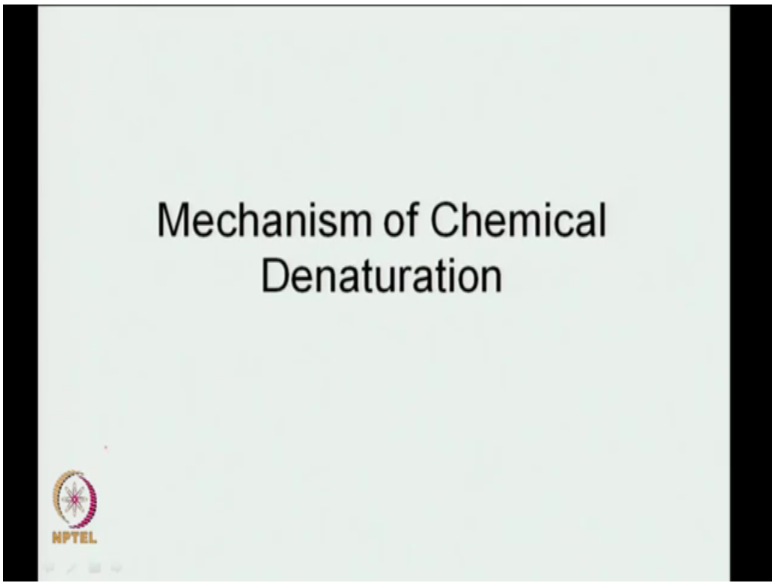
Bio-Physical Chemistry
Dr. Pramit Chowdhury
Department of Chemistry
Indian Institute of Technology, Delhi

Lecture - 18
Mechanism of Chemical Denaturation

(Refer Slide Time: 00:37)



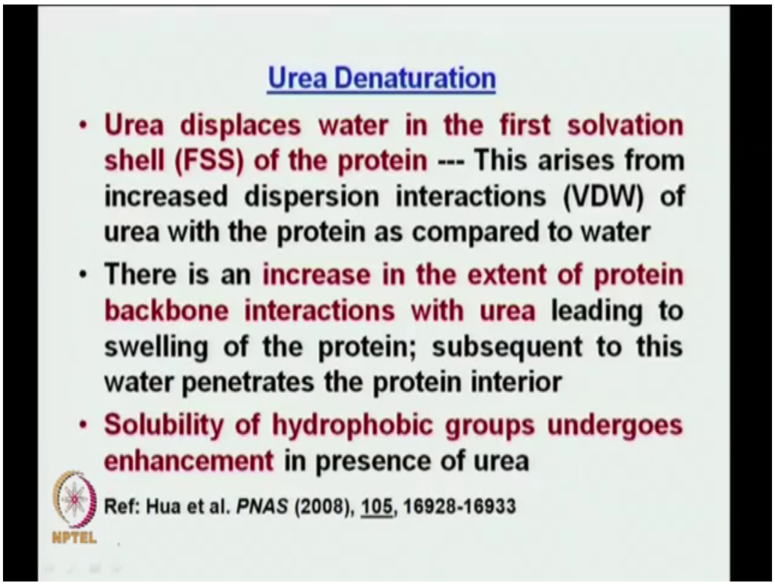
Mechanism of Chemical Denaturation

So, we will continue our discussion on Denaturation. Last class we looked at some details of chemical denaturation. So, I will just briefly mention some of the mechanisms or you know a few mechanistic insights that people have provided over how actually denaturation is brought about by say urea.

Now, let me tell you this whatever I am you know whatever sentences I am going to write or you will be seeing on the slides, those are from very recent research papers. So, that means, that no matter this denaturation whether chemical, I mean chemically whether by urea or guanidine hydrochloride is so commonly used. But, still there is you know lot of discussion about how actually these two compounds bring about the denaturation of a protein or a peptide.

I mean there are two separate schools of thoughts like this, and you will try to appreciate that. One is that the denaturant directly interacts with the peptide backbone ok, and opens it up. The other school of thought is that the chemical denaturant changes the structure of the water near the peptide, and thereby bringing about denaturation. There are two different schools of thought ok.

(Refer Slide Time: 02:00)



Urea Denaturation

- Urea displaces water in the first solvation shell (FSS) of the protein --- This arises from increased dispersion interactions (VDW) of urea with the protein as compared to water
- There is an increase in the extent of protein backbone interactions with urea leading to swelling of the protein; subsequent to this water penetrates the protein interior
- Solubility of hydrophobic groups undergoes enhancement in presence of urea

Ref: Hua et al. *PNAS* (2008), 105, 16928-16933

But this is from a very recent paper. And, what it says about urea denaturation is this. Look at the first sentence urea displaces water in the first solvation shell, FSS is the First Solvation

Shell of the protein, and this arises from increased dispersion interactions which means Van Der Waals interactions of urea with protein as compared to water.

Now, this is we have not exactly looked at urea you know peptide interaction, but we know what Van Der Waals interactions are. And what it says is that urea just gives rise to more dispersive interactions as compared to water right that is number 1.

The next is there is an increase in the extent of protein backbone interactions with urea leading to swelling of the protein; subsequent to this water penetrates the protein interior right. And then the solubility of the hydrophobic groups undergoes enhancement in presence of urea.

So, if you think about these three statements, you see these are very logical statements right, I mean this is the way you would think about a protein denaturing. One – it interacts with the protein directly right, which is your backbone interaction. Two – it has more dispersive interaction, so that means, in the first solvation shell where you will be having water molecules, you replace this water molecules gradually by what urea.

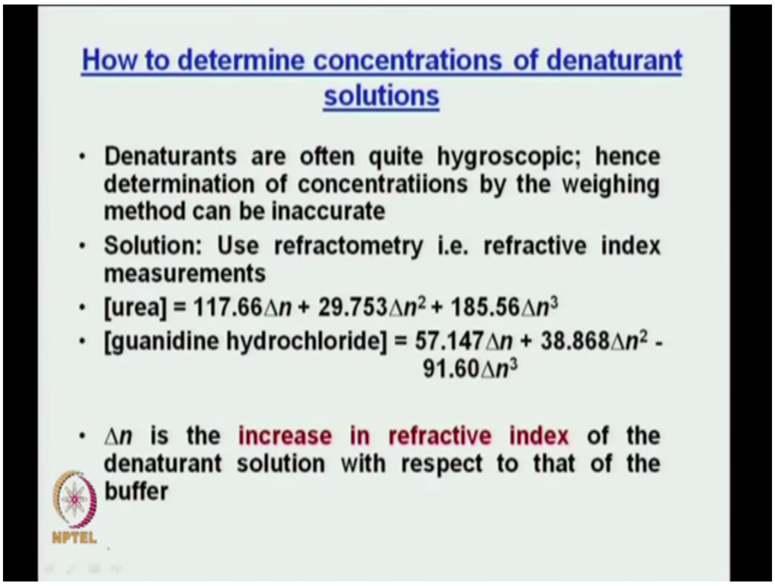
And that is thermodynamically favourable, because, why, because the dispersion of the dispersive interactions of urea with the protein or peptide backbone is thermodynamically more favourable than that with water and the protein or peptide backbone.

And the last one, obviously, make sense because remember when you are exposing proteins, that means, you are unfolding proteins you are exposing more and more hydrophobic groups, and you better have this thing that the hydrophobic groups are more soluble. Because if they are not soluble in this urea solution then they are going to fold back and form the folded structure again. So, intuitively thinking you know these three do make logical sense right.

So, there have been lot of computational studies, theoretically I mean experimentally these things had been little bit hard to prove, but still there are experimental studies, we will not go into that.

But just I mean keep this mind about what you know sort of interactions or what sort of lines that people would think about when they try to look at the mechanistic insight of a denaturant or chemical denaturant actually unfolding a protein ok. And you can see the reference at the bottom this is a PNAS paper 2008 right. It is from Dave Thirumalai's group at University of Maryland, and they have been doing a lot of work with regards to this ok.

(Refer Slide Time: 05:11)



How to determine concentrations of denaturant solutions

- Denaturants are often quite hygroscopic; hence determination of concentratiions by the weighing method can be inaccurate
- Solution: Use refractometry i.e. refractive index measurements
- $[\text{urea}] = 117.66\Delta n + 29.753\Delta n^2 + 185.56\Delta n^3$
- $[\text{guanidine hydrochloride}] = 57.147\Delta n + 38.868\Delta n^2 - 91.60\Delta n^3$
- Δn is the increase in refractive index of the denaturant solution with respect to that of the buffer

Now, this is one thing I would like to tell you before I go further. See if you would be doing chemical denaturation, that means, unfolding a protein with help of urea guanidine hydrochloride, how would you actually determine the concentration of the denaturant solution?

Now, one way is the most straight forward way is you know what volume of sample you have, you know what concentration of denaturant you want, whether urea guanidine hydrochloride, and you weigh it out on analytical balance right, and then dissolve the same amount in water and to the corresponding stuff.

But the problem is as you will see the first sentence will tell you, denaturants are often quite hygroscopic, hence determination of concentrations by the weighing method can be inaccurate right. So, that means, you take urea, but the actual amount of urea is less because you have more water adsorbed on to it. So, are essentially you are over estimating the denaturant concentration.

Now, why is this so important? Again try to realize this suppose you have done a measurement say on a certain; on a certain day of the week say on Monday right and that day the humidity level was not that high ok. Now, you are doing the same measurement say on Thursday where the humidity level is very high.

Now, what will happen, the amount of water that is absorbed or adsorbed by this denaturant would be different. So, essentially on both these days your actual concentration based on your weighing out will be different right, because you have different amounts of water adsorbed.

So, what will happen is if you are trying to do a reproducibility if you are trying to reproducible what will happen your experiments might not be reproducible, because your actual concentration of urea though by balance you have taken the same concentration, but because of the differential amounts of water adsorbed, your actual concentration of urea is different right. So, if you are ever see that definitely you would think that is must you know that something that must have never happened ok.

Then what do people do? People try an alternate thing. What they do is, it does not matter you weigh out something you put it in the solution you dissolve it in your buffer right and then you take the refractive index measurement, because in the refractive index measurements you are taking in solution right it is not you are using solid sample and in solution mostly is water so does not matter. So, refractive index is actually going to tell you the concentration of urea actual urea you have dissolved in water right.

And, obviously, there are relations. So, I will just give you the relations we will not go into those derivations. So, you can see the concentration of urea is given by this expression where you have the first term is the constant times delta n plus another constant times delta n square plus another constant times delta n cube, I will tell you what delta n is very soon. And then again the concentration of guanidine hydrochloride is given by a very similar expression right.

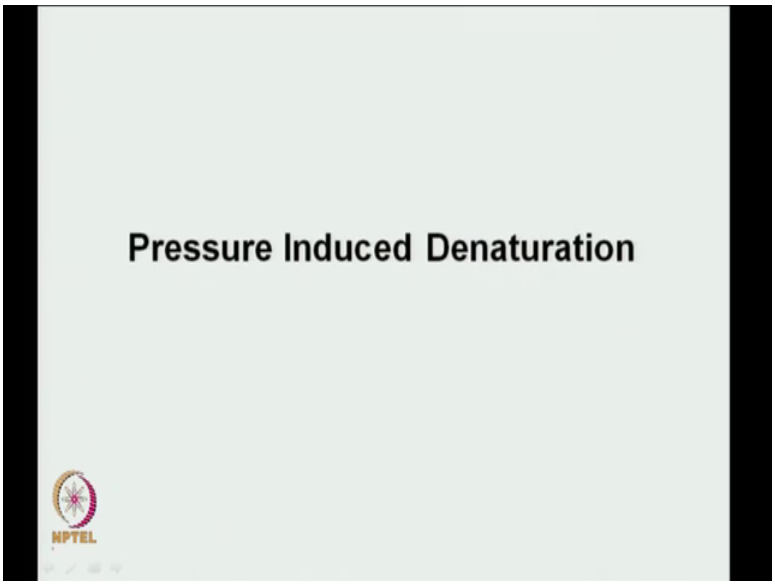
And you can see what delta n is, delta n is increase in refractive index of the denaturant solution with respect to that of the buffer. That means, what you do is you prepare your say you are preparing denaturant concentrations from say 0 from say 0 to 8 molar urea right.

Now 0 molar urea is essentially what your buffer. So, you take the refractive index measurement of your buffer right, then you go to 1 molar urea there will be a change in refractive index, so that is your delta n. Then again you go to 2 molar urea, delta n, and then you put it in this equation and correspondingly that delta n will tell you what the concentration of urea is ok.

This is how you do it and this is the best way of doing it because as I said in our country where humidity levels differ a lot you know in Delhi it is probably not a problem that you know that much of because if you take in winter or say in summer months essentially very dry. But if you go to a place like you know say Calcutta where I am from what will happen the humidity levels are really high, and they can really fluctuate and then you will be running into a problem.

So, it is best to create an alternate or to go by an alternate method which is this refractive index measurement ok. So, if you are ever doing this make sure you do that to crosscheck your measurement, because this is going to give you the actual concentration of urea all the time ok.

(Refer Slide Time: 09:20)

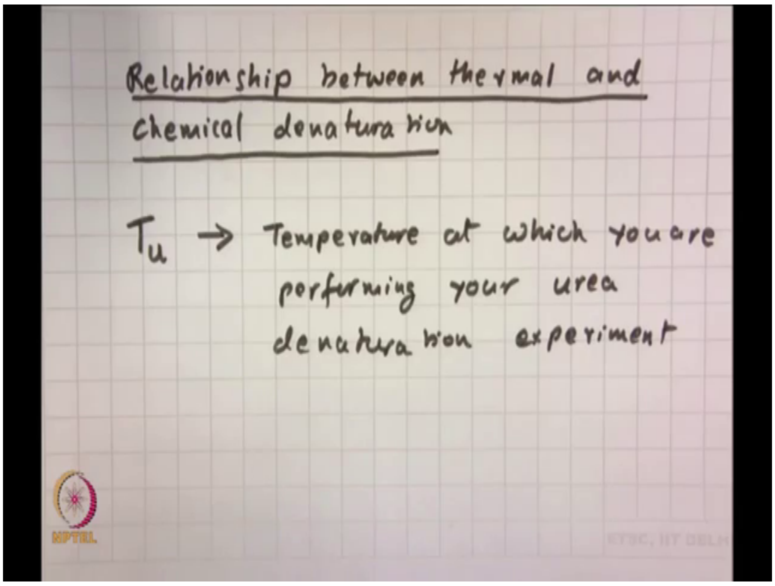


Pressure Induced Denaturation

So, the last topic would be pressure induced denaturation. But before I do that, before I actually do pressure induced denaturation, I will you know try to give you a small relationship between chemical denaturation and thermal denaturation. See, this is a very logical question that someone can ask or it can come to your mind. We have studied thermal denaturation; we have studied chemical denaturation right; both the denaturants right. Both are you going to denature them denature the protein.

But at any point of time, how are these two related, because we are doing a denaturation I will be by two different methods one is you are providing thermal energy, the other one is providing a compound you know which does something with the protein and denatures it. But what is can we have a relationship between say the T or between the denaturant concentration and the temperature at which your thermal melting is occurring. So, what we are going do is, we are going to try to derive a very small relationship.

(Refer Slide Time: 10:17)



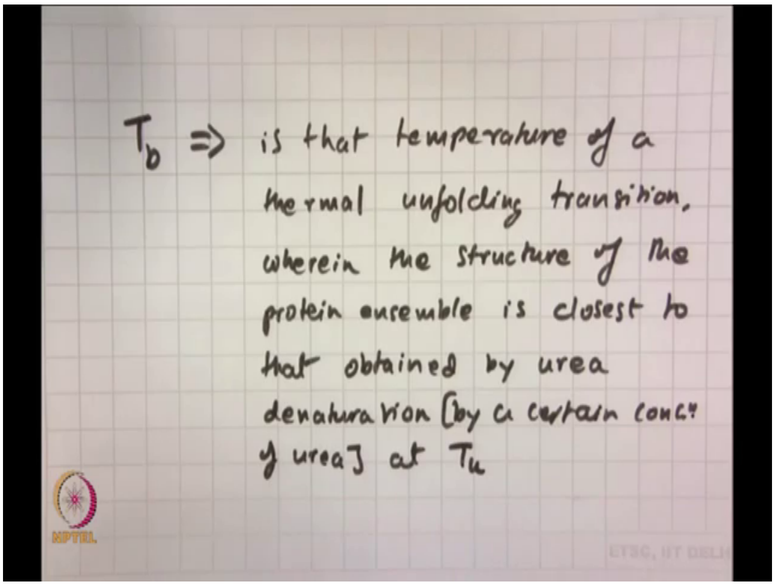
Relationship between thermal and chemical denaturation

T_u → Temperature at which you are performing your urea denaturation experiment

So, a relationship between thermal and chemical denaturation right. Now, before we start with the actual derivation, what we will do is, we will just define two temperatures. One is T u. What is T u? T u is the temperature, T u is the temperature at which you are performing your urea denaturation experiment, urea denaturation experiment right.

And see we discussed this before right, the difference between thermal and chemical is, thermal is you gradually change the temperature, chemical is you do not change the temperature, it is an isothermal transformation. Transformation in the sense is done isothermally, you keep the temperature fixed, and then you change the denaturant concentration. So, that temperature we are taking as T u ok.

(Refer Slide Time: 11:53)



$T_b \Rightarrow$ is that temperature of a thermal unfolding transition, wherein the structure of the protein ensemble is closest to that obtained by urea denaturation [by a certain concn of urea] at T_u

Now, what we do is we define another temperature. Now, this temperature, listen to carefully. I define T b. What is T b? Now, first I will tell, and then write it down. What T b is this is how I look at it. See, at T u at any given denaturant concentration you have you will be having a certain structure of the protein right depending upon what denaturant concentration you have.

If it is low, it is more towards a native; if it is high, it is more towards a unfolded state; if it is in between, then you know it is in the transition region. It will be mixture of both. And we are always taking into account the fact that it is a two state right native, unfolded or denatured.

Now, the way I define T b is remember I am trying to find a relationship. So, the way I am, so I have to find a point of similarity between these. But what is my what is you know what is my what should I say scale of similarity, the scale of similarity would be that temperature or something relate to the structure, that means, I chemically denature it at a certain concentration C at temperature T u, I get a certain structure. So, this is certain structure.

So, to get a relation, I have to find another structure in my thermal denaturation profile at a certain temperature which or where or at that point the structure is closest to the structure I have in urea. Is it clear or not? So, that means, I am trying to compare two different things in chemical denaturation at certain concentration see I have a structure right.

If I am going to compare these two, I better have the same structure. And I say that I take a certain temperature that temperature is T b where the structural similarity of my thermally denatured state is most to that of my chemical denatured state right.

So, then in brief I can write T b is that temperature of a thermal unfolding transition, wherein, wherein the structure of the protein ensemble is closest to that is closest to that obtained by urea denaturation, urea denaturation ok, by a certain concentration of urea I mean by a certain concentration of urea at T u, my certain concentration urea at T u. Just go through the statement and see whether it is clear or not.

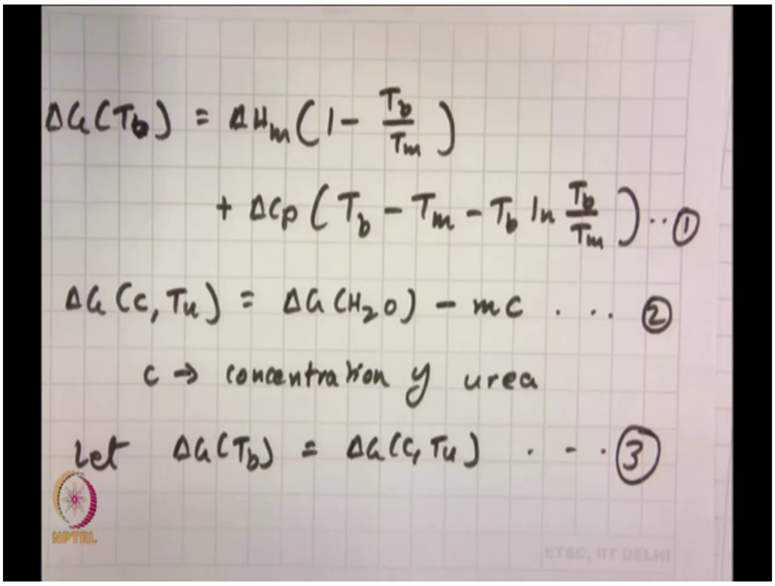
Again it says T b is that temperature of a thermal unfolding transition, wherein the structure of the protein ensemble is closest to that obtained by urea denaturation where I am assuming a certain concentration of urea say C, but done at T u, because T u we are not changing for chemical denaturation right. So, this is the starting point we have to find two different temperatures right T u and T b.

So, we go to, now, now think about this. What we can do? What we can do is this is what we are going to do, both these at both these temperatures you will be having delta G. Now, both these structural equivalent now they are closest. So, the delta G should be the.

Student: Same.

Same. And I know my delta G dependence of thermal, I also know my delta G dependence of urea. So, what I would do is, I will just equate these two, and that is essentially I am going to do ok. So, let us do that.

(Refer Slide Time: 16:00)



$$\Delta G(T_b) = \Delta H_m\left(1 - \frac{T_b}{T_m}\right) + \Delta C_p\left(T_b - T_m - T_b \ln \frac{T_b}{T_m}\right) \cdot\cdot \text{①}$$

$$\Delta G(c, T_u) = \Delta G(H_2O) - mc \;\; . \; . \; . \;\; \text{②}$$

c → concentration of urea

$$\text{let} \;\; \Delta G(T_b) = \Delta G(c, T_u) \;\; . \; . \; . \;\; \text{③}$$

So, from before we have seen that delta G say from thermal as a function of temperature. So, I write here T u delta G T u. Well, let me write T b for the time being because T b is my thermal unfolding the temperature is equal to delta H m 1 minus T b over T of m plus delta C p T b minus T m minus T b natural log T b over T of m.

So, what I have done here is if you remember before we did not have T b we had just T. So, delta G of T is equal to delta H m we know what m stands for, and we just had T. Now, we are saying is we have defined this temperature T b, and we our reference temperature is T m always we have defined temperature T b and that is what we are just putting it right ok.

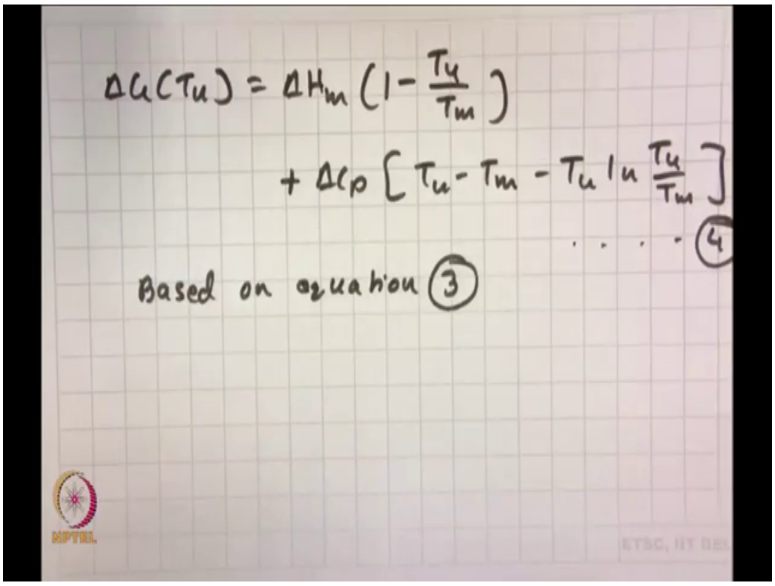
Now, from your chemical denaturation, what you know, from a chemical denaturation, this is your thermal right. From your chemical denaturation, what you know is delta G ok, delta G, I can say as a function of a certain concentration of urea c at temperature T u is equal to what is equal to delta G, remember it was H 2 O right minus m times c. What is c? c is the concentration of your urea or denaturant, isn't it? So these things we know now.

So, what now what I am going to say is that because I am looking at structural equivalence right then I can say that my delta G T of b should be equal to what delta G c, T of u ok. I can say let this be there. I can give these equations as this is number 1, this is number 2 and this is number 3. So, you are clear up to this one right.

Now, if you look at delta G c, T u, delta G c, T u, that c is taken care of by m c in equation 2 right. But what is delta G H 2 O? Delta G H 2 O is that free energy change where you have zero denaturant. So, this now remember this experiment is done at T u. So, what I can do is, this for this delta G H 2 O, what I can do is I can take this delta H delta G H 2 O. And in my original I mean in my original relation where I have T b is equal to T, I can replace the T by what T u, the same thing you realize this.

See, this is delta G H 2 O right at a specified temperature. My specified temperature is T u right. Now, what I also know is that delta G at any given temperature is by this relation. So, what I just do is, since I have already done T b, I replace 1 T b with T u that is it because that there is no denaturant concentration.

(Refer Slide Time: 19:39)



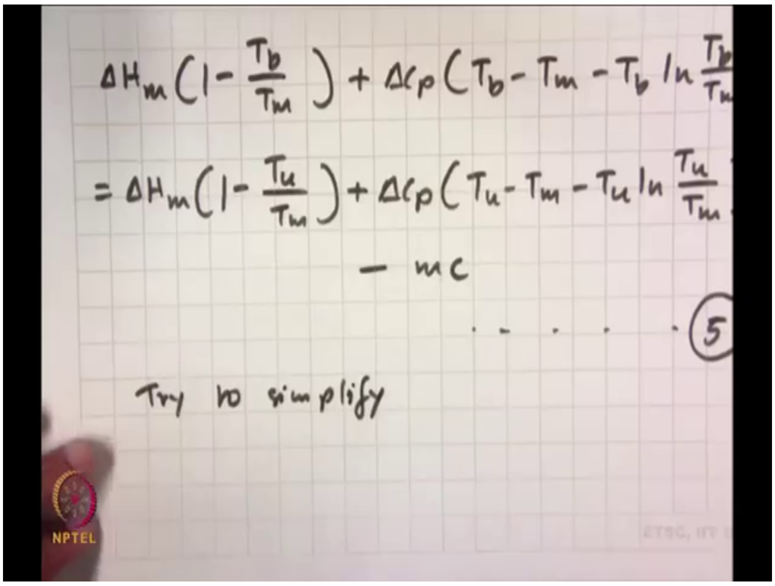
$$\Delta G(T_u) = \Delta H_m\left(1-\frac{T_u}{T_m}\right) + \Delta C_p\left[T_u - T_m - T_u \ln\frac{T_u}{T_m}\right] \quad \ldots\ldots ④$$

Based on equation ③

So, then what I can do is I can write delta G T u, I will just write it down is equal to delta H m 1 minus T u over T of m plus delta C p it will give me T u minus T m minus T u natural log T u over T of m, and this is say equation 4. Is it clear this far what we did?

Now, because based on equation 3; based on equation 3, that means, this one where we have equated these two ok, what can I say now, now this is what I can write down ok. I will go to the next page, because this will not give me much space based on equation 3, this is what I can write.

(Refer Slide Time: 20:43)



$$\Delta H_m \left(1 - \frac{T_b}{T_m}\right) + \Delta C_p \left(T_b - T_m - T_b \ln \frac{T_b}{T_m}\right.$$

$$= \Delta H_m \left(1 - \frac{T_u}{T_m}\right) + \Delta C_p \left(T_u - T_m - T_u \ln \frac{T_u}{T_m}\right.$$

$$- mC$$

. . . . . . ⑤

Try to simplify

What I can write is delta H m 1 minus T b over T of m then plus delta C p T b minus T m minus T b natural log T b over T of m is equal to now equal to what?

Student: (Refer Time: 21:03).

Is equal to delta H m 1 minus now I should instead of T b I should be having what?

Student: T u.

T u right T of m plus delta C p T u minus T m minus T u natural log T u over T of m ok. But is this all?

Student: Minus m minus mc.

Minus m c isn’t it? So, this should be minus m c and this is equation number, what should the equation number be?

Student: (Refer Time: 21:43).

Equation number 5, I guess say equation number 5, ok, right. See we are trying to get a relationship between T b and T m. And if you are going to get a rather T b and T u, and if you are going to try to get a relationship between that, you must make sure that it has a dependence on the denaturant concentration, because depending upon denaturant concentration your structure is going to change. Isn’t it? So, we have to have the dependence, so that is minus m c.

So, then if I just cover this from here to here, this portion is delta G H 2 O done at temperature T u right, and this is

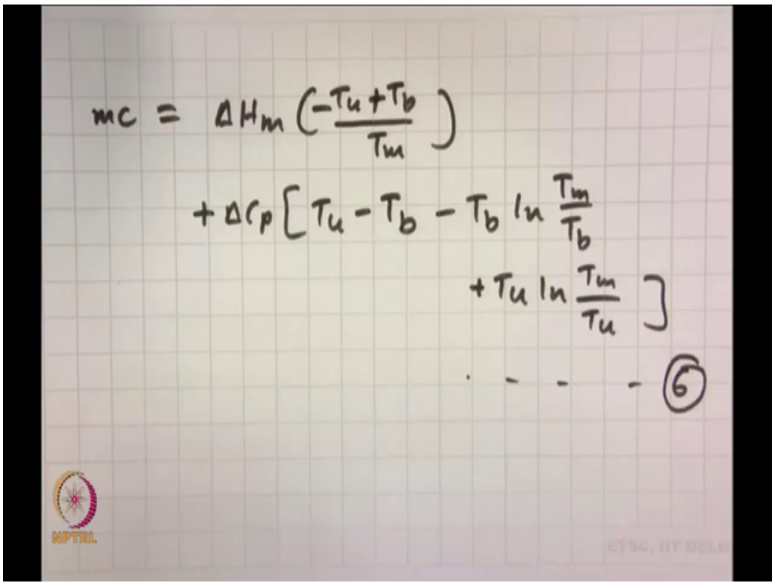
Student: m.

Just.

Student: m c.

m c, the free energy dependence linear ok. So, what we will do is now we will try to simplify it. And the way you do it is we take m c to the left hand side and then bring the whole thing to the right hand side ok. And, this is what we should be getting finally. Just correct me if I am writing anything by mistake.

(Refer Slide Time: 22:52)



$$mc = \Delta H_m \left(\frac{-T_u + T_b}{T_m} \right) + \Delta C_p \left[T_u - T_b - T_b \ln \frac{T_m}{T_b} + T_u \ln \frac{T_m}{T_u} \right] \quad \cdots - ⑥$$

I can write then m c is equal to delta H m, then I can write T u minus T b over T of m. Is the first term ok? Then I can write delta C p, then it would be T u minus T b ok, because T m and T m cancels right. We can see the T m cancels. Now, what are we left with is if I get, so let me get this again if I get this minus T b natural log T b over T m on this side it, it becomes what plus T b natural log T b over T m right.

But what I will do is, I will change the logarithmic now that means I will go to ln T m over T b ok. So, the hence this one I can write as minus T b natural log T m over T of b ok. Then what were the next term be plus T u natural log T m over T of u ok. And let this be equation number 6.

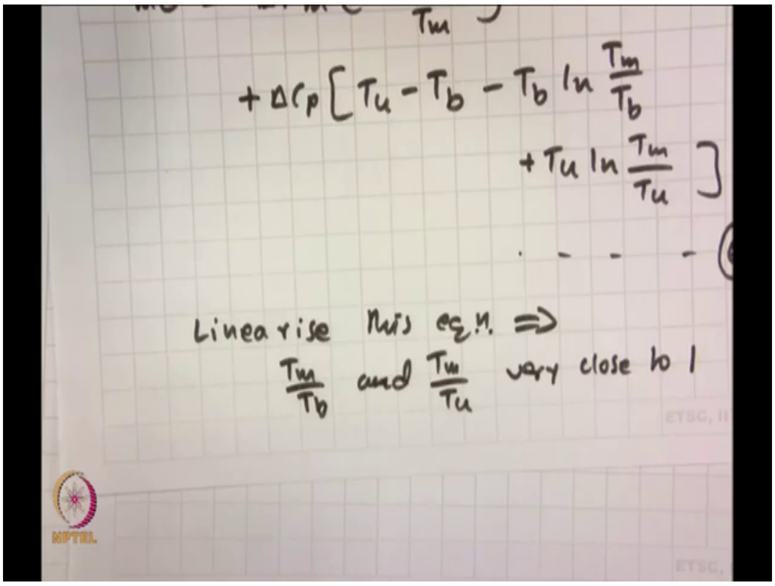
Student: (Refer Time: 24:14) sir it will be T b minus T u.

Which one?

Student: Sir the first term delta H m.

You are right. It would be T b minus T u isn't it. You are absolutely right. Well, what I will do is this T u minus T u, thank you, absolutely right ok. So, this is you know the simplified form we have up till now.

(Refer Slide Time: 24:46)

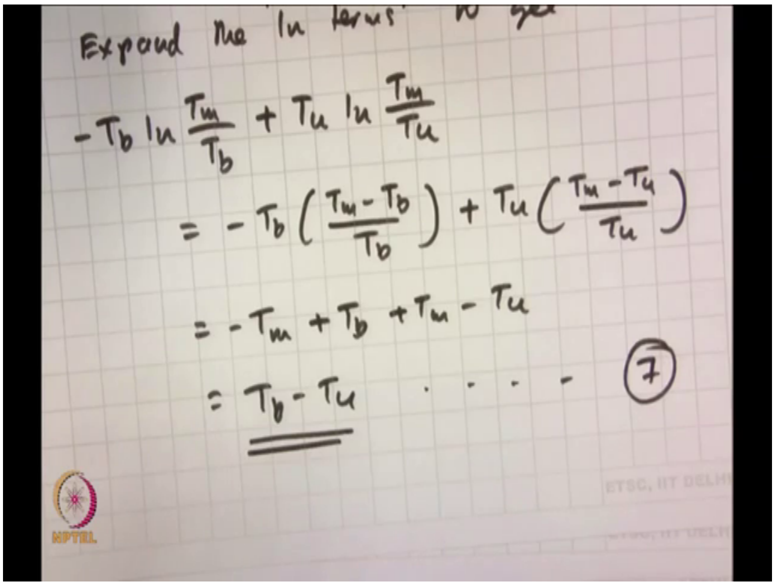


$$+\Delta C_p\left[T_u - T_b - T_b \ln\frac{T_m}{T_b} + T_u \ln\frac{T_m}{T_u}\right] \quad \text{- - - -}$$

Linearise this eq.n ⇒ $\frac{T_m}{T_b}$ and $\frac{T_m}{T_u}$ very close to 1

Now, what we will do is, the problem is again we have this logarithmic term, we will try to linearize this equation. We will try to linearize this equation. And we assume for this one what we assume is T m by T b and T m by T u are very close to 1, very close to 1 ok. T m by T b and T m by T u are very close to 1 that is what see that was the reason why we change this logarithmic division term. So, now I will use another approximation and write down the approximation for you.

(Refer Slide Time: 25:34)



Expand the ln terms to get

$$-T_b \ln \frac{T_m}{T_b} + T_u \ln \frac{T_m}{T_u}$$

$$= -T_b\left(\frac{T_m - T_b}{T_b}\right) + T_u\left(\frac{T_m - T_u}{T_u}\right)$$

$$= -T_m + T_b + T_m - T_u$$

$$= \underline{\underline{T_b - T_u}} \quad \ldots \ldots \quad ⑦$$

So, we will expand the natural log terms, we will expand the natural log terms right to get and I can write this one down now minus T b natural log T m over T of b plus T u natural log T m over T of u is equal to minus of T b, then T m minus T b over T of b plus T u T m minus T u over T of u ok. And if we simplify this, what do we get, if we simplify this, what do we get? You see T b, T b cancels; T u, T u cancels. What am I left with, what am I left with?

Student: (Refer Time: 26:34).

Minus T m.

Student: (Refer Time: 26:36).

Plus T b. Then?

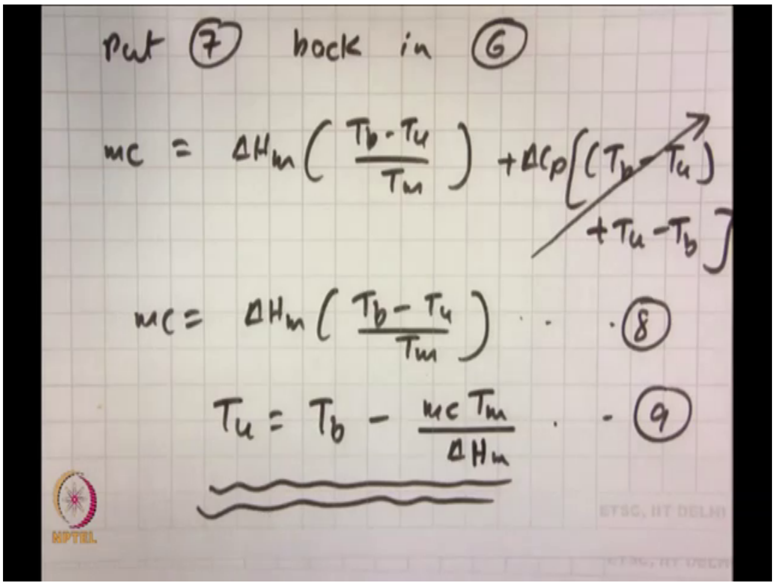
Student: (Refer Time: 26:37).

Plus T m minus T u. So, it is

Student: T b minus T u.

T b minus T u ok. And this one let this be equation number what 7 ok. So, see, you have taken you know complicated term a series of terms or combination of terms, you applied a linear approximation right, you linearised it, and then you get a very simple term like this.

(Refer Slide Time: 27:15)



Put ⑦ back in ⑥

$$mc = \Delta H_m \left(\frac{T_b - T_u}{T_m} \right) + \Delta C_p \cancel{\left[(T_b - T_u) + T_u - T_b \right]}$$

$$mc = \Delta H_m \left(\frac{T_b - T_u}{T_m} \right) \quad \cdots ⑧$$

$$\underline{T_u = T_b - \frac{mc\, T_m}{\Delta H_m}} \quad \cdots ⑨$$

So, I can go forward and put 7 back in 6, I guess that is what it was right. So, then I can have m c is equal to delta H m T b minus T u over T of m, then I should be having plus delta C p T b minus T of u right, so that is what we get. Now, you can see the relation is very simple.

We always we are trying to get a relationship between what between T u and T b essentially, because T u is where we are doing this unfolding, and T b is where the thermal unfolding transition where this it was structural equivalent ok. So, from here what should I be getting? I should be getting this is this is m c is equal to this, then I can write down I will just give you the final form, yes, ok.

Student: (Refer Time: 28:19).

Then let me write this down T b minus T u which was this one, I forgot this. Then

Student: Plus t.

I can write plus T u minus T of b right yes see that is why I lose track of this big equations ok. So, T b minus T u T u minus T b where I get rid of this term totally goes to 0.

So, therefore, I have m c is equal to delta H m T b minus T u over T of m ok. And therefore, finally, I can write down the last expression for you. Then T u is equal to T of b minus m c T of m over delta H m, say this is equation 8, then this is my final equation.

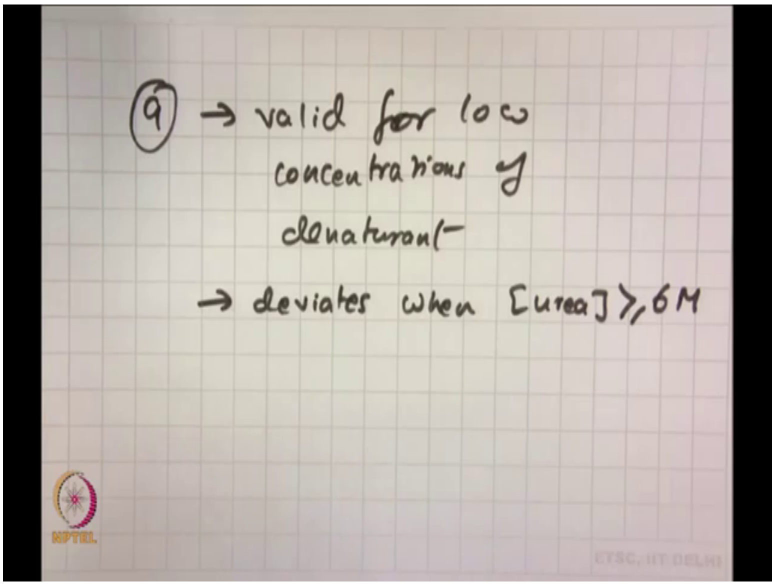
So, you can see, that means, there is a very simple relationship I repeat under the condition, under the approximation that you are assuming a linear approximation right. If you take higher orders then; obviously, relation will be complicated right, you will be having problems.

But then what it says is T u is equal to T b minus m c T m over delta H m you know what T b and T u are you have defined, then m is a slope, you can get from your free energy measurements, c is the concentration you know and delta H m and T m are also known to you from how we discussed about fitting your thermodynamics data, especially for thermal transition ok

Now, let me tell you this, again this we have derived using a linear approximation, but the problem is this equation only holds for low concentrations of denaturant. That means, when you are in the regime of say one to; say one to like may be 4 or 5 molar of denaturant then this condition will hold or this equation will hold. If you go to very high concentration of denaturants especially 7, 8 molar you know even 8 molar urea, then you will see deviations from this linear relationship.

The reason being your linear approximation does not holds at the time, you have to bring in higher orders. And only after you bring in higher orders would you be able to model the actual relationship between T u and T b ok. So, please keep this in mind.

(Refer Slide Time: 30:48)



⑨ → valid for low concentrations of denaturant

→ deviates when [urea] $\geq 6M$

So, this equation, so, equation 9, it is a equation 9 valid for low concentrations of denaturant right. Deviates when concentration of urea or denature or denaturant is greater than or equal to 6 molar, then deviations are observed ok. So, finally, we have come to a point where we have been able to derive through approximations and analytical relationship between T u and T b.

And you would know given a certain T u temperature T u and a certain temperature T b, how they would be related if we are talking about structural equivalence because that is something we always have to keep in mind ok. We cannot about talk about two different structures, because then the free energies would not be in the same. The free energies have to be the same hence we have to talk about the same structure.

So, this brings us to the end of our discussion on chemical and thermal denaturation. I do not want to well you will see that there are many other variations, there are many other insights, there are many other models to it specially for chemical denaturation.

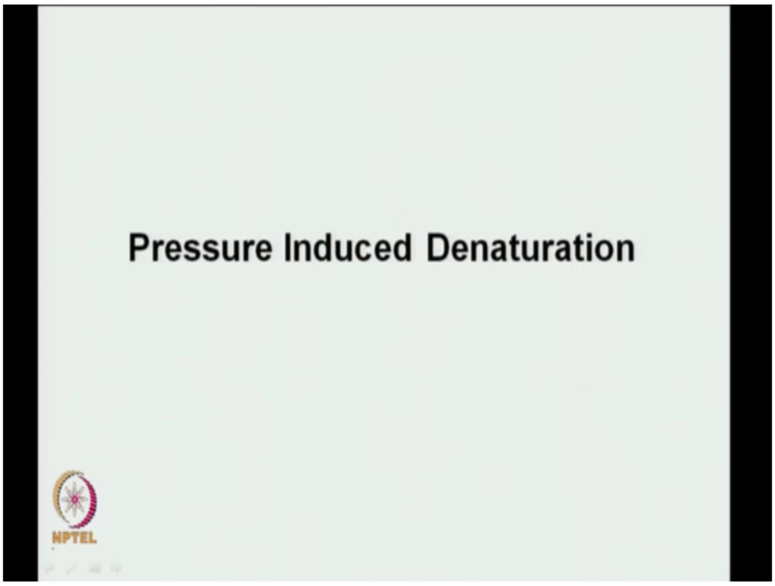
There is something known as the binding model, but you know we will not go into that because the idea is not to look at the different models, but the idea is at least for you to able to be able to appreciate what all you know goes in when we look at interactions between say a thermal transition and a protein when you are putting in thermal energy or a molecule which is able to denature your protein ok.

And one thing you must have seen is no matter what you do in both the cases your accessible surface area plays a huge role. Because accessible surface area determines your delta C p and delta C p is very strongly related accessible surface area. And you have also seen that your m is also related to your accessible surface area.

So, m and delta C p typically go hand in hand because they are highly dependent upon your accessible surface area. And it only makes sense because the more you denature a protein, the more you increase the accessible surface area, because you are opening up the protein and in the more are your opening up hydrophobic groups into the surrounding solvent. Hence delta C p does play a huge role no matter what you are talking about ok.

Now, the last topic that we would be talking about in denaturation is pressure denaturation. Now, pressure denaturation why would we care about that or you know why did we not talk about it before?

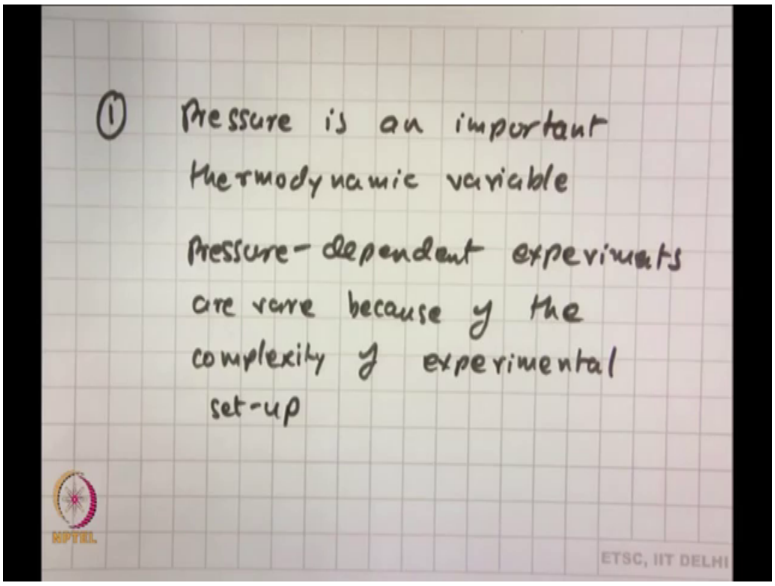
(Refer Slide Time: 33:40)



Pressure Induced Denaturation

See, if you talk about pressure induced denaturation, so pressure induced denaturation is important. Why?

(Refer Slide Time: 33:51)



① Pressure is an important thermodynamic variable

Pressure-dependent experiments are rare because of the complexity of experimental set-up

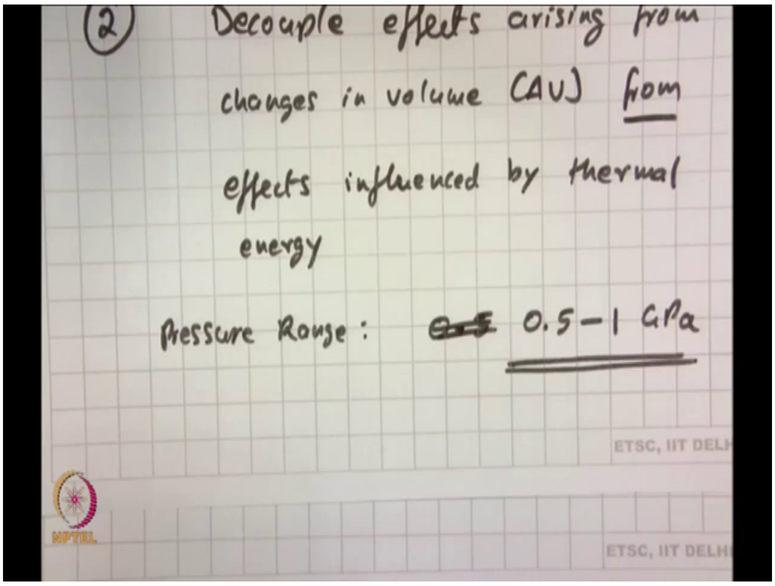
So, number 1, pressure is an important thermodynamic variable ok. So, pressure is a very important thermodynamic variable. But whatever you have seen up till now is temperature, one it was a thermal transition, because it is easy for you to do. And the next one was the urea at a specific concentration of or a specific temperature. Then why are pressure temperature or pressure experiments so rare, because pressure dependent experiments are rare because of the complexity of experimental setup.

See everyone realizes, everyone realizes that pressure is a very important variable, a very important thermodynamic variable. But the one that has been the bottleneck the one that is in the bottleneck or that is be in the real obstacle is that if you would be trying to apply a huge amount of pressure, then the amount of you know the type of experimental setup you need would be kind of sophisticated.

Sophisticated in the sense that it would be able it should be able to withstand that amount of pressure, because you have to apply a pretty high pressure I will tell you what the pressure is in approximate range. And that is why you know people did not want to invest so much time in it because they were easily looking at denaturation through what thermally or chemically ok.

So, this was the main thing behind it, but you know nowadays with people trying to probe in different things you know taking on different very topics, people have actually started looking at pressure denaturation right in details, though not so many groups few select groups have actually started.

(Refer Slide Time: 36:09)



② Decouple effects arising from changes in volume (ΔV) from effects influenced by thermal energy

Pressure Range : ~~0.5~~ 0.5 – 1 GPa

Now, what is the other importance of pressure denaturation? The other importance of pressure denaturation is you can decouple; you can decouple effects arising from changes in volume which is delta v from effects influenced by thermal energy right.

That means, if you are applying pressure, then the direct effects would be what in terms of volume. And if we have a temperature dependence experiment, what you do? In temperature dependence you not only increase the temperature what you also do you also do expansion

Student: Expansion.

Or if you are going the other way you do compression. So, in that case, the delta v changes also inherently involved though we are not looking at it.

So, what does pressure do? Pressure helps you separate the thermal energy component from the change in volume component that is why again pressure is so important. But the fact is that these things are hard to do because of proper experimental setups right. And as I was telling you the pressure range we are interested in for proteins. So, the pressure range typical is from 0.5, let me write this again 0.5 to 1 giga Pascal, 0.5 to 1 giga Pascal ok. G stands for giga.

Now, you can understand how higher pressure this is, just normal pressure is not sufficient you have to have pretty high amounts of pressure. And to have this you have to have a very dedicated experimental setup, and that is what people have not been able to do so frequently, it is one of the reasons or that is the major reason.

Now, if you all going to look at your free energy change because of both pressure and temperature, see logically what should we have. Whatever delta G we have seen up till now especially what thermal it only included temperature right. So, somehow I would have to bring in a change in delta v right.

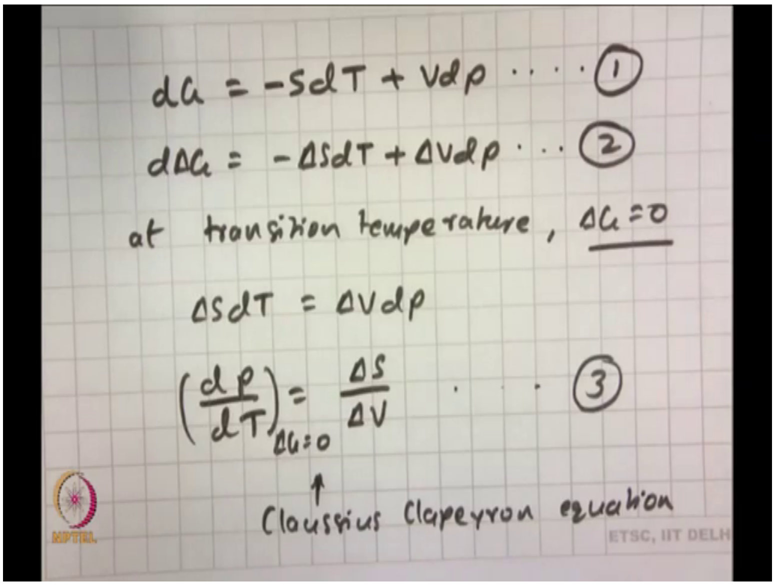
And if I am going to bring a change in delta v, then instead of measuring delta v directly, what I can do is I can do it by some other parameters if you guys can recall in thermodynamics, there is alpha, coefficient of thermal expansion

Student: (Refer Time: 38:53).

And beta remember isothermal compressibility. So, what are these? See, if we can somehow in del G incorporate these two, then we can have a situation where I have information from both from pressure dependent and from temperature dependent. And the temperature dependence you know plus volume I have from the thermal transition. So, somehow I can relate these two ok.

So, you know let us try to do a small derivation the reason I am doing this I know these derivations take some time you know, ut if you do it from the basic principle you just have a better feel of it right. So, let us do that and let us see where we go with that ok.

(Refer Slide Time: 39:42)



$$dG = -SdT + Vdp \cdots ①$$

$$d\Delta G = -\Delta SdT + \Delta Vdp \cdots ②$$

at transition temperature, $\underline{\Delta G = 0}$

$$\Delta SdT = \Delta Vdp$$

$$\left(\frac{dp}{dT}\right)_{\Delta G=0} = \frac{\Delta S}{\Delta V} \quad \cdots ③$$

↑

Claussius Clapeyron equation

So, what the our starting point is we know this d of G is equal to see this is where we start from a free energy, what is it equal to minus S d T then plus.

Student: V dP.

V dP isn’t it? So, this one I will say is, I will start the equations again numbering. So, this is 1 for me this is under pressure denaturation ok. But we are not looking at G, what are we looking at we are looking at

Student: (Refer Time: 40:06).

Delta G. So, can we write as d of delta G is equal to minus delta S d of T plus delta V d of P, and this is something you know right ok. Now, what can we have, at transition temperature, at transition temperature, you remember what your transition temperature was like T g, T m, this is the transition temperatures. At the transition temperature, what about delta G?

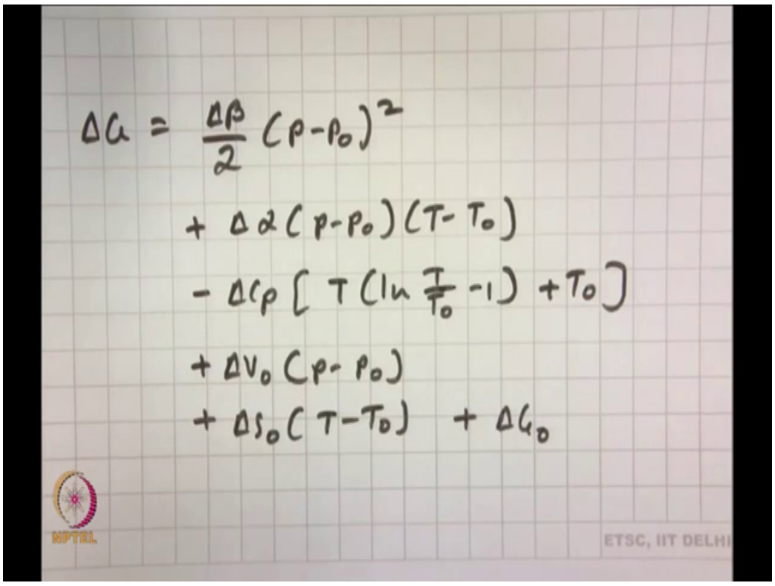
Student: 0.

Delta G equal to 0 ok. If you have delta G equal to 0, then what can we write I can write delta S d of T is equal to delta V d of P. Or I can write d of P over d of T is equal to what delta S over delta V right. And this is equation 3. What equation is this?

Student: Clausius-Clapeyron.

Clausius-Clapeyron equation ok. So, I can write this as delta G is equal to 0. So, this equation is your typically Clausius-Clapeyron equation ok. So, these are three equations we start with and nothing is new to you right these you know. Now, what I will do now is I will give you the final expression of delta G what people have derived or what we are going to derive at least to a certain part or to a certain extent, and then go about see to see how we can derive it.

(Refer Slide Time: 41:51)



$$\Delta G = \frac{\Delta\beta}{2}(p-p_0)^2$$
$$+\ \Delta\alpha(p-p_0)(T-T_0)$$
$$-\ \Delta C_p\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right]$$
$$+\ \Delta V_0(p-p_0)$$
$$+\ \Delta S_0(T-T_0)\quad+\ \Delta G_0$$

So, I will give you the final expression. The final expression of delta G, the final expression of delta G is equal to delta beta by 2 p minus p 0 squared or we just write it down I will explain it to you, then plus delta alpha p minus p 0 T minus T 0 ok, then minus delta C p T natural log T over T naught minus 1 plus T naught then plus delta V naught p minus p naught plus delta S naught T minus T naught plus delta G 0 or delta G naught. So, just look at these the first one has what beta, the second one has what

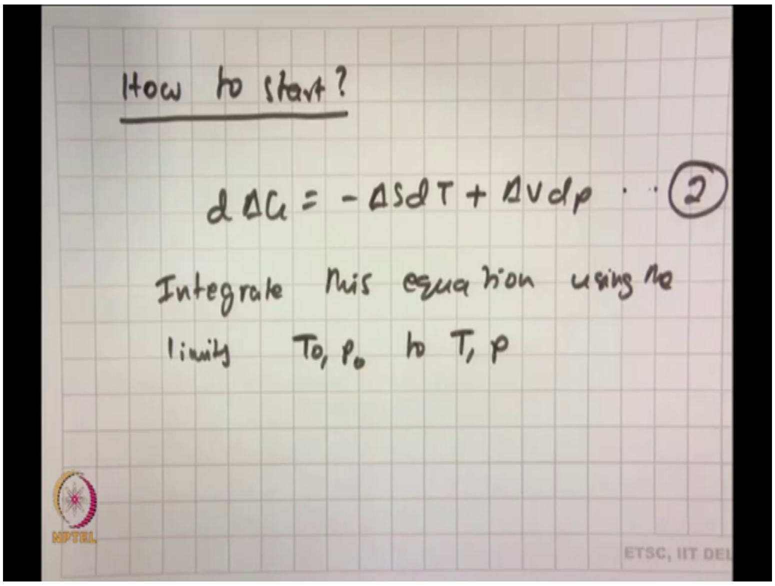
Student: (Refer Time: 42:58).

Alpha. So, this is alpha is here thermal expansion, the other one is here isothermal compressibility. The next one has delta C p right, this is we had seen for your thermal transition. Then we have delta V 0, because V naught rather I will tell you what these are p minus p naught, then delta S naught T minus T naught plus delta G naught ok.

See, now you can realize this. The way we derive the thermal transition, we did d G is equal to minus S d T plus V d p right or d of delta G is equal to minus delta S d T plus delta V d p. When we derived the thermal, what did we say we actually said that d p essentially was 0, because there was no pressure dependence that was the assumption we took and that is how we derived it ok.

But you know, but you know when you are increasing temperature, you can play with pressure right. So, here what you are doing is you are integrating the full equation. So, what you do, how did we get this? So, let me write this as equation 4.

(Refer Slide Time: 44:04)



How to start?

$$d\Delta G = -\Delta S dT + \Delta V dp \quad \cdots \quad ②$$

Integrate this equation using the limits T_0, p_0 to T, p

How to start to get this relationship? So, how to start? What we start from is we start from again d of delta G the same thing this is the equation minus delta S d T plus delta V d of p this was the equation I think what it was 2 right, so equation 2. And we say we integrate, we integrate this equation, we integrate this equation using the limits T naught p naught to T, p ok. We integrate this equation.

What are the lower limits? T naught for temperature, p naught for pressure; what are the higher limits T for temperature p for pressure. Again because in this case we are not assuming anything, we are saying that free energy is dependent on both temperature and pressure that is what G T p is right, G depends upon temperature and pressure ok.

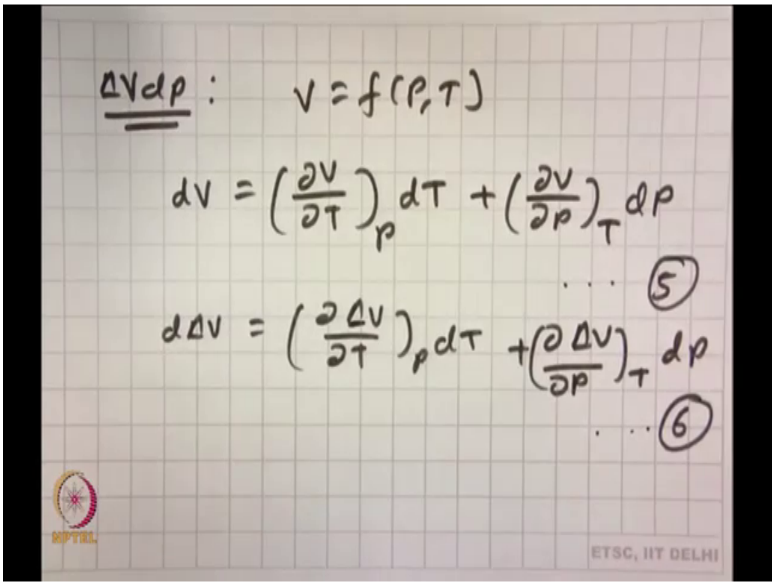
So, let us try to see how we can do it ok. But remember this can you integrate this equation just in a straight forward manner? That means, can I just integrate d of delta G and write delta S T minus T 0 plus delta C p minus p 0, can I write that? One thing is I am not going to get the equation, I showed you that is for true, but the thing is you cannot do that. Why?

Because both these delta S and delta V would be having the dependence of the others, so these are not independent right, you cannot consider them to be independent of each other. Why? Because S is a function of what p and T, V is also a function of.

Student: p and T.

p and T. So, hence you would have to take the variation of delta V or delta S as a function of these two variables that is what we have to do first. And then we come back and plug it back into this equation, and then do the rest. Is it clear? Because delta v and delta s are not independent of these, they are dependent on this variables we are changing are the limits between which we are integrating right. So, then what we now do is this is also very simple, and you guys all know what we say that ok.

(Refer Slide Time: 46:24)



$\Delta V dp$: $V = f(p, T)$

$$dV = \left(\frac{\partial V}{\partial T}\right)_p dT + \left(\frac{\partial V}{\partial p}\right)_T dp \quad \ldots ⑤$$

$$d\Delta V = \left(\frac{\partial \Delta V}{\partial T}\right)_p dT + \left(\frac{\partial \Delta V}{\partial p}\right)_T dp \quad \ldots ⑥$$

So, let us look at delta V dp first right. Let us look at delta V dp. When we are looking at delta V dp what I am saying is I am saying V is a function of p and T right, you can straightaway say that right, V is a function of p and T ok, if p V is equal to n R T.

And if I say that V is a function of p and T, and remember these all functions which are what functions?

Student: State function.

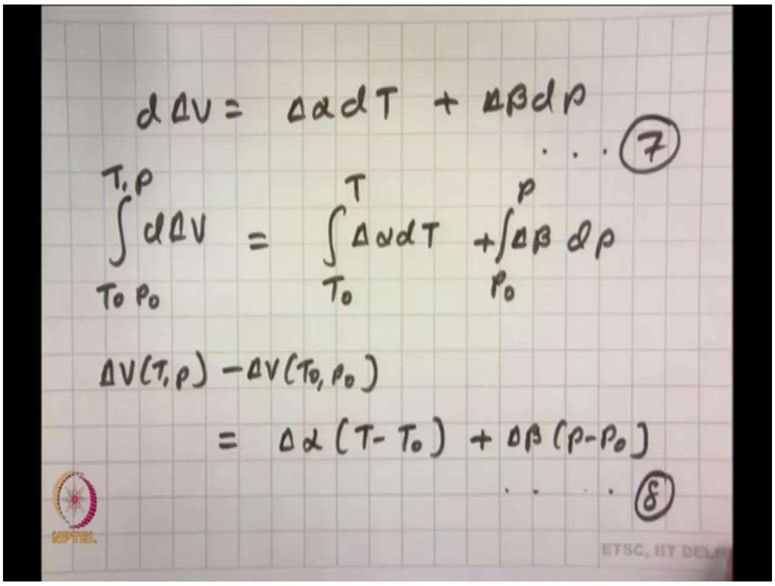
State functions, they define the state of the system. So, I can write exact differentials of the state functions. If I can write exact differentials of the state functions, what should I write, for dV this should be equal to del V over del T, then I can write at constant pressure d of T plus del V over del p at constant temperature d of p ok. So, this I would keep as what was the last equation number do you remember?

Student: 4.

4, was not it? I will keep as 5. But remember we are not interested in V like we were not interested in G, we were interested in what delta G. So, here what we were doing delta V. So, what I will just then do is d of delta V is equal to del of delta V over delta T p d of T plus del of delta V over delta p temperature d of p, and this is equation number 6.

See you already have a differential relation for delta V which is dependent upon what T and p that is what we wanted, because you know that the delta V was dependent upon this right.

(Refer Slide Time: 48:24)



$$d\Delta V = \Delta\alpha\, dT + \Delta\beta\, dp \quad \ldots ⑦$$

$$\int_{T_0\, p_0}^{T,\, p} d\Delta V = \int_{T_0}^{T} \Delta\alpha\, dT + \int_{p_0}^{p} \Delta\beta\, dp$$

$$\Delta V(T,p) - \Delta V(T_0,p_0)$$
$$= \Delta\alpha\,(T-T_0) + \Delta\beta\,(p-p_0) \quad \ldots ⑧$$

Now, we do a small change I hope you will appreciate this. This d delta V, I can rewrite as delta V, I can rewrite as delta alpha d of T plus delta beta d of p. Yes or no?

Student: Yes sir.

See the way you define alpha is what, remember the way you define alpha?

Student: 1 by.

1 by V, then?

Student: Del v.

Del V by del t at constant pressure. But you know what why you take 1 by V, what was the reason you take 1 by V? There is a reason. Is alpha or beta and beta intensive or extensive?

Student: Intensive.

Intensive properties. How does how do you make it intensive? By dividing by V right, that is why you have the V, like 1 by V times this is actually alpha you know that is why in that sense I should not be calling this alpha.

But I am trying to tell you if you take 1 by V it is say V m essentially it is a V molar volume you are saying that expansion per unit volume, that means, you can never have an extensive variable now you already have like its per mole right. When you define per mole, it is no longer extensive it is intensive, and the same thing I have done here. Is it clear and that is why we are not taking 1 by V here.

So, now if I have this, if I have this now, remember our delta V is dependent upon temperature and pressure. So, what I can do is, I can integrate I can integrate I can do d of delta V between what limits T naught, p naught, T, p. Isn't it? And this should now should give me now what now this is only a function of temperature I can write delta alpha d T T naught to T plus delta beta then this is dp, this should be what

Student: p naught to p.

p naught to.

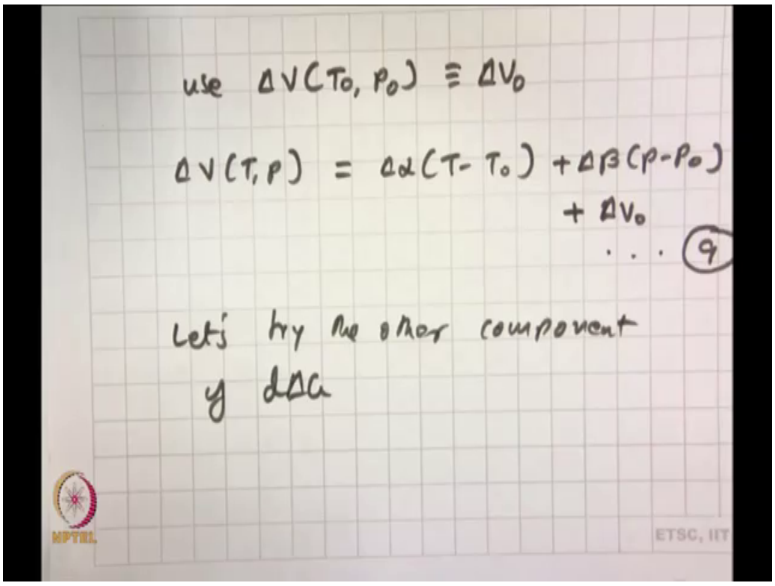
Student: p.

p.

Student: d of.

d of P ok. Or I can rewrite or I can rewrite as now check this I can write delta V T, p minus delta V T naught p naught ok, I have come them into 1 is equal to what is my first term? Taking into consideration the fact that delta alpha and delta beta are constants ok, they are not varying. This should be delta alpha T minus T naught, then what do I have I should be having plus delta beta p naught. So, let this be equation 8 ok.

(Refer Slide Time: 51:35)



use $\Delta V(T_0, p_0) \equiv \Delta V_0$

$$\Delta V(T,p) = \Delta\alpha(T-T_0) + \Delta\beta(p-p_0) + \Delta V_0 \quad \ldots \text{⑨}$$

Let's try the other component

of $d\Delta G$

And the way I can rewrite is what I will do is I will make a small change I will make a small changes, I will use delta V T naught p naught to be delta V naught, it is like your delta G naught right. It is your delta V naught at a certain temperature ok. So, then this would ah] change as delta V T p is equal to delta alpha T minus T naught plus delta beta p minus p naught, then what I have is plus delta V naught which is equation number 9. Is it clear?

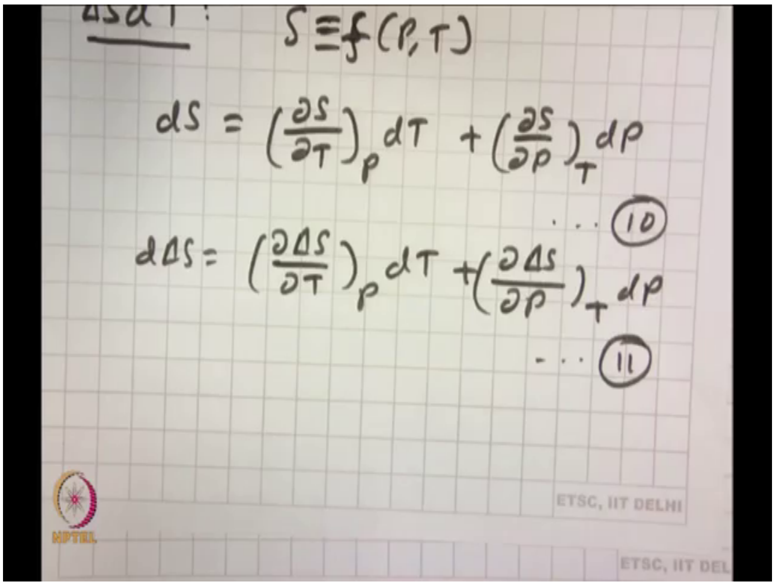
See, we started from very first principles, and we just took into account the dependence of delta V on T and p. Now, you think about this if you go to the original equation, that means, if you go to the original equation which we had to start with look at this original equation. If we are going to integrate now, d delta G between what? T naught p naught to T and p, you already have an expression for delta V which I have just derived.

This delta V naught depends upon what? It depends upon T which is T minus T 0, it depends upon p, the p minus p 0, and then there is one constant term which is delta V naught. So, you can just instead of delta V, you can just put that in right and integrate that portion out ok. This is for you to do right.

And for you to do, but one thing you can realize if you go back to your previous equation very first equation that I wrote down I said I will come back to it this equation number 4 look at this. You already have an expression with delta beta coming from delta V; you already have something which is p minus p naught ok. Look at the second term. You already have delta alpha from delta V, because these are relate your volume changes isn't it, then you already have T minus T naught ok.

The p minus p naught is missing, but you know if you would do delta alpha dp T minus T 0 is a constant then you will get p minus p 0 ok. Then the rest we do not have except we have delta V naught delta V naught is also already there ok. So, keep this in mind. So, this is important. Now, what about the other one, what was the other one? The other one was let us try the other component of d delta G ok.

(Refer Slide Time: 54:32)



ΔSdT : $S \equiv f(p,T)$

$$dS = \left(\frac{\partial S}{\partial T}\right)_p dT + \left(\frac{\partial S}{\partial p}\right)_T dp \quad \ldots ⑩$$

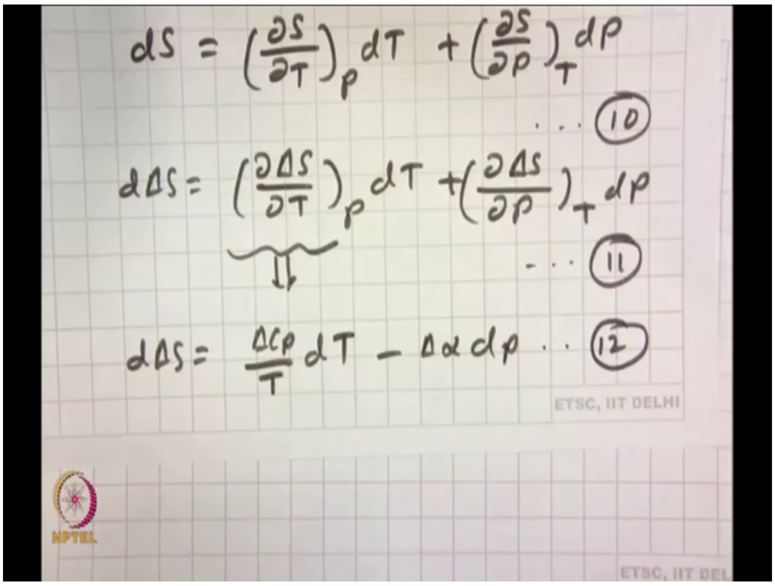
$$d\Delta S = \left(\frac{\partial \Delta S}{\partial T}\right)_p dT + \left(\frac{\partial \Delta S}{\partial p}\right)_T dp \quad \ldots ⑪$$

That means now what we are going to try is we are going to try delta S d T ok, we are going to try delta S d T. I am avoiding the negative sign, now we will put in the negative sign later ok. Now, this delta S d T again, what do we start with we start with S is equal to a function of S right or a function of p, T right.

And again all these are exact I mean straight functions exact differential is valid. So, I can write d of S is equal to del S over del p T at constant pressure d of T plus del S over del p at constant temperature d of p and let this be equation 10.

But we are not interested in S we are interested in what delta S, then d of delta S is equal to del of del S over del T at constant pressure d of T plus del of delta S over delta p at constant temperature d of p. Just let me finish this one. This is 11 ok.

(Refer Slide Time: 55:53)



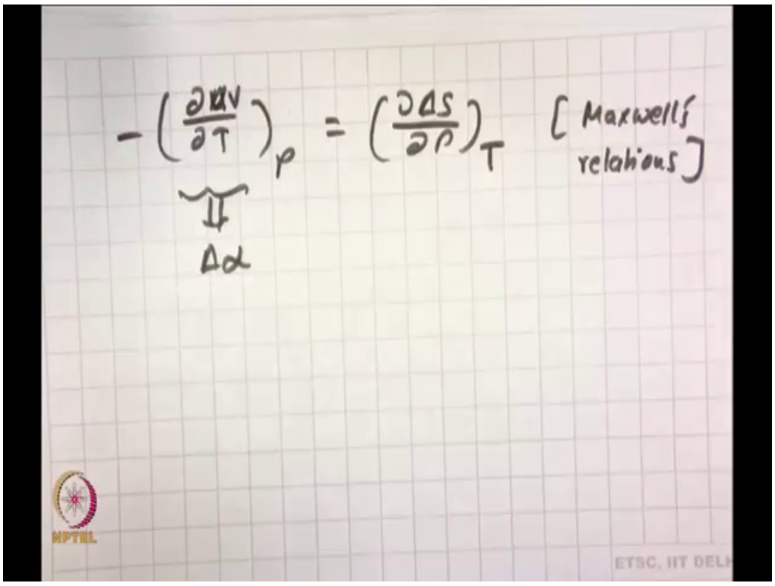
$$dS = \left(\frac{\partial S}{\partial T}\right)_p dT + \left(\frac{\partial S}{\partial p}\right)_T dp \quad \dots ⑩$$

$$d\Delta S = \underbrace{\left(\frac{\partial \Delta S}{\partial T}\right)_p}_{\Downarrow} dT + \left(\frac{\partial \Delta S}{\partial p}\right)_T dp \quad \dots ⑪$$

$$d\Delta S = \frac{\Delta C_p}{T} dT - \Delta\alpha\, dp \quad \dots ⑫$$

Now, what I can write for this one? For example, this first term what can I write I can write d of delta S is equal to delta C p by T d of T ok. And this one, this one I can write as minus delta alpha d of p ok. I will tell you where I am coming from. So, let this be equation 12. Where am I coming from? I am coming from this. Remember, there was something referred to as Maxwell’s relations ok.

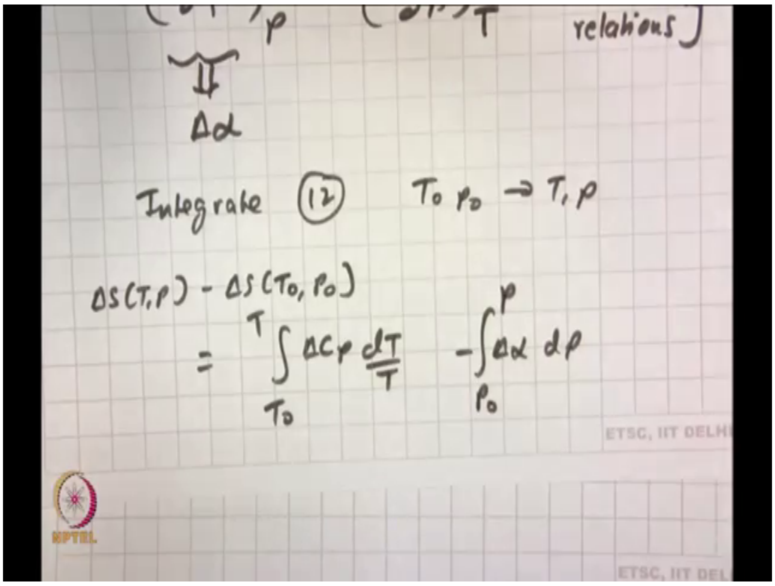
(Refer Slide Time: 56:31)



$$-\underbrace{\left(\frac{\partial \Delta V}{\partial T}\right)_p}_{\substack{\parallel \\ \Delta\alpha}} = \left(\frac{\partial \Delta S}{\partial p}\right)_T \quad \text{[Maxwell's relations]}$$

So, delta V by delta T at constant pressure let me put in a well delta V, let me put in at constant pressure would be del of delta S by delta p at constant T ok. And we know already that this one is alpha. So, this one is what? Delta alpha we already know that, and hence you get equation number 12 ok. So, this is based on Maxwell's relations. Please go back and check right.

(Refer Slide Time: 57:20)



relations]

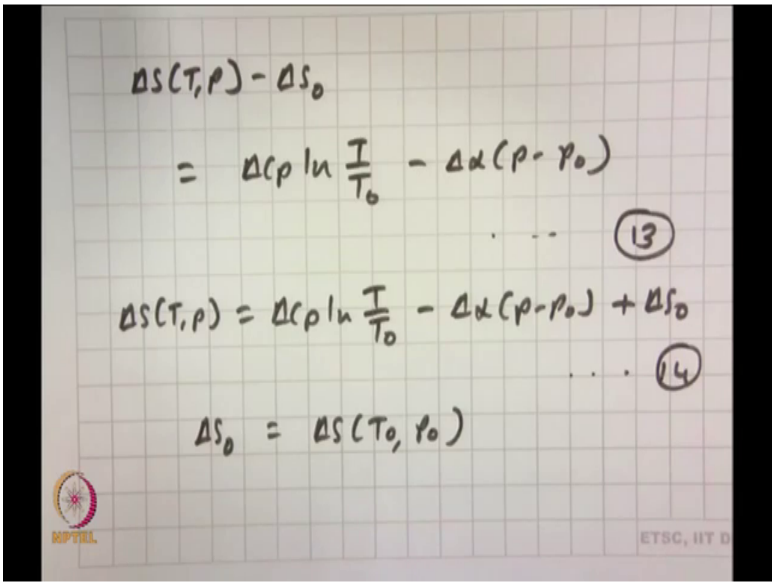
$\Delta\alpha$

Integrate ⑫ $T_0\, p_0 \rightarrow T, p$

$$\Delta S(T,p) - \Delta S(T_0,p_0) = \int_{T_0}^{T} \Delta C_p \frac{dT}{T} - \int_{p_0}^{p} \Delta\alpha\, dp$$

Now, what I just do is just let me do this, because I am almost to the end. I integrate equation 12, limits T naught, p naught to T, p. And what do I get? I get delta S T, p minus delta S T naught, p naught is equal to delta C p d T over T, T naught to T minus delta alpha dp p naught to p ok. I am integrating equation 12 is just like that. And let me write down the final form before I end the class. Up till this you are right? I just integrated that is it nothing more.

(Refer Slide Time: 58:17)



$$\Delta S(T,p) - \Delta S_0$$

$$= \Delta C_p \ln \frac{T}{T_0} - \Delta\alpha (p - p_0) \quad \ldots \text{⑬}$$

$$\Delta S(T,p) = \Delta C_p \ln \frac{T}{T_0} - \Delta\alpha (p - p_0) + \Delta S_0 \quad \ldots \text{⑭}$$

$$\Delta S_0 = \Delta S(T_0, p_0)$$

Then I can write delta S T p minus delta S naught this is like same like delta V naught is equal to taking delta C to be your C p to be constant, I can write delta C p natural log T over T naught minus delta alpha p minus p naught right.

And this would be equation 13. And lastly then delta S T, p is equal to delta C p natural log T over T naught minus delta alpha p minus p naught plus delta S naught. This is equation 14, where delta S naught is equal to delta S T naught p naught ok. I know I have run out of time, but this is what I had to complete today because otherwise I would have to start a fresh in the next class.

But you can see by doing this again you have delta S where you have the other remaining terms, one was delta C p is you already have right, and well delta alpha you already have and the other one is delta S naught ok. These things are common. So, what you now do is you take this put it back into the d of delta G is equal to that delta S d T minus delta S d T plus delta V dp. Replace delta S and delta V by the these expressions like equation 14 and what was the other equation? I forgot.

Student: 9.

Equation 9. 14 and 9 and you should be getting the final expression of delta G which has both volume changes and also temperature changes that is the most general equation you can derive ok.