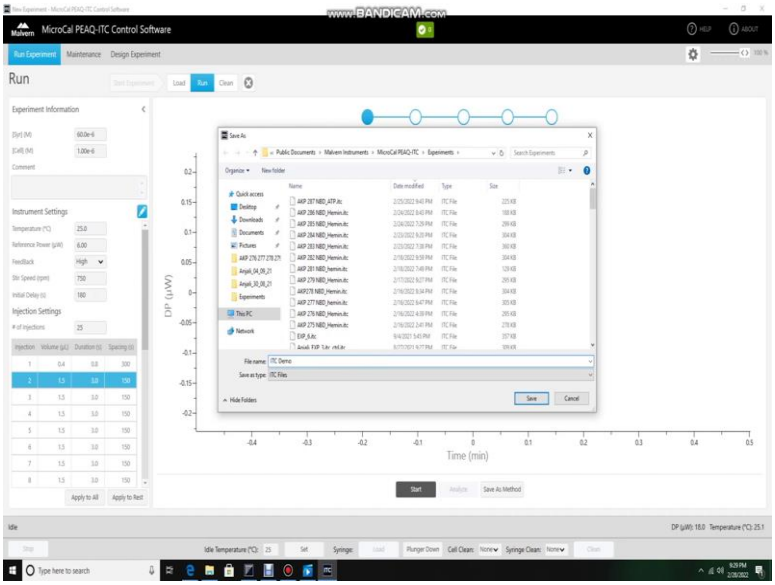

So, after setting these parameters we can click on the start to start this experiment before starting it will ask to save the experiment. So, we can save it as ITC demo. Now, it has started the temperature is already out at 25. So, it has moved to the third step here we can see the steps which will be followed during the experiments. Set idle then setting a temperature and then equilibrating.

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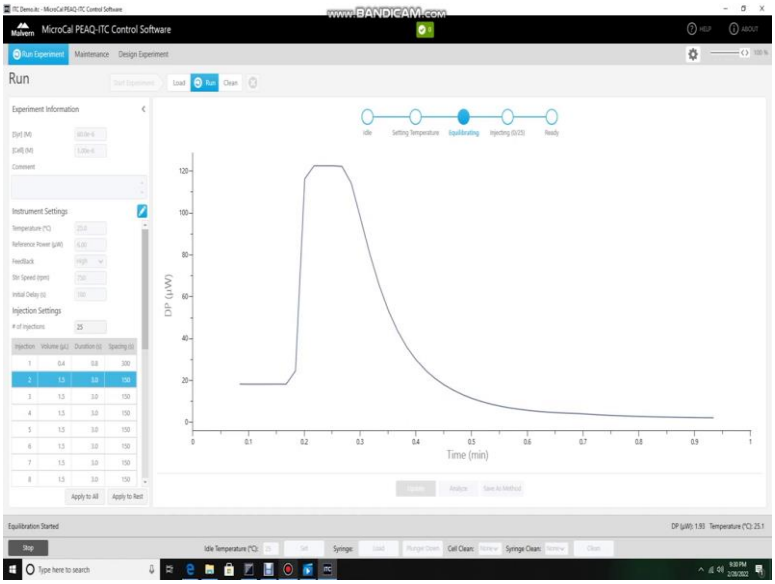

Equilibrating means it will bring up the reference power to 6 micro watt as set by us. After the equilibrating is then it will start after it will start giving the injections the first injection will start after 180 seconds in the initial delay of after equilibrating and after that the consequent injections will follow till 25 injections are completed after 25 injections are completed it will show this ready it will become blue and our experiment will be done.

So, after the experiment is done, we will move to the analysis software of our experiment.

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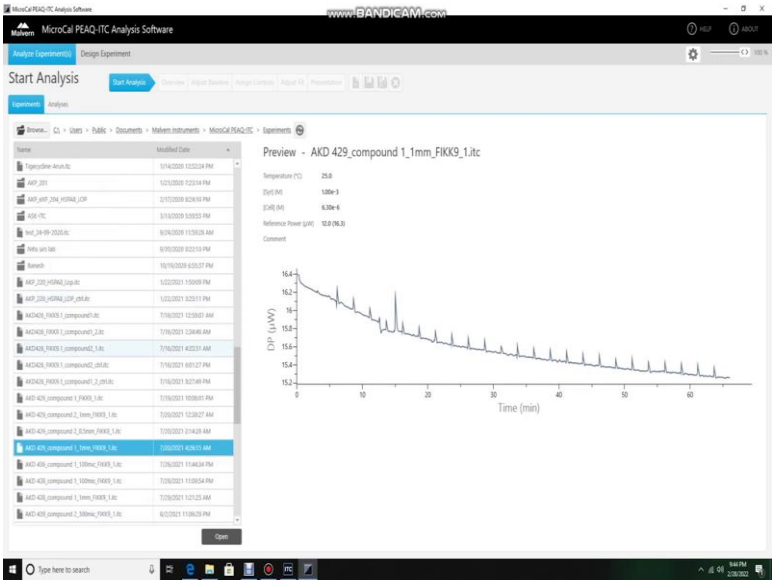

So, now we will analyze our experiment using the analysis software first we will open our experiment we can search it by the name will be put that is ITC demo. So, it is this one. So, here it is this one ITC demo. Ok.

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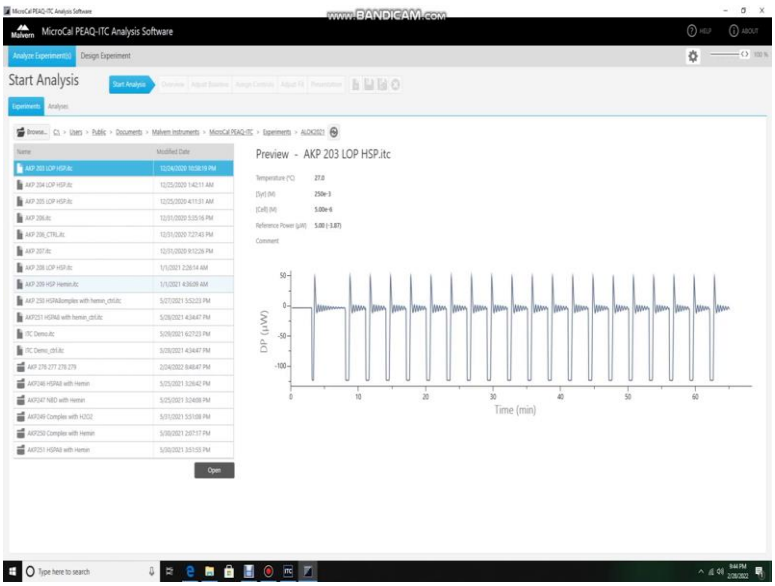

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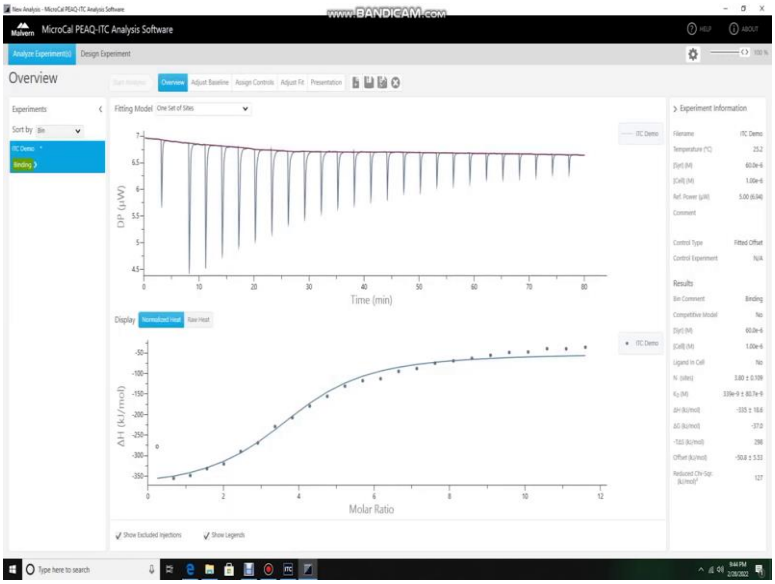

So, here we can see we can see the with consequent injection the differential power is decreasing and finally, it is reaching to a around 0 and its constant in last few injections and here we can see the instrument it showing binding; that means, it is there is a binding between the ligand and our protein.

So, after running an experiment it is important to run a control experiment which is run in the same way, but in the cell only buffer is used and no protein is there. So, we have to add that control experiment to this experiment while analyzing. So, I have already run that control experiment. So, I will add that experiment here.

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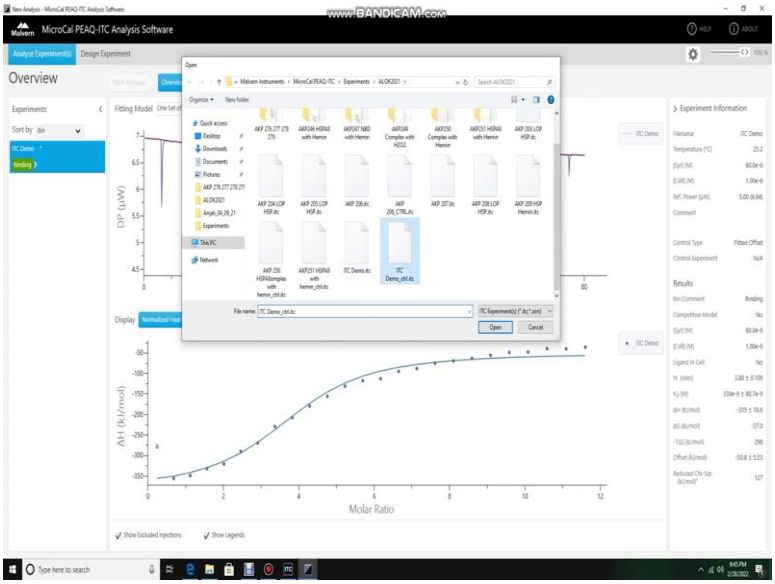

So, it is this one ITC demo control experiment. So, this exponent is now added and it is already written here control why because, while saving this experiment, we need to add underscore CTRL after the experiment name then it will automatically take it as a control experiment.

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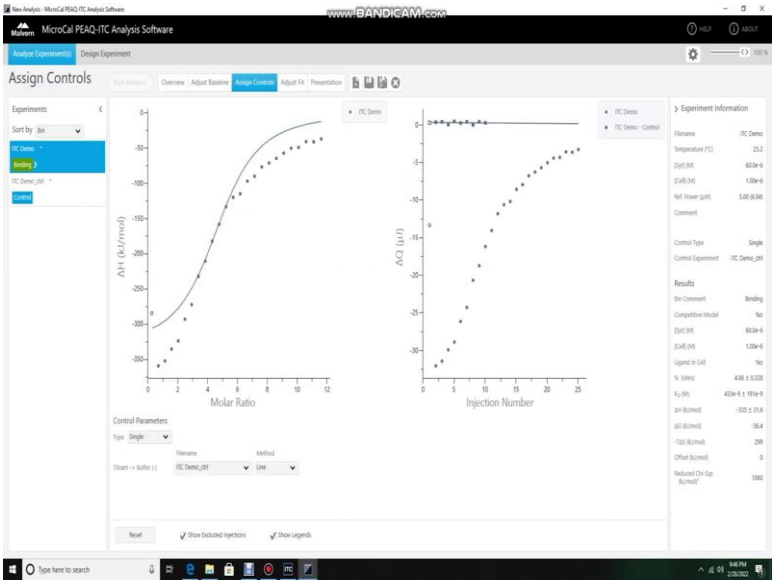

Now, we can see we are now we can assign controls here where we can subtract the control from our experiment.

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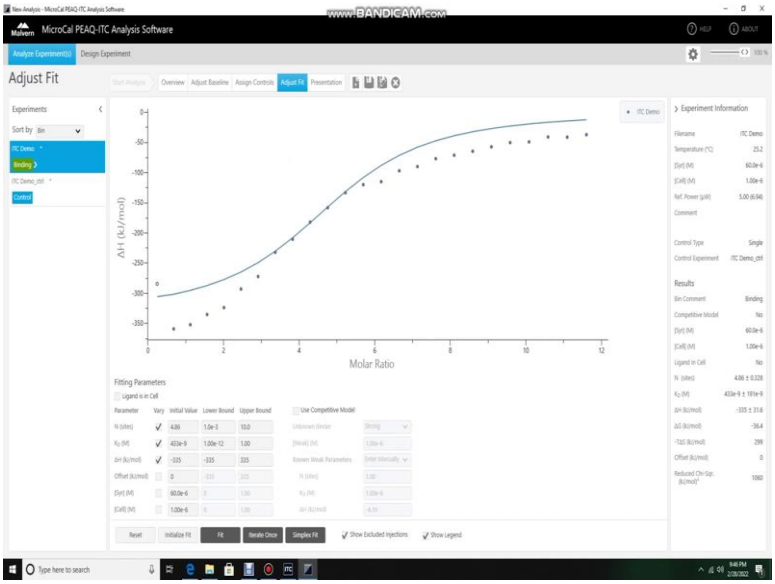

Here we can see it is one titrant to the titrated with buffer this is the file name and the method of control subtraction here it we have three methods mean, point to point and line here we have selected line.

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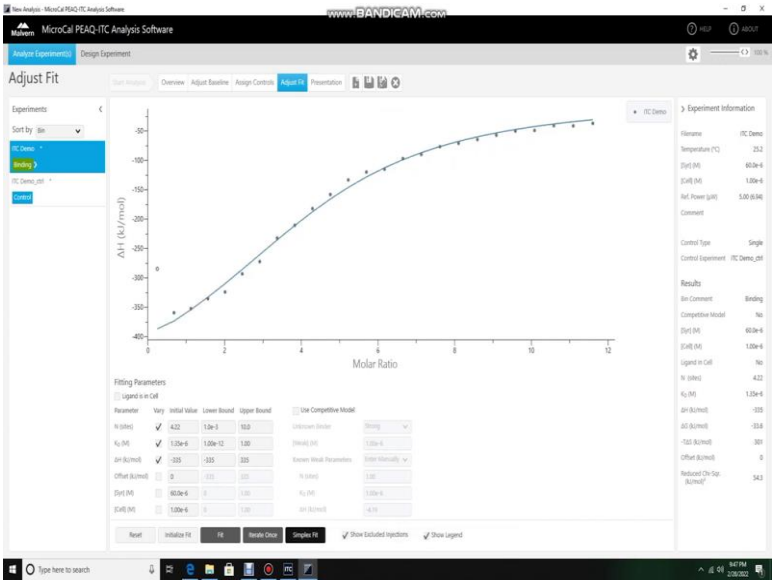

So, now we will go to the adjust fit and here we will try to fit this with this data to get a good sigmoidal curve.

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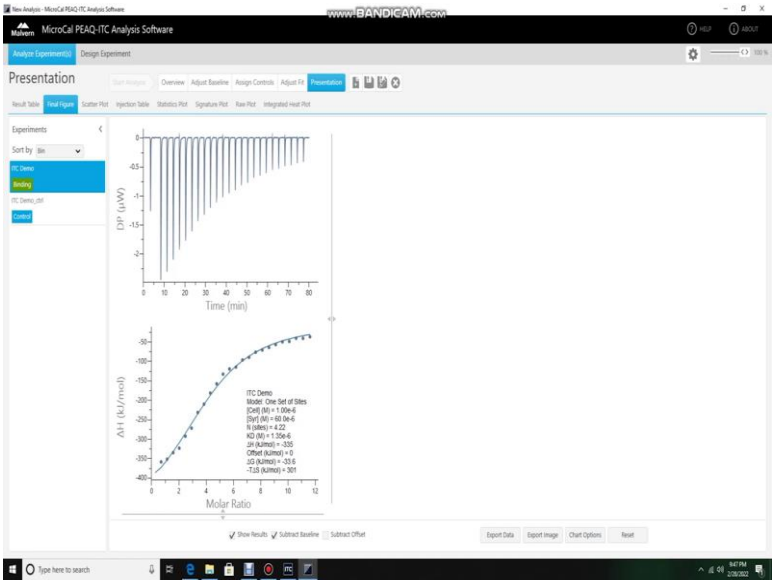

So, I will its a simplex fit we can see now it is nicely fitted all the points are closer to this line. So, it is nicely fitted and now we can move to the presentation part and go to the this final figure this will give us the final data for our experiment. Here we can subtract the here we can see the baseline is continuously going down.

So, we can subtract the baseline to bring it to normal and then we here we can click show results which will give us the values like N is the number of sites then K D value which is here 1.35 micromolar then delta H which is minus 335 kg per mol and other values can be found here.

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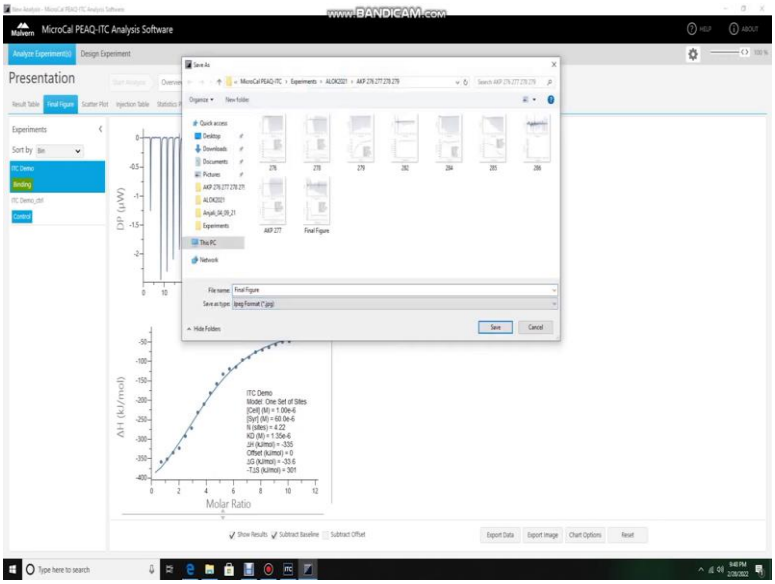

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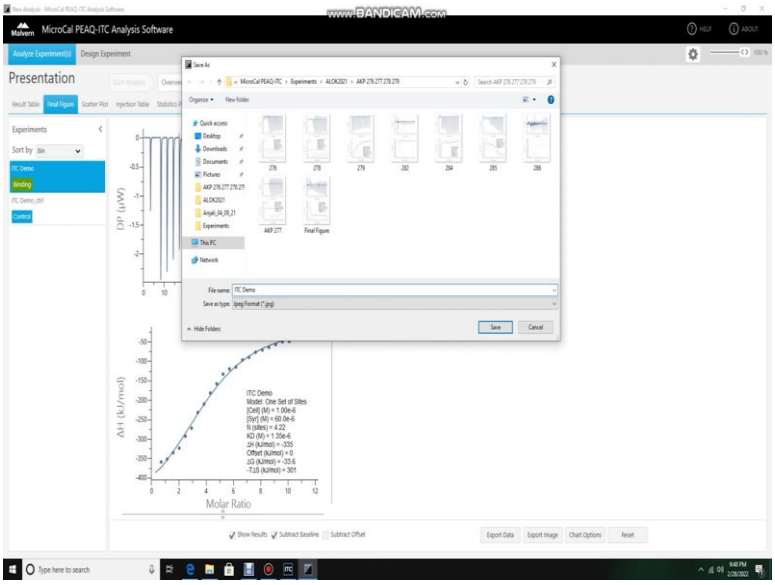

So, now this is the final figure which we can export it from here in our required format suppose we are doing here in jpg format and we gets we can save it as ITC demo and now it is safe and our experiment is completed.

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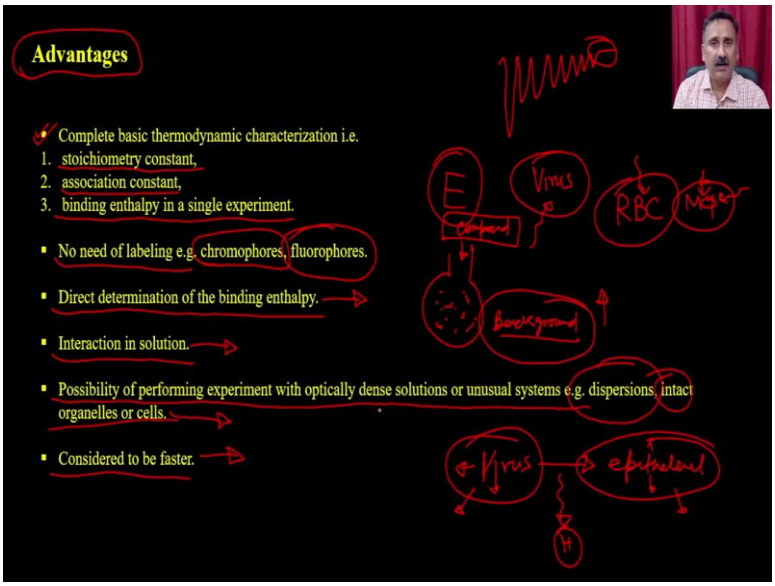

Now, what are the different advantage of the ITC? Ok its it is a very is you know sensitive method. So, it actually can be able to help you in terms of you know determining the all the thermodynamics parameters.

So, it actually can give you the stoichiometry can actually be able to give you the association constant or dissociation constant, it actually can give you the binding enthalpy in a single experiment and most important is there no need of labeling that is the chromophore is not required or fluorophore is not required.

So, without the help of the chromophore and fluorophore you can be able to determine whether the ligand is binding to the enzyme or not. Then it actually gives you the direct determination of the binding enthalpy. So, there is no indirect measurements there are no you know.

So, its actually gives you the direct measurement of the binding enthalpy and say whether the binding is exothermic or endothermic interaction occurs in solutions. So, it actually mimics the biological system. So, its mimics the biological environment because most of the biological reaction occur in a aqueous environment or actually occurs in a solution. So, its actually can mimic that.

Then the possibility of performing the experiment with optically dense solution or unusual system like the dispersion, intact, organelles or the cell is also possible. So, you

can actually not only going to you cannot not only use the enzyme you can actually be able to use like the cells or virus for example. So, you can actually use the virus particle as long as the system is you know homogeneous or it is actually remains in suspension, you can be able to use that.

So, you can actually be able to use even the full cell like for example, you can use the RBC, you can use the macrophages and you can say whether this particular molecule is binding to macrophage or RBC or something or not right and you can easily calculate some of the drug molecules whether they are binding to virus or not right.

So, what you can do is in the reaction vessel what you can do is you just take the virus particles and first you are actually going to do a background corrections because as soon as you will take the larger molecules as soon as you will take the organisms for example, the organism will have many types of proteins, it is not going to have only one protein right.

So, it is not pure system it is actually a impure system where you are going to have the for example, in the virus you are going to have the core proteins you are going to have the reverse transcriptase, you are going to have DNA, RNA and all that kind. So, background is going to be very high and that could be one of the challenge what you have to face.

So, then what you can do is you can actually be able to inject the compound and as soon as you will inject the compound and if the virus is binding to this particular compound, it will actually going to show you a dip ok and this dip will actually going to be keep reducing and ultimately you are going to see a saturation.

And that is how you can be able to calculate whether the compound is binding to the virus or not you will not be able to answer whether the compound is binding to which protein or which molecule because that is very difficult to say, but and other thing what you also can do is you can for example, if I want to know whether the virus is infecting the epithelial cell or not right.

So, if I want to do this kind of experiment, here is a both the virus and as well as epithelial cells are containing the sum amount of you know many proteins right and that is why there will be a problem of background because when you add a virus right there

will be a heat exchange within the virus right. So, within the virus there will be heat exchange and then within the epithelial cell there will be a heat exchange and there will be a heat exchange when they are interacting as well.

So, you want to know this you do not want this right. So, this has to be subtracted this has to be subtracted and then you are actually going to see a subtracted data and then from subtracted data you can actually be able to make out when the you are adding the virus whether there will be a significant change in heat exchange or not.

So, this is little more complicated and little more difficult to perform. It is very fast. So, you can be able to do a binding study within one hour or two hours depending on how frequently or how much time it requires for the substrate to interact with the enzyme molecules or not.

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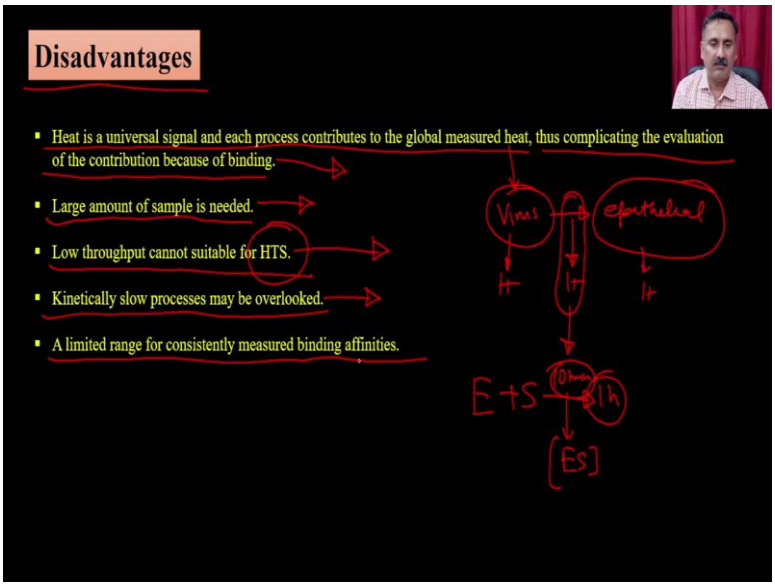

What is the disadvantage? So, there are several disadvantages heat is a universal signal and each process contribute to the global major heat thus complicating the evaluation of the contribution because of the binding. So, this is I have already explained with the help of a reaction a example like virus and epithelial cells right. So, you are only measuring the heat right you are not saying that heat whether the heat exchange is from the virus particle or whether from the epithelial cell or whether from the their interactions.

So, it you cannot distinguish from this H, this H and this H right the only way you can actually be able to distinguish is that if you subtract this one and this one from the original data, but sometime they that may not be also accurate and that may be also misleading. So, heat is not only the parameter which actually changes when the ligands are interacting with the enzyme and that is why it is not very you know its actually going to be misleading sometime ok.

Because if there are complicated system like this, it may actually results into the change in heat, but that may be because of the internal heat exchanges within the system itself. The large amount of sample is required because you have to fill the reaction vessel with the enzyme you require a large amount of ligand and so on.

Low throughout cannot be suitable for H T S. So, this kind of measurements cannot be done for multiple ligands. So, because for (Refer Time: 47:34) ligand you are supposed to prepare the enzyme for the reaction vessel and so, on. So, it is it cannot be done on a H T S mode actually. Kinetically slow process and may be overlooked. So, sometime if you are actually having the enzyme substrate interactions and the, but the kinetics is very low right.

So, it requires actually 1 hour for example, or it requires 10 minutes for example, to bind the complex and then now it is only going to form the complex right. So, these kind of reactions are actually going to be a problematic and a limited range is consistently measuring the binding affinity. So, it is actually also you know a limited range is consistently being measured and binding affinities.

So, what we have discussed so, far? We have discussed about the isothermal titration calorimetry and how you can be able to use that for measuring the enzyme substrate interactions and how you can be able to calculate the different types of binding constant and the different types of thermodynamical parameters.

So, with this I would like to conclude my lecture here in the subsequent lecture we are going to discuss some more techniques related to enzyme substrate interactions.

Thank you.