**Chemical and Biological Thermodynamics: Principles to Applications**

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**Lecture No – 39**
**Calorimetry**

In the previous lecture, we talked about that calorimeters have existed since 18th century; however, with time it was felt that there is a need to develop more sensitive calorimeters because if we are interested in understanding the systems of biological interest in which the heat change is not very large then much improved technology which can detect very small amount of heat changes is required and there has been tremendous amount of development in the field of calorimetry.

Let us discuss different type of calorimeters and also discuss that they can be used to understand what type of processes ultra sensitive micro calorimetry by ultra sensitive literal meaning is that we want a micro calorimeter which can detect very very small amount of heat changes.

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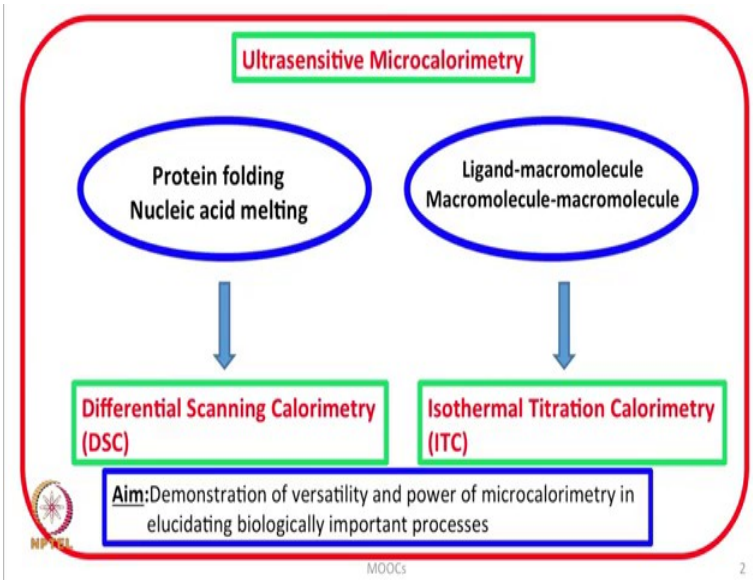


Let us take a look at the slide we will be talking in this course mostly on protein folding issues protein stabilization etcetera in the area of protein folding and nucleic acid melting differential scanning calorimetry is very very useful and when it comes to understanding

the guest host interactions like Ligand macromolecule interactions or macromolecule macromolecule interactions isothermal titration calorimetry becomes very very useful.

So, there are 2 types of ultra sensitive micro calorimeters available which can address these biological issues one is differential scanning calorimeter and the other is isothermal titration calorimeter the aim of this discussion is demonstration of versatility and power of micro calorimetry in elucidating biologically important processes.

So, let us discuss about these calorimeters one by one first we will talk about differential scanning calorimetry and let us try to take the literal meaning of the word differential scanning calorimetry and it is abbreviated as DSC. Usually we will call DSC for differential scanning calorimeter differential means there has to be some differential property scanning means we have to scan what it should be scanned is it temperature or is it time that we will discuss and calorimetry means we are talking about the heats.

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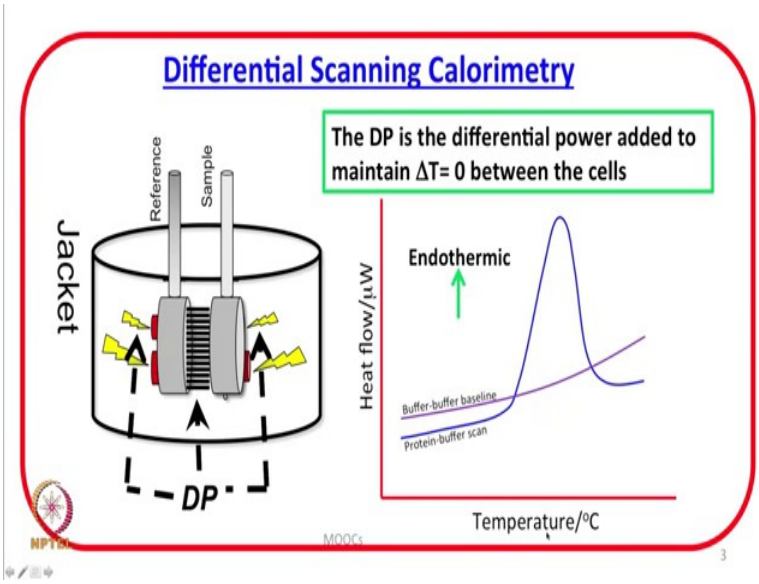


Let us take a look at the block diagram of a differential scanning calorimeter I am going it to discuss the principle of differential scanning calorimetry the principle remains same whether it is a differential scanning calorimeter for the measurement of large amount of heat changes or it is a micro differential scanning calorimeter the differences between the different type of differential scanning calorimeters will be the cell design. Even though I am discussing the differential scanning calorimetry specifically for biologically important systems, but the principle remains same what is the principle let us discuss.

Let us take a look at this block diagram in a differential scanning calorimeter what you have is a reference cell and you have a sample cell as I mentioned earlier the cell design will vary from one calorimeter to another calorimeter depending upon the type of applications, but the principle remain same. So, there is a reference cell and there is a sample cell this is enclosed in a adiabatic jacket constant power is supplied to the sample as well as the reference cell same power is supplied. So, once you fill this reference cell and the sample cell suppose in biological systems we take a protein solution then the sample cell will be filled with the protein and the reference cell will be filled with the solvent in which the protein is dissolved which usually is buffer.

So, initially a constant power is supplied and it is heated at constant heating rate or a constant scan rate we call in DSC heating rate is usually termed as a scan rate. So, you increase the power; that means, same amount of power in a programmed way increase with time the temperature in both the cells will start increasing by equal amount.

In reference cell we take the solution or material in which there is no change in its state during the entire scan; however, in sample cell if it is a protein at certain temperature the protein will start unfolding.

Now, let us try to understand that why there will be changes in the sample whether it is a phase change or an either kind of change with temperature when you take a solid and you start heating a solid at certain temperature the solid will melt and that is a phase transition, but when you take a protein you take a protein in a solution. And initially when the temperature is low the protein is in the folded form and when you start increasing the temperature the interactions which are holding the protein together in the three dimensional stable conformation form the interactions will start getting weakened and once the temperature is large enough to disrupt all those interactions the protein will open up.

This is also like a phase transition and for this transition the sample will absorb heat because in order to carry out the transition heat is required heat is required to break those intra molecular interactions heat is required to disrupt those interactions which are holding the structure together.

So, let us go back to the slide when the reaction or the transition starts in the sample cell what will happen since it absorbs energy additional power needs to be supplied to the sample cell so that the temperature of both the cells remain constant.

Please note down simultaneously you are heating it at a constant heating rate, but you want that the temperature difference between the 2 cells should remains 0 in the sense that if temperature tends to fall in this means if it starts absorbing heat additional power needs to be supplied to the sample cell and that differential power as a function of temperature or as a function of time is noted down why I am saying as a function of temperature or as a function of time because we will be heating it at a constant scanning rate scanning rate is so many Kelvin per minute.

So, therefore, whether we record as a function of time or we record as a function of temperature we have the requisite information. So, this is what is commented over here that the DP; DP is differential power added to maintain delta T equal to 0 between the cells is noted and what we have as an output is heat flow microwatt that is the differential power as a function of temperature and when it is proteins a typical output will be as seen in the blue curve.

What you have initially here is the protein let us say this temperature is about 25 degree centigrade at 25 degree centigrade let us assume that the protein is in the native conformation and when you start heating it initially you see ideally the DP should be 0, let me explain it over here.

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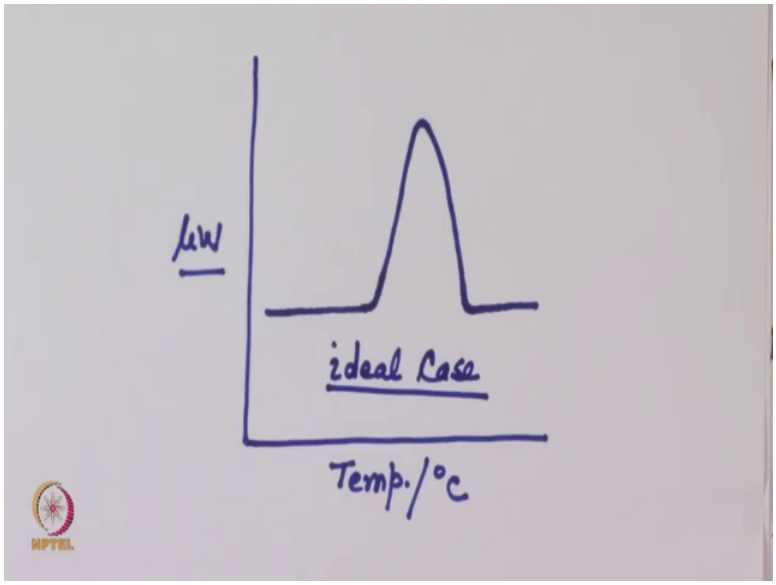


Here let me right microwatts that is the power and here I have temperature let us say I put in degree centigrade initially when no transition is taking place in the sample cell let me go to the slide you have protein sample here you have buffer over here initially if there is no transition happening the DP; that means, there is no additional power to any of the cells the DP is equal to 0; that means, the output you do not require any additional watt to be given it should be like this and once the transition starts then additional power needs to be given to the sample cell because transition is an endothermic process therefore, that additional power is recorded and it will eventually come back to the same line this is an ideal text.

So, what is shown over here is that this additional power is given to the sample cell until the transition is complete and then slowly and slowly that power is gone back to the same that is the same power which is being given to the sample and reference cell there is no difference in the power given to the sample and reference cell at the end, but this is an ideal case.

Let us take a look at the slide back here there is some slope and the origin of the slope I will be discussing a bit later, but let us concentrate on this blue line that this transition or we call it as a peak arises because the sample in the sample cell is undergoing a transition it needs heat to carry out that transition and since it needs heat the temperature will tend to fall. Therefore, additional power is supplied and that is noted in the form of a

deviation from here and once the entire process is over it comes back and this process has to be endothermic.

Please also note that I have written over here buffer; buffer baseline why does one need to do a buffer, buffer baseline I will explain.

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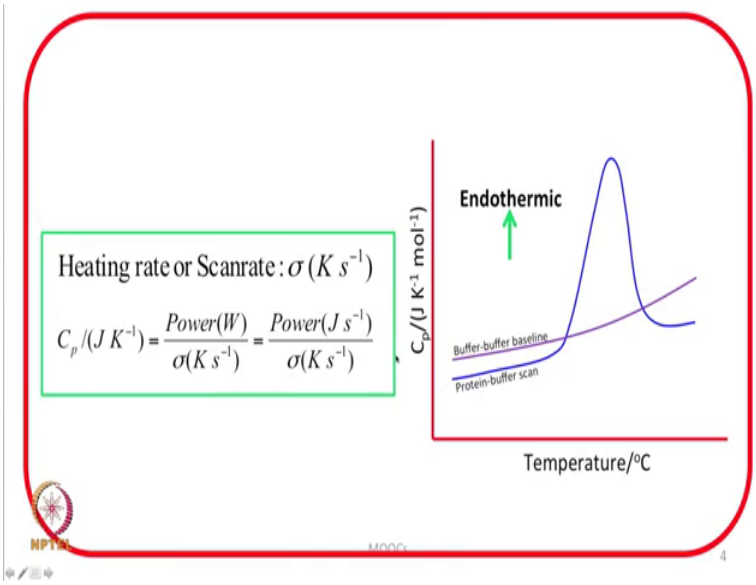


Next, but first of all you see over here the output is actually in terms of the microwatts that is the additional power which is supplied as a function of temperature, but I want to convert this power into a thermodynamic quantity such as the heat capacity how we can convert power into heat capacity let us look at the slide.

Let us say the heating rate or scan rate in Kelvin per second or per minute whatever way you want to describe is sigma and if I divide power divided by the scan rate the units of power is joule per second and the units of scan rate are Kelvin per second per second per second will cancel. So, the unit is joules per Kelvin joules per Kelvin are the units of heat capacity.

So, therefore, you can convert the power into heat capacity by dividing by the scan rate and as a result now I can show the final output in terms of heat capacity that is if you normalize the power with respect to the scan rate and or divide the power with respect to scan rate you will get heat capacity and eventually I have a plot of variation of heat

capacity as a function of temperature and since I have heat capacity and let us recall our discussion on heat capacity.

Heat capacity is very important thermodynamic connector between various thermodynamic properties at one temperature and another temperature the information on heat capacity is essential if we want to connect a thermodynamic property at one temperature to a thermodynamic property at another temperature. But in this context as we will see that heat capacity provides a lot of information and let us slowly and slowly discover that that what that information is.

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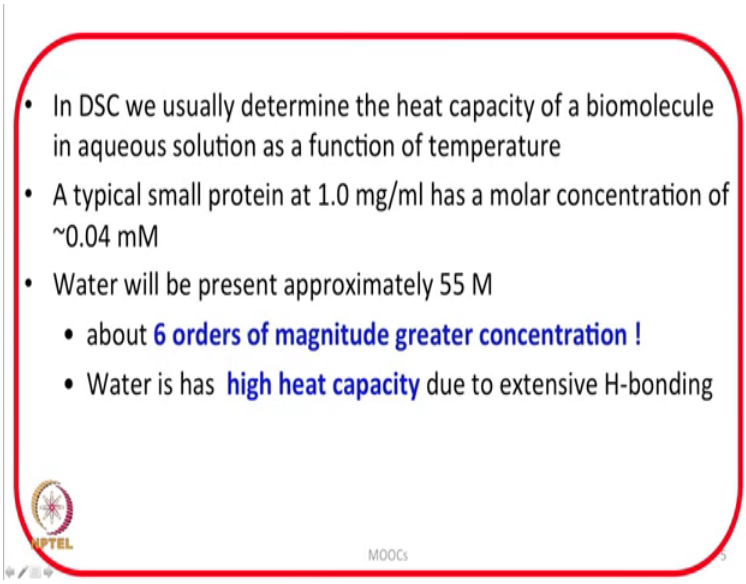


So, as commented over here in DSC we usually determine the heat capacity of a biomolecule in aqueous solution as a function of temperature and typically when you prepare a protein solution the molecule or weight of protein is generally very large compare to your buffer or other small molecules and the amount of protein taken for conducting the experiments is usually very small. Let us say about 1 milligram per m l a 1 milligram per m l for a typical small protein will correspond to 0.04 millimolar and protein you are going to take in the sample cell and protein is prepared in a buffer recall our discussion on buffers.

I specifically emphasized on the significance important of buffer when it comes to biologically important systems because we want the protein to remain stable and the

conformational stability of a protein is largely dependent upon PH and that is why there is a need of buffer.

Buffer is prepared in water and what is the concentration of water; water is about 55.5 molar.

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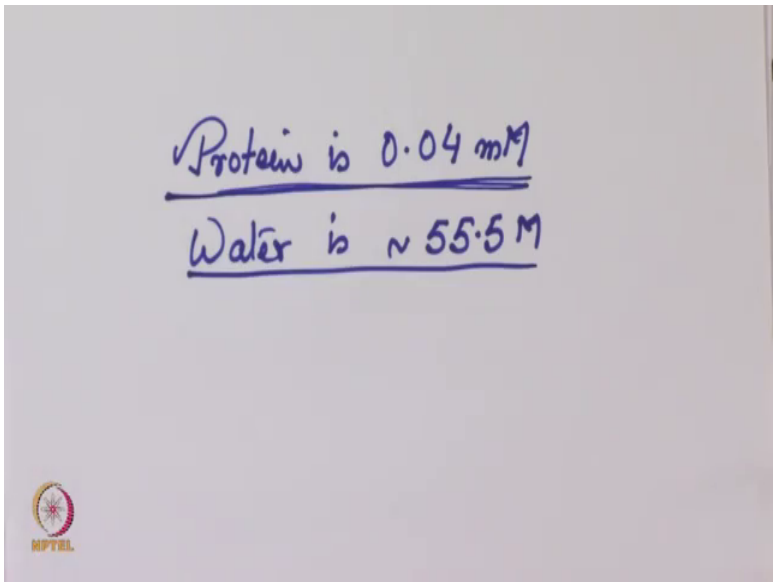


And now you compare if protein in this particular case that we are discussing in this particular example if protein is 0.04 milli molar and water is about 55.5 molar look at the imbalance in the concentrations.

Water has high heat capacity because there is an extensive amount of hydrogen bonding in water and we are preparing protein solution in water or buffer which has large amount of water which is actually prepared in water and therefore, how do we actually get information on heat capacity changes which are exclusively because of protein and get rid of water contributions to a large extent and that is done by putting the reference solution in the reference cell we will discuss more about that.

So, that is what was I was commenting upon that water will be present approximately at 55.5 molar level which is about ten raise to the power 6 times the magnitude compared to the concentration of the protein and also water has high heat capacity because of extensive amount of hydrogen bonding.

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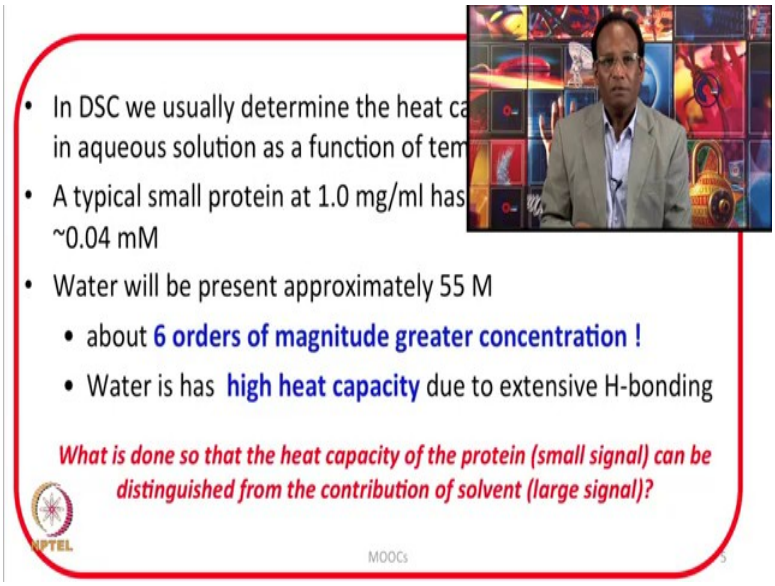


So, the question is what is done. So, that the heat capacity of protein which is actually a small signal can be distinguished from the contribution of solvent which is a large signal let us discuss about that.

Let me first show you the type of cells which are usually used in differential scanning calorimetry or calorimeter especially for biological systems 2 types of cells.

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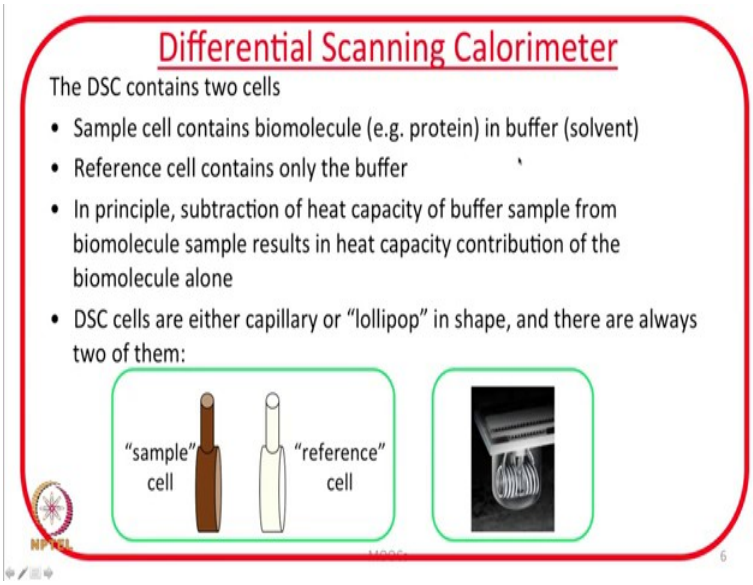


Are used this cell sample cell reference cell pair this is like a lollypop and the other one is a thin capillary type what is the advantage of any one of these over the other we will

discuss a bit later, but you do not want any additional reactions to take place when the sample is filled in the cells.

We will discuss a little more about this later and let us take a look at the comments the DSC contains 2 cells sample cell contains bio-molecule protein in buffer solvent reference contains only buffer in principle subtraction of heat capacity of buffer sample from bio-molecule sample results in heat capacity contribution of bio molecule alone and this is precisely the reason for making the differential scanning calorimeter with 2 cells one is a sample cell. Other is a reference cell because you want to determine the thermodynamic properties which reflect on the changes happening in the sample if the sample is protein for example, and you want to minimize the contributions to the major thermodynamic parameters from the reference and that is why that reference is filled into the reference cell.

And the next DSC cells are either capillary this is the capillary type or lollypop in shape and we will discuss later on that why and which design is better, but in addition to this there are some technical issues for example, cell geometry is important it is not always possible to prepare a pair of cells in which the cell geometry is 100 percent similar even if there is a small difference in the cell geometry and since we are talking about micro calorimeters we are talking about the calorimeters which are capable of detecting minute quantities of heat. Therefore, matching of cell geometry between the sample cell and reference cell becomes very very important and if there is no match then what will happen let us take a look at that.

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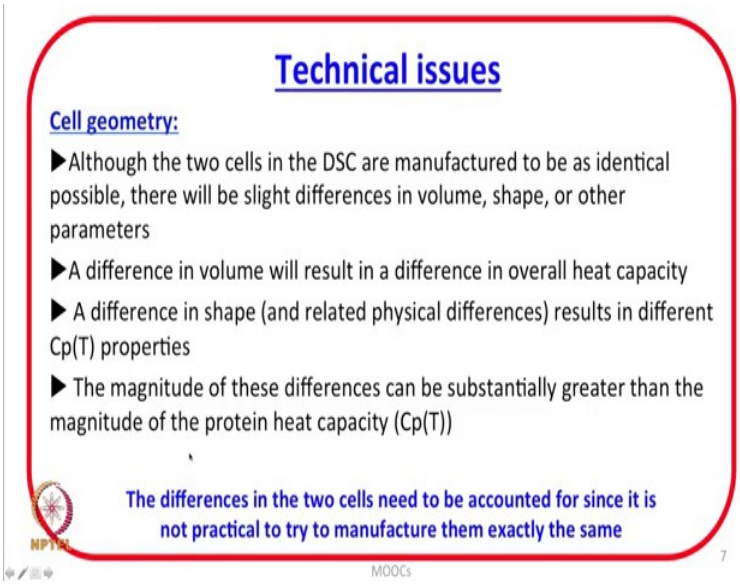


As commented over here although the 2 cells in DSC are manufactured to be as identical as possible still there will be slight differences in volume shape or other parameters moreover if there is a difference in volume that is definitely going to result in a difference in overall heat capacity.

Similarly, as I was talking about geometry a difference in shape and of course, and related physical differences will also result in different heat capacity verses temperature properties and the magnitude of these differences can be substantially greater than the magnitude of protein heat capacity.

If the cell geometry is largely different therefore, as commented over here the differences in the 2 cells need to be accounted for since it is not practically feasible to manufacture the 2 cells exactly the same and these are the reasons for doing a separate scan for just filing the reference material in both the cells.

What you need to do is for example, if we are talking about the protein solutions a separate experiment needs to be done in which the buffer is filled both in the sample cell as well as in the reference cell let us take a look at.

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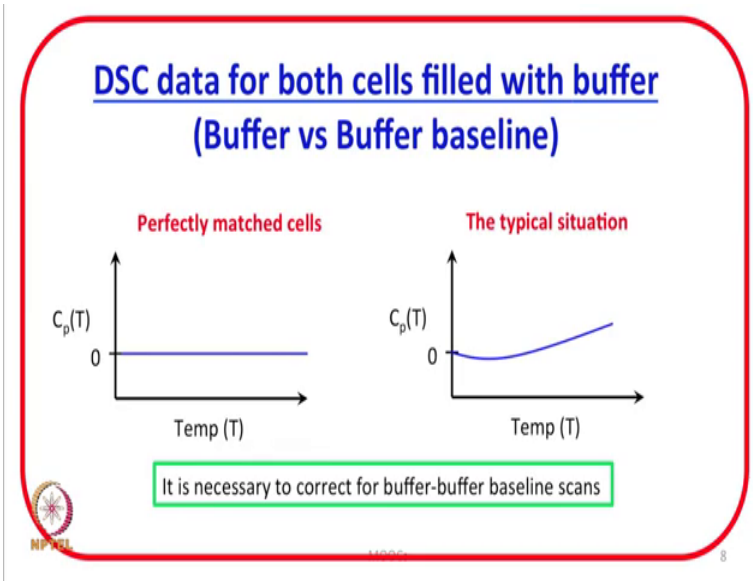


If we are able to get a pair of cells which are perfectly matched there is no difference in the geometry we will expect a straight line when do we expect a straight line when we fill buffer in the sample cell as well as in the reference cell and when you heat since in both the cells you have the same material you have the same solution you do not expect any phase changes you do not expect any reactions to happen. Therefore, there will be no additional power supply to any of the cells whether it is reference cell or the sample cell and the resulting signal will be or differential power will be 0 throughout.

However in reality what we get is not a straight line the shape the final shape of the buffer verses buffer scan or water verses water scan will be dependent upon the type of difference or the extent of difference in the cell geometries.

A typical output is like this it can be curvature downwards it van be curvature upwards and if the matching of the cell is better it can be even better therefore, it is very necessary to correct for buffer buffer baseline scans.

So, therefore, if we want to study the unfolding of a protein by means of heat and we want to use the differential scanning calorimeter to get relevant information we will need to do 2 experiments 1 experiment will be in which we take the protein solution into the sample cell and buffer in the reference cell and the second experiment will be you take buffer in both the cells and then we subtract the 2.

So, let us go back to the same slide where explained the principle of differential scanning calorimetry and discussed that the output for the protein sample taken in the sample cell will be like this and when you just take buffer in both that is called the baseline scan buffer buffer baseline scan that if it turns out to be like this what we need to do is subtract this from this to get a final differential scanning calorimetric output and that is the output which is to be used for the analysis of the data.

So, what we have discussed in this lecture is the principle of differential scanning calorimetry why the output like the output is in the form of a peak we also discussed that what problems can arise if there is a difference in the geometry of the cells and we also discussed that how to account for or how to minimize the effect of the heat capacities of the solvent used in differential scanning calorimetry and that is done by using the same solution in the reference cell.

In the next lecture we will discuss what type of what kind of thermodynamic properties we can extract from this output and then later on we will discuss the applications of those derived thermodynamic properties in the context of proteins.

Thank you very much.