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

Lecture - 35
Experimental Tools

So, what we will start from today and this is what we will end with; I mean the course is some spectroscopy tools. You know what is spectroscopy a little bit of that and absorption fluorescence IR CD ok.

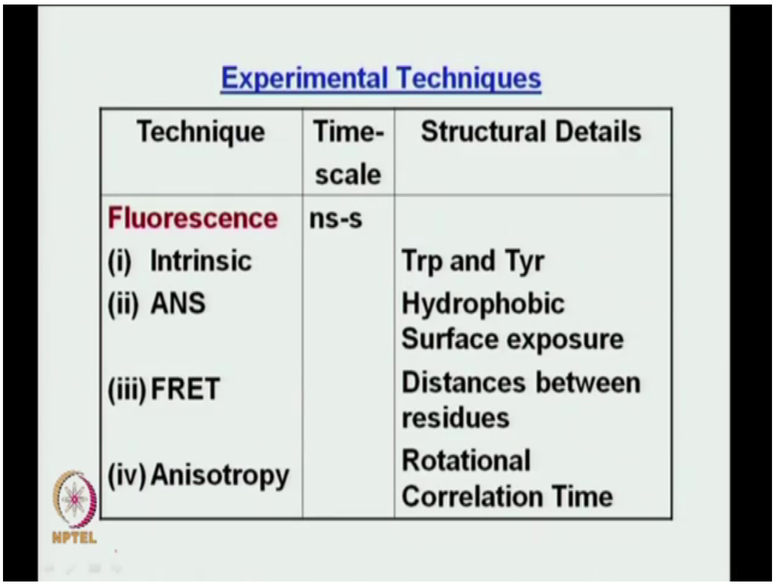
(Refer Slide Time: 00:51)

Experimental Tools

So, that is why this class starts with this heading Experimental Tools. That means, the tools you would need these are not the techniques these are the tools you would need to probe the folding and unfolding of proteins right.

But remember these tools are very general; because we are talking about, because this is a course on Biophysical Chemistry our focus is mainly on proteins or bio macromolecules or biomolecules. But as such you take any you take any other compound or molecule which is not a biomolecule, still these tools would be equally applicable right. So, that is you know that is the real good thing about the generality of this thing or this subject called spectroscopy.

(Refer Slide Time: 01:35)



Experimental Techniques

Technique	Time-scale	Structural Details
Fluorescence	ns-s	
(i) Intrinsic		Trp and Tyr
(ii) ANS		Hydrophobic Surface exposure
(iii) FRET		Distances between residues
(iv) Anisotropy		Rotational Correlation Time

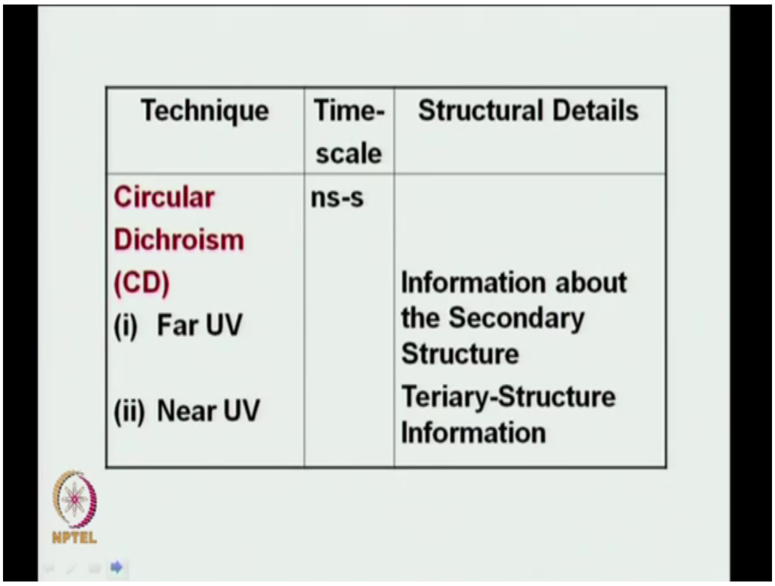
So this you have seen before, but I will just quickly scan through it. So, some of these experimental techniques one is fluorescence, look at the relevant time scale you can go from nanoseconds to seconds right and what do you get out from it? So, a fluorescence is as says under intrinsic, intrinsic means something which is present in the protein itself. So, some of the intrinsic amino acids are tryptophan tyrosine also fell alanine, but Trp and tyrosine are the ones which are mainly used in the fluorescence emission of.

Then ANS I told you before that ANS is a certain dye called 1 anilino 8 naphthalene sulfonic acid. Now this dye is commonly used to look at exposure of hydrophobic patches in proteins ok. So, you can also look at the change in fluorescence intensity of ANS to figure out what is going on as you change conditions. Then third is FRET you know we I have talked about this a little bit FRET is about how the transfer of energy occurs between a donor and acceptor and this depends upon the distance between these two, right.

So, you can understand if you are looking at a conformational transition, you start from an unfolded state and you come to a folded state and you have two chromo poses donor acceptor. What will happen is in unfolded state they would be really far off, so fluorescence or rather energy transfer efficiency would be really low when they come very close to each other. The energy transfer efficiency would be really high and that is how you can track it and you can even use it for kinetics right.

And then there is something known as anisotropy, if you remember this thing we had done this protein apomyoglobin did you remember apomyoglobin when we talking about this ribosomal tunnel. And, we said that apomyoglobin starts getting some structure and there was something known as rotational correlation time or rotational spectroscopy. Where you would look at the rotation of apomyoglobin fragment and see whether it is bound to the peptide tunnel or not, so that is essentially what it is anisotropy.

(Refer Slide Time: 03:31)



Technique	Time-scale	Structural Details
Circular Dichroism (CD)	ns-s	
(i) Far UV		Information about the Secondary Structure
(ii) Near UV		Teriary-Structure Information

So, these are some of the main techniques that fall under fluorescence, then a very important technique called circular dichroism. See for example, if you would get if you would get a protein right, if you would get a protein and the first thing someone asks you does it have a structure. If it does have a structure what structure does? It have I am talking about the secondary structure?

So, then immediately what you can do is you can take the sample put it in the CD spectrometer and take our accurate spectrum right. And, that will that is one of the easiest ways of finding out whether a protein has an alpha helical structure or a beta sheet structure or a mix type you know you can do fittings and all these things.

Not only the secondary structure you can also look at the tertiary structure. So, that is why you see there are two regions wavelength regions: one is the Far UV and one is a Near UV.

So, what is this far and near about, near and far with respect to what? Why do we call near, why do we call far?

Student: (Refer Time: 04:20).

Which what wavelength

Student: Visible (Refer Time: 04:23).

Visible right. So, if anything is near UV that means this is in the UV region, but near to the visible.

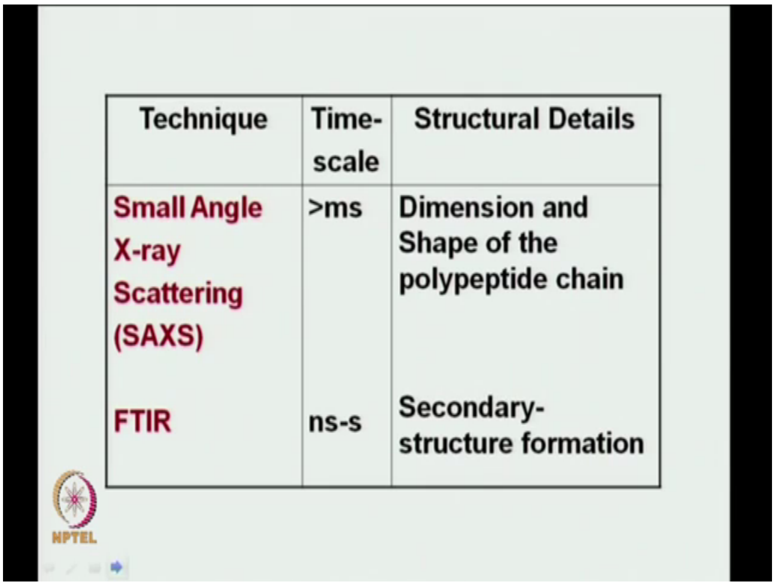
Student: Visible.

So, where it is a visible say is from say 400 to 700 nanometers right. So, anything which is close to 400 would be?

Student: Near UV.

Near UV, because it is near to the visible region the more you, the farther you move lower from 400 you go to the far UV. So, typically for CD the far UV looks at secondary structure that is why it says you can see the far UV secondary structure. So, that is alpha helix beta sheet and all these things right, even random call the near UV looks at the tertiary structure ok. So, there will also be tertiary structural changes which happen in the near UV. So, near UV region would as you see 350 400 and all these things.

(Refer Slide Time: 05:09)



Technique	Time-scale	Structural Details
Small Angle X-ray Scattering (SAXS)	>ms	Dimension and Shape of the polypeptide chain
FTIR	ns-s	Secondary-structure formation

So, then there is something known as small angle X ray scattering. So, small angle X ray scattering popularly known as SAXS. Now what does it tell you? It gives you the dimensional shape of the polypeptide chain. Now this is what we need it for; say for example suppose you unfolding a protein right; that means, you are destroying the structure in a protein it goes from folded to native to a non-native or denatured state.

Now someone asks you, what is the change in the dimension? Now you know that if you are going to open a protein now the dimension is going to change, because you are disrupting forces and the protein is now slowly opening up. Now all proteins would not open up exactly the same manner, some proteins might undergo a huge change in dimension some proteins might not be undergoing that much of a change in dimension. But how would you know what is happening?

So, one of the techniques is small angle X ray scattering which tells you which tells you or gives you an idea about the approximate dimension of your protein state, whether it is in the unfolded or whether it is in the folded. And remember whenever we are talking with this SAXS you are doing in solution, so you always look at what? You do not; you always essentially look at the hydrodynamic radius right; because you always have water molecules associated with these things ok.

The next one is a FTIR is called Fourier Transform Infrared. Now, I know that in one of your physical chemistry practical's you have done this infrared spectroscopy what was that experiment, was it this was not that dimer formation or something like that you did?

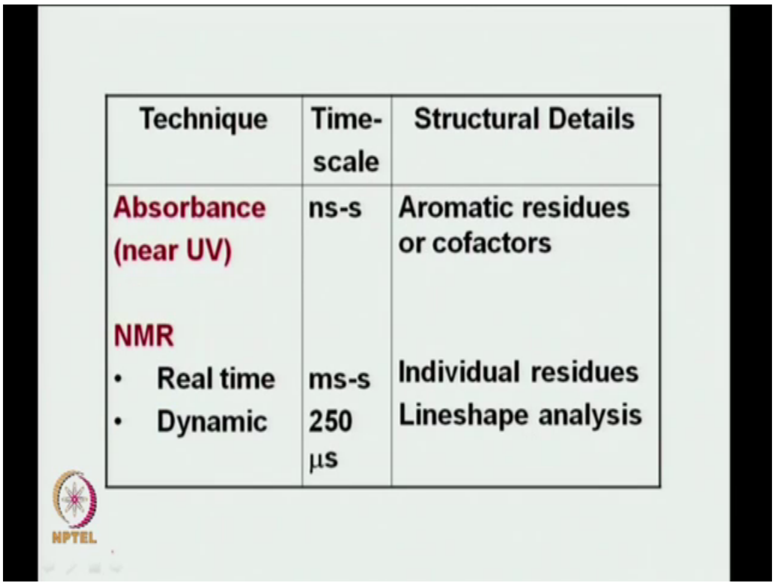
Student: (Refer Time: 06:27).

It was in your first practical you used the.

Student: (Refer Time: 06:34).

Where IR instrument right in an instrumentation lab. So, that is the principal, proteins have remember we were talked when we were talking about the laser induced temporary jump we talked about this IR absorption band 1631 for an alpha helix. So, this is essentially what you are talking about. So, if you take FTIR then you would be getting that information of your secondary structure as well. Now, what is the timescale for this? The timescale is nanoseconds to seconds.

(Refer Slide Time: 07:00)



Technique	Time-scale	Structural Details
Absorbance (near UV)	ns-s	Aromatic residues or cofactors
NMR		
• Real time	ms-s	Individual residues
• Dynamic	250 μs	Lineshape analysis

So, then the other one is something which you know very well it is called absorbance, this is a essentially Lambert-Beer's law right. Absorbance in the UV visible region, FTIR was absorbance in the what? IR region, because you are looking at bond stretching bond vibration. This is your electronic transition you are looking at absorbance in the near UV.

So, it is nanoseconds to second that is a timescale it can even actually even go lower, what essentially look at is aromatic residues or cofactors ok. So for example, cofactor; how do we define a cofactor? Well, let us take this myoglobin right or hemoglobin, hemoglobin has what? There is a heme prosthetic group in the middle. So, this one has a characteristic transition; do you know what the transition is known as or that band is known as?

Student: pi (Refer Time: 07:46).

Pi test pi to pi transaction what is a band known as? It starts with an s.

Student: (Refer Time: 07:50) band.

Yes, it is called a solid band. So, this solid band is something which you can look at absorbance of and see what changes are going on at the at some structural level right. Then obviously, NMR there are two types: one is real time one is dynamic. So, you can look at the you know the breadth of timescale in one case it is millisecond to second, in the other case it is about 200 microseconds or so.

So, you know that is pretty much about the common tools or the tools that are used commonly when people are looking at proteins or investigating proteins, no matter for what reason right. But, before we go into spectroscopy we have to know some very basic things right.

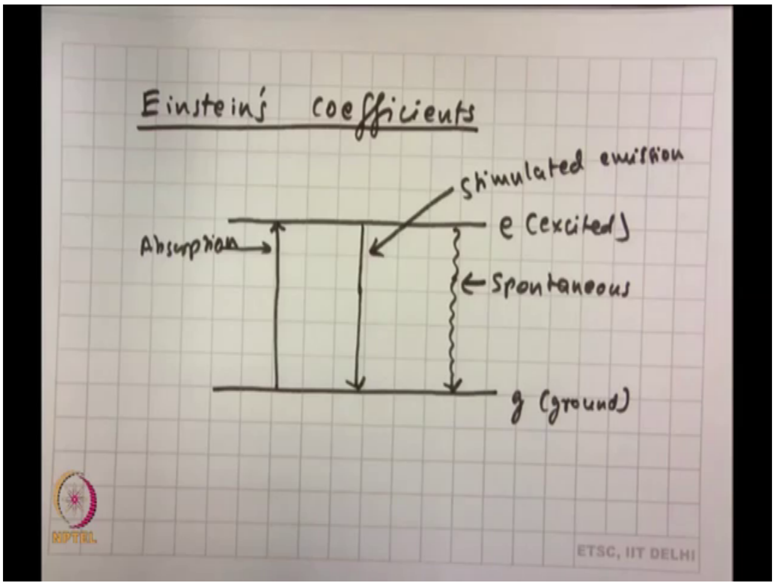
So let us look at this, the first basic thing obviously is if you are interested in spectroscopy, I do not know how much was covered in groups in spectroscopy in your class, but that was mostly from the group theory point of view. But if you would be able interested in spectroscopy view or interested in spectroscopy you want to take spectroscopy up as your you know future PhD project or whatever.

Then please do look up time dependent perturbation theory if you are not taught ok, at any subsequent time of this. So, time dependent perturbation theory we cannot go over during this class, because it would take some time and we do not have much time left. So, time depend perturbation theory is the thing which from where essentially spectroscopy originates.

Remember perturbation is a certain perturbation what do you have, in this case if you are doing spectroscopy what do you are perturb in the system with? You perturb in the system with the electromagnetic field which is a radiation and that is why that comes into your perturbation ok. It is like h 0 plus lambda h prime do you remember that was your perturbation theory.

That h prime is an oscillating function of your I mean it is your essentially your radiation ok, oscillation function of time. So, please look that up. So, that is extremely essential. But what we will do is, we will consider the fact that time depend perturbation theory is there we have to look that up.

(Refer Slide Time: 09:56)



We will move forward and we will talk about something which is very fundamental, this is referred to as Einstein's coefficients; referred to as Einstein's coefficients ok. Now let us consider this two level system ok. So, two level means I have a two level system that means, this is my ground state; g stands for ground and this is my excited state, e stands for excited ok.

Now what I will do is, I will throw in some radiation that is some energy density, this system whatever system looking at would absorb that and then move from the ground state to the excited state. So, the first process is absorption ok. So, the first process is absorption. So, this is absorption ok; so, here after you have excited the molecule from the ground state to the excited state. What will happen? The molecule cannot stay the excited state for infinite time, what it would try to do is it would try to come down to the ground state.

Now, it can come down to the ground state in two ways, what I mean by two ways? One is it can just spontaneously by itself come down right, spontaneously by itself come down. That means, let me give you a wiggly line; so this is referred to as a spontaneous downward transition ok, from the excited state to the ground state. The other one is if one is spontaneous what is the other way of coming down, can you tell me?

Student: (Refer Time: 12:12).

Yeah, what does that give rise to? That is what happens in lasers right, what is the full form of laser?

Student: Light amplification by stimulated emission of radiation.

Right. So, if this is spontaneous; if this is spontaneous then what is the other form?

Student: Non-spontaneous (Refer Time: 12:29).

Yes, if it is spontaneous the other one is non-spontaneous right, very good what you just said (Refer Time: 12:36) what is that light amplification by.

Student: Stimulated (Refer Time: 12:37).

Stimulated emission.

Student: (Refer Time: 12:38).

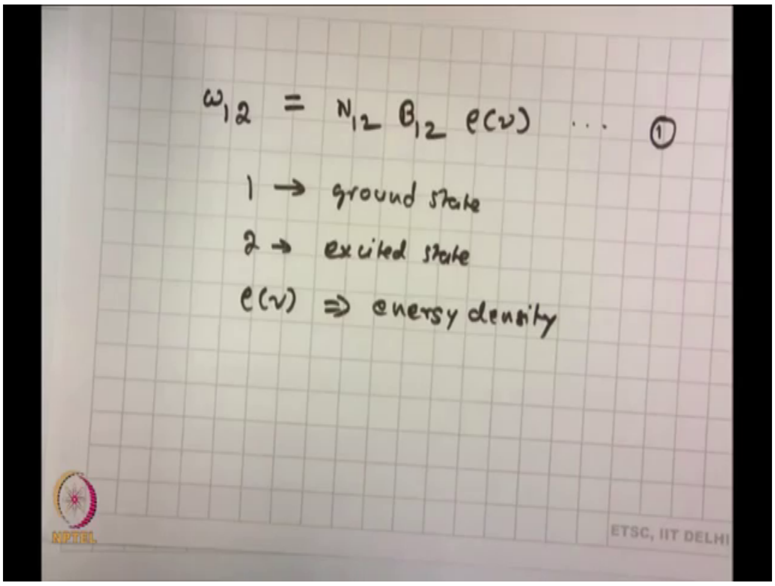
That means ok, good understood. So, if one is spontaneous that means it just comes down by itself, it does not have to be told to come down to the ground state. The other one is stimulated that means there is something which stimulates its transition from the upper level which is the excited state to the ground state right. So, then this is the other one we have.

So, this is your stimulated emission this stimulated, one is spontaneous transition one is a stimulated transition. Now, stimulated emission of radiation I just pointed out is the principle of laser right, light amplification by stimulated emission of radiation right. But, remember these are three distinct processes. But, also try to realize one thing, when you are; when you are coming down you can have two types of transitions right; spontaneous and stimulated.

But when you are going to have an absorption are you going to have any spontaneous absorption, you are not going to have a spontaneous absorption. Why? Because, for absorption you need to supply energy right and only after it absorbs the energy would it go to the excited state, there is no way you can just go like that right. It just cannot it just cannot happen that it was sitting on it on it is sheer one find the and suddenly jumps to the excited state cannot do that. It has to be hit with a certain electromagnetic radiation which it put adsorbed right ok.

So that is why there are three exclusive processes in very general terms.

(Refer Slide Time: 14:33)



$$\omega_{12} = N_{12} B_{12} \rho(\nu) \quad \ldots \quad ①$$

1 → ground state

2 → excited state

$\rho(\nu)$ ⇒ energy density

Now what I can do is I can say that ok, let us try to write some equations down, how do we do that? What I say is based on this I say that my rate of transition; my rate of transition from the ground state to the excited state, where 1 refers to the ground state and 2 refers to the excited state ok. So that means, 1 is the ground state and 2 is the excited state ok.

So, this is equal to that means I am looking at the rate of transition; where w 1 2 is the rate of transition from the ground state to the excited state. It would depend upon what? Now, think about your rate in your chemical reaction, what does it depend upon? It depends upon rate constant, it depends upon a?

Student: Concentration (Refer Time: 15:18).

Concentration very good. So now, in this case instead of concentration what we will use is the number of molecules N 1 2 ok. Then, I will use a rate constant which I write as B 1 2, the rate constant which I write as B 1 2. But please remember this; please remember this I have not yet done, why? Because, absorption cannot occur spontaneously right. What would it depend upon? It would depend upon the energy I am supplying it with, the electromagnetic field right. So, what we say is we take this and we multiply as a term which is rho nu. So, this is the energy density with which you are hitting your molecule that is finally absorbing the energy you are hitting it with.

Now rho nu is your energy density, that is energy per unit volume over a per unit frequency interval or over a defined frequency range. Where have we seen this before rho nu? You have seen this before tell me where? What was the first thing you did in quantum mechanics, before you actually went on to the postulates?

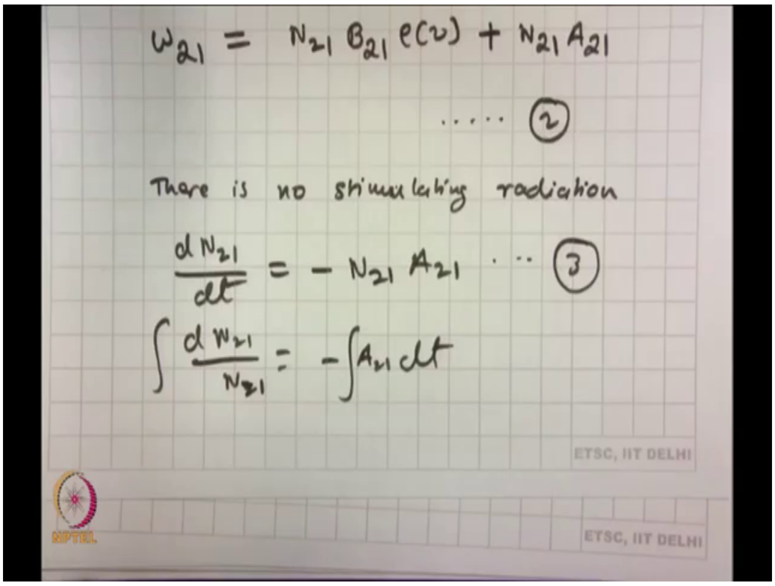
Student: (Refer Time: 16:43).

Yes you did that you did blackbody radiation, remember in blackbody radiation there was something known as rho nu. Where rho nu d nu was equal to this times by over this times d nu, where we had this you know Planck's expressions coming in. So, rho nu essentially is this this is exactly the same rho nu it is your energy density ok. It is your energy density over a defined frequency interval or per unit frequency interval. So, rho nu is your energy density.

So that means what is your assumption? Your assumption is that means your treating this whole process the treating this whole process as the molecule being a what blackbody emitter or absorber ok. That means, your molecule is being treated as a black body system that is what you are using it does not matter, because you know what the energy density is you know the distribution that is what you are using it for right. So, this is equation number 1.

Now remember this was for the up going process right, that is from 1 to 2. So, I should also have one rate equation which comes down the other way right.

(Refer Slide Time: 18:11)



$$w_{21} = N_{21} B_{21} \rho(\nu) + N_{21} A_{21} \quad \ldots\ldots ②$$

There is no stimulating radiation

$$\frac{dN_{21}}{dt} = -N_{21} A_{21} \quad \ldots ③$$

$$\int \frac{dN_{21}}{N_{21}} = -\int A_{21}\, dt$$

ETSC, IIT DELHI

So that means, if this is from the ground to the excited state. That means, I am looking at an absorption process. What would happen if I want to come down that is w 2 1? There is the rate of coming down to the ground state from the excited state that is from 2 to 1 this is equal to. How many factors how many times would I be having here now, how many independent terms? In the first case equation 1, I had only one term right which was n times B times rho nu. In the second case how many would I be having tell me?

Student: 2 (Refer Time: 18:37).

2 all we having 2, why? Because I have two processes one is a stimulated, one is a spontaneous right. So, the stimulated please understand would just be the reverse of absorption. So, in this case what I would write is N 2 1 there is a number of molecules in the excited state which is 2 times B 2 1 that is a rate constant of this downward transition.

Remember I am talking about stimulated and because this is stimulated what extra should come along with it?

Student: Energy density (Refer Time: 19:14).

Energy density right because absorption was also stimulated in the sense right, you had to do that absorption. So, again you will be having the same rho nu ok. So, this is your stimulated.

Student: Lifetime is (Refer Time: 19:27) also (Refer Time: 19:28) lifetime.

Lifetime is coming will lifetime will be coming from here, but lifetime is a result of that.

Student: (Refer Time: 19:34).

Lifetime this you will get the lifetime hold on right, lifetime will come from here because we are talking about rates right and what is lifetime? Lifetime is the time it takes to decay right. So, once we get the rate constant we get the lifetime too simple. Then the next term; the next term has to be of your or for your spontaneous, see it would be N 2 1. Now I define a new constant; new constant is A 2 1 ok, N 2 1 times A 2 1 where A is a rate constant for what? Spontaneous transition or spontaneous emission down to the ground state. Now tell me along with this should I write rho nu or not.

Student: No.

I would not write rho nu right because, this is not stimulated this is spontaneous right. So, that is why then this is your total expression for the rate of downward transition and this is 2. Now let us go to the next step. So, what we have seen is we have two different equations in one case we have just one way of going up and the other case we have two very general ways of coming down right, one is the spontaneous one is stimulate right. One in which you do not have to hit the other one which you have to stimulate peg along to move down.

So, suppose this happens suppose I say that there is no stimulating radiation. Suppose there is no stimulating radiation, that means it has absorbed but for it to come back it does not get any stimulating radiation. That means, for from 2 to 1 your rho nu is essentially what 0 ok. If that is the case; if that is the case remember you have to have radiation for absorption to occur right, otherwise it will not go up. But what I am saying now is once it goes up everything comes down by spontaneous emission, there is no stimulating emission.

So then what I can write is if this is the case what I can say then d N 2 1 over d of t; d N 2 1 over d of t should be equal to; should be equal to; now what should I write now here?

Student: (Refer Time: 22:06).

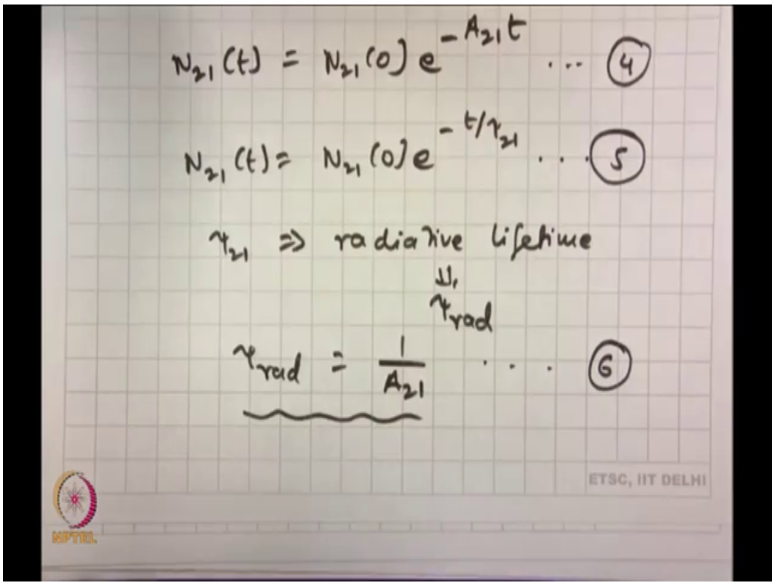
What should I write minus?

Student: (Refer Time: 22:10).

N 2 1 times A 2 1, that is what you do right, rate constant times concentration that same here it is rate constant a times the number of molecules so this is 3. So now, what you can do is, you can integrate this you can integrate this. So, you will be having d of N 2 1 over N 2 1 is equal to minus A 2 1 d of t and then you carry out the integration ok. Then you carry out the integration is very simple.

So, what we will be getting is this right. So, this is essentially what type of process is not it you are is not it your characteristic first order process you are looking at?

(Refer Slide Time: 22:54)



$$N_{21}(t) = N_{21}(0)\, e^{-A_{21} t} \quad \ldots \text{④}$$

$$N_{21}(t) = N_{21}(0)\, e^{-t/\tau_{21}} \quad \ldots \text{⑤}$$

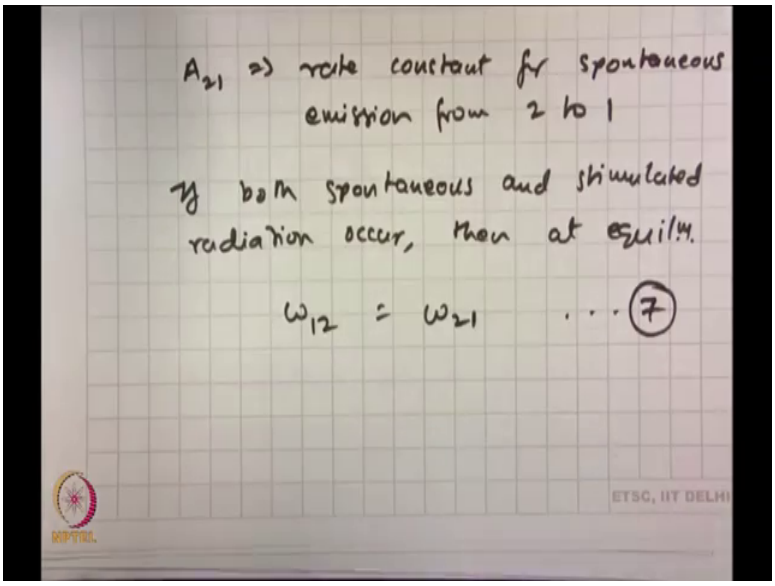
$$\tau_{21} \Rightarrow \text{radiative lifetime} \Downarrow \tau_{rad}$$

$$\underbrace{\tau_{rad} = \frac{1}{A_{21}}} \quad \ldots \text{⑥}$$

So, then what will happen is you can write N 2 1 t is equal to N 2 1 0, that means a 0 time e to the power minus A 2 1 times t ok. That means, at t is equal to 0 the number of molecules is N 2 1 0 and then you are looking at it is decay as a function of time and it is an exponentially decaying function right. So, let this be 4 ok.

Now A 2 1, what was A 2 1? A 2 1 was a rate constant was not it? So, I can write this one again as N 2 1 of t is equal to N 2 1 0 e to the power minus t by tau 2 1. So now, you can understand Pancham, where your lifetime is coming; there is right is coming right. Do you know what this lifetime is known as or this tau what is it known as? See we had encountered this tau before there was a relaxation time and all these things before right. But what is this tau known as? This tau I will tell you what this tau is known as radiative lifetime ok. This tau is known as radiative lifetime, that is why it is also referred to as tau radiative and I can write tau radiative is equal to 1 by A 2 1. This is number 6 ok.

(Refer Slide Time: 24:57)



$A_{21} \Rightarrow$ rate constant for spontaneous emission from 2 to 1

If both spontaneous and stimulated radiation occur, then at equil^m.

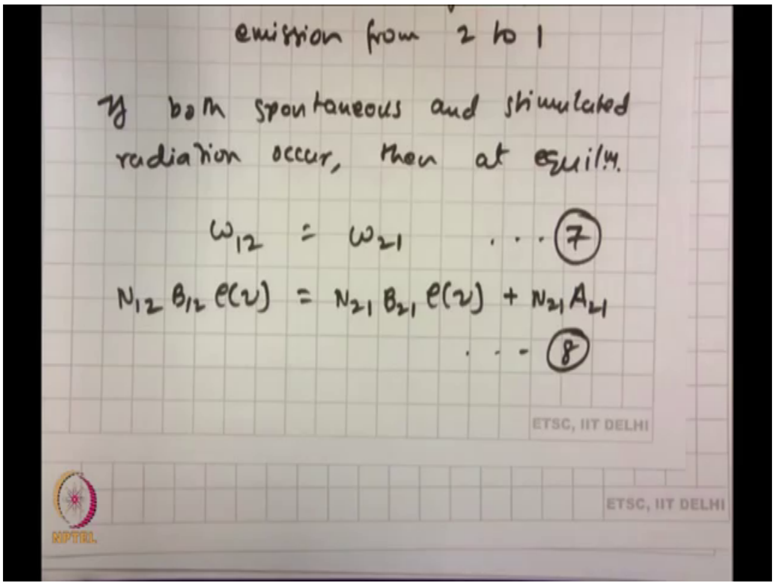
$$\omega_{12} = \omega_{21} \quad \ldots ⑦$$

ETSC, IIT DELHI

Now, this is a very important equation, where A is where A is A 2 1 is a rate constant for spontaneous emission from 2 to 1; that is from state 2 or level 2 to level 1. So, this why again this tau is called the radiative lifetime and it is tau radiation and this is actually the inverse of your rate constant for spontaneous emission good. Now think about this, this is where we had considered there was no stimulating rotation to start with.

Now what happens if you have both, that means you also have spontaneous radiation occurring you also have stimulate radiation occurring. That means, if both spontaneous and stimulated radiation ok, then at equilibrium then at equilibrium that means my equilibrium has been attained. If the equilibrium has been attained then what will happen? The rate of upper transition would be equal to the rate of downward transition right and then I can write w 1 2 is equal to w 2 1 is not it? I can write w 1 2 is equal to w 2 1 ok.

(Refer Slide Time: 26:53)



emission from 2 to 1

If both spontaneous and stimulated radiation occur, then at equilm.

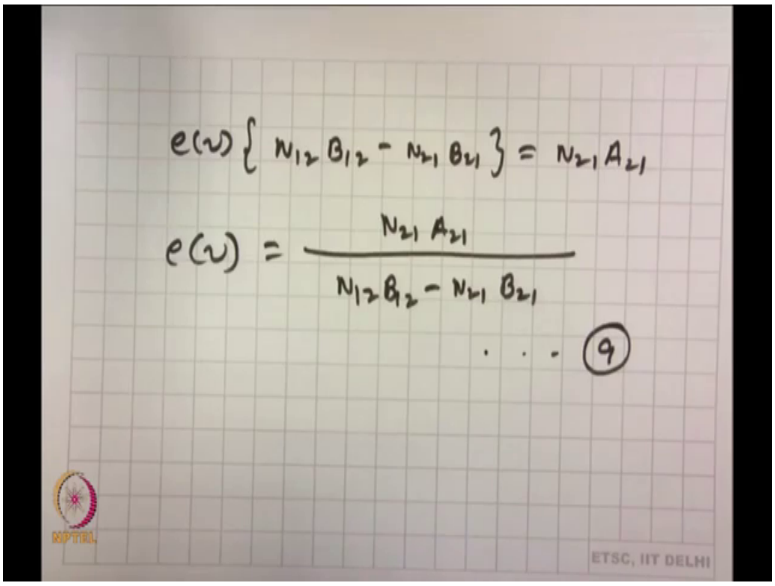
$$\omega_{12} = \omega_{21} \quad \ldots ⑦$$

$$N_{12} B_{12} \rho(\nu) = N_{21} B_{21} \rho(\nu) + N_{21} A_{21} \quad \ldots ⑧$$

ETSC, IIT DELHI

So, from here then I can again write N 1 2 B 1 2 rho nu is equal to N 2 1 B 2 1 rho nu plus N 2 1 A 2 1. So, I have just put in the expressions for w 1 2 and w 2 1 that is what we had written before ok. What am I solving for? What I am solving for is rho nu, that means from here you find the expression for rho nu.

(Refer Slide Time: 27:31)

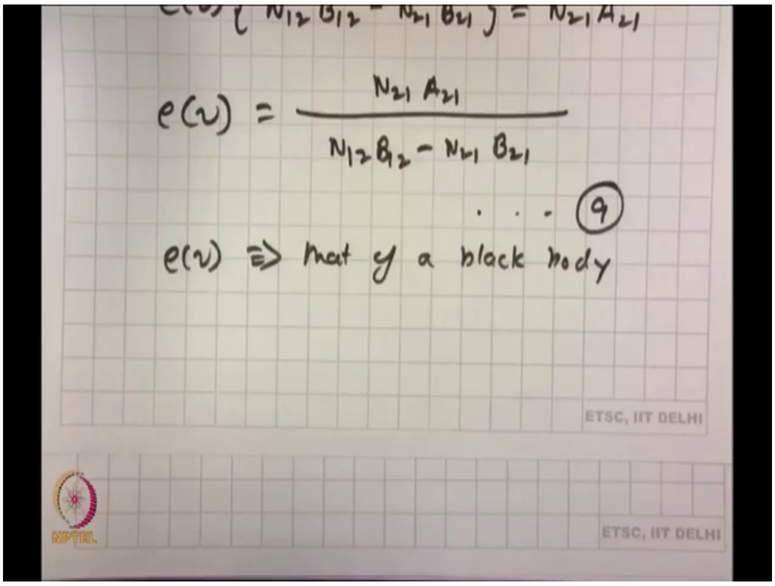


$$\rho(\nu)\left\{N_{12}B_{12} - N_{21}B_{21}\right\} = N_{21}A_{21}$$

$$\rho(\nu) = \frac{N_{21}A_{21}}{N_{12}B_{12} - N_{21}B_{21}} \quad \ldots ⑨$$

So, then from here I can write rho nu N 1 2 B 1 2 minus N 2 1 B 2 1 is equal to N 2 1 A 2 1 or rho nu is equal to N 2 1 times A 2 1 over N 1 2 B 1 2 minus N 2 1 B 2 1 and let this be question number 9. Let this be equation number 9.

(Refer Slide Time: 28:16)



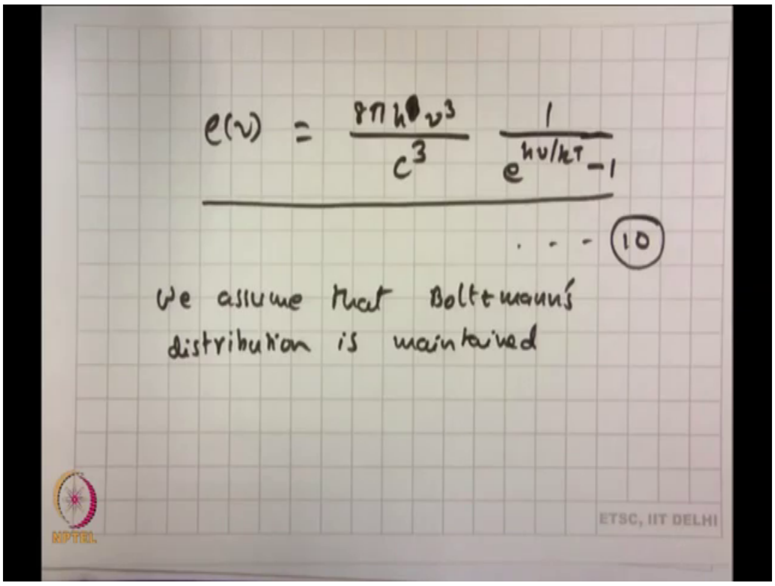
$\{N_{12}B_{12} - N_{21}B_{21}\} = N_{21}A_{21}$

$$\rho(\nu) = \frac{N_{21}A_{21}}{N_{12}B_{12} - N_{21}B_{21}} \quad \ldots ⑨$$

$\rho(\nu) \Rightarrow$ that of a black body

Now please remember, the assumption we started with was rho nu is that of a black body rho nu is that of a black body. If rho nu is that of a black body, then what we can now do is we already have an expression for rho nu from Planck’s hypothesis.

(Refer Slide Time: 28:46)



$$\rho(\nu) = \frac{8\pi h \nu^3}{c^3} \frac{1}{e^{h\nu/kT} - 1}$$

$\dots$ ⑩

We assume that Boltzmann's distribution is maintained

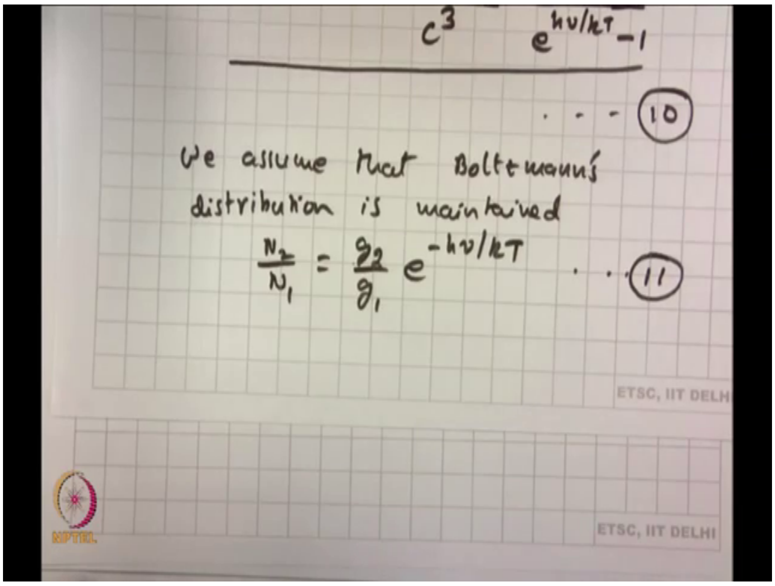
ETSC, IIT DELHI

So, we just use that expression for rho nu if and if you would remember that; if you would remember that from before, rho nu is equal to 8 pi nu cube sorry h nu cube over C cube times 1 over e to the power h nu over k T minus 1; this is what you know from before.

See essentially what you are trying to do is, you have this rate constants B 2 1 and A 2 1 right, you have this rate constants B 2 1 A 2 1. We are started with these but we do not know what A 2 1 and B 2 1 are. We have to get expressions for these in terms of certain parameters we know like say a Planck's constant like a frequency which is something very inherent when we are talking about spectroscopy right.

So, can we make a comparison between this one and the one we had already derived which was equation number 9. But before going there; before going there another assumption we will take is we assume that Boltzmann’s distribution is maintained. So, we assume that Boltzmann’s distribution is maintained.

(Refer Slide Time: 30:25)



$c^3 \quad e^{h\nu/kT}-1$

. . . (10)

We assume that Boltzmann's distribution is maintained

$$\frac{N_2}{N_1} = \frac{g_2}{g_1} e^{-h\nu/kT} \quad \ldots (11)$$

ETSC, IIT DELHI

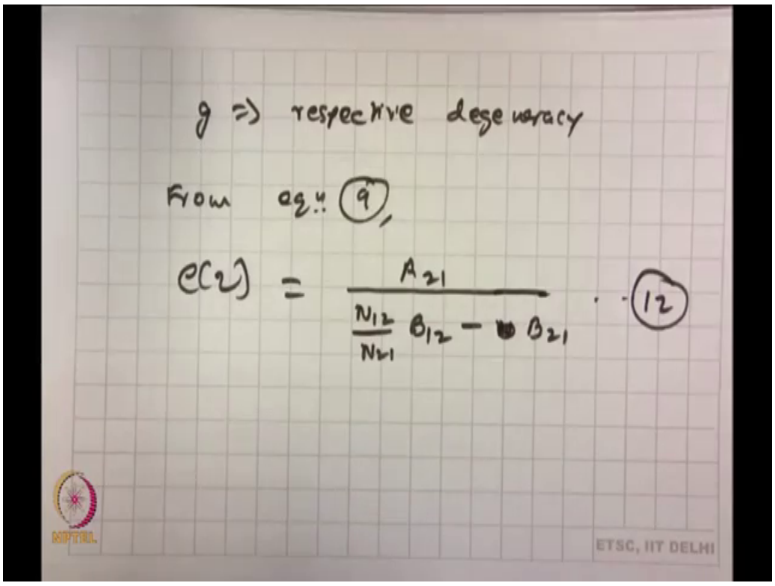
ETSC, IIT DELHI

And if that is the case; if that is the case then I can write N 2 over N 1 is equal to e to the power minus h nu over k T, e to the power minus h nu over k T. Here I am sure you have seen this I can also write g 2 over g 1. Can you tell what g refers to, what as you refer to?

Student: Degeneracy.

Degeneracy right, so g refers to degeneracy. So, what this g 2 and g 1 they take care of the fact how many same energy levels you have of that specific level we are talking about right.

(Refer Slide Time: 31:13)



$g \Rightarrow$ respective degeneracy

From eq.^{n} ⑨,

$$\rho(\nu) = \frac{A_{21}}{\frac{N_{12}}{N_{21}} B_{12} - B_{21}}$$ ⋯ ⑫

ETSC, IIT DELHI

So, then from here I can write g is a respective degeneracy right and where 11 came from please remember 11 came from it came from if you would remember, if ever you would be doing this Boltzmann population N 2 over N 1 it would be e to the power minus h or e to the power minus e to the minus e 1 over k T remember. So, that is e to minus e 1 is what? So delta e which is equal to h nu and that is what you are looking at ok. So, that is what is your Boltzmann population is right, so that was number 11 right.

Now, going back so from equation 9 ok, let us go back to equation 9. From equation 9 what I can write is rho nu is equal to; rho nu is equal to now I will do two small algebraic things, one is I will divide throughout there is a numerator and the denominator by N 2 1 ok. I will divide throughout by N 2 1, so what I can do is, I can write A 2 1 by N 1 2 N 2 1 times B 1 2 minus

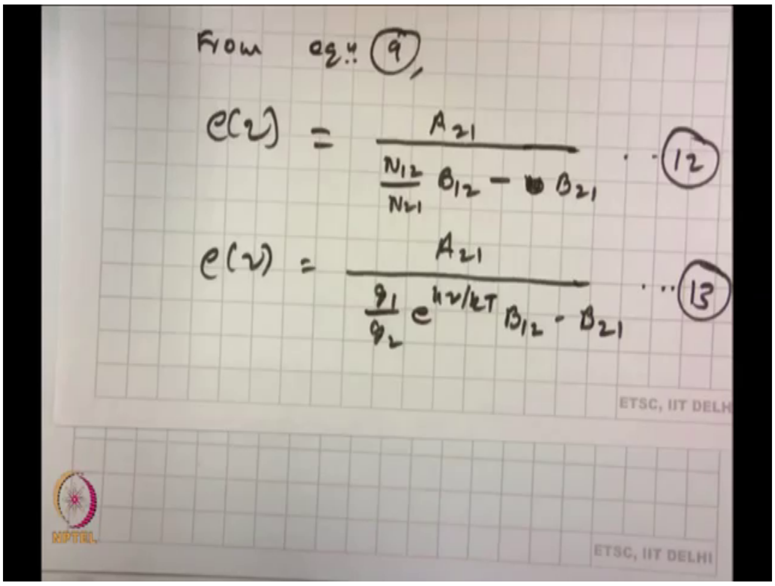
N 2 1 will be canceled actually minus B 2 1 this is number 12. So, I am dividing throughout by N 2 1 ok.

Now the next step, the next step is see I have two somehow get rid of the N's ok, I have to somehow get rid of the N's; one N I have already get rid of gotten rid of in terms in front of B 2 1 there was I divided throughout by N 2 1. Now what can I substitute in in place of N 1 2 over N 2 1, what can I do?

Student: D 2 1 (Refer Time: 33:22).

Right we just did that right. So, here N 1 2 over N 2 1 is essentially N 1 over N 2, because N 1 2 is the number of molecules in the ground state going to the excited state. And, N 2 1 is the number of molecules in the excited state coming down to the ground state ok.

(Refer Slide Time: 33:41)



From eq.ⁿ ⑨,

$$\rho(\nu) = \frac{A_{21}}{\frac{N_{12}}{N_{21}} B_{12} - B_{21}} \quad \ldots ⑫$$

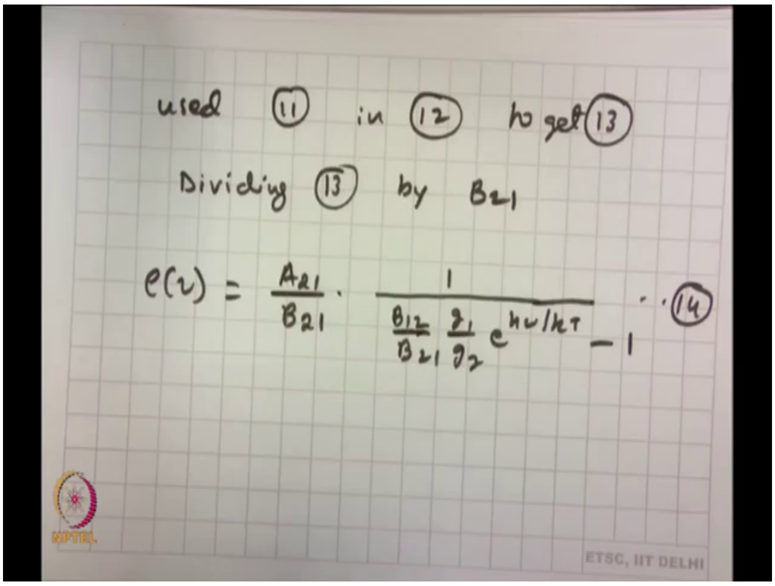
$$\rho(\nu) = \frac{A_{21}}{\frac{g_1}{g_2} e^{h\nu/kT} B_{12} - B_{21}} \quad \ldots ⑬$$

So, then we do that so this is rho nu is equal to A 2 1 over. So, here if N 2 by N 1 was g 2 by g 1 e to the power minus h nu over k T. What would this be?

Student: g 1.

g 1 by g 2 then e to the power h nu over k T right, then I will be having B 1 2 minus B 2 1 right. Let this be 13 ok.

(Refer Slide Time: 34:18)



used ⑪ in ⑫ to get ⑬

Dividing ⑬ by B_{21}

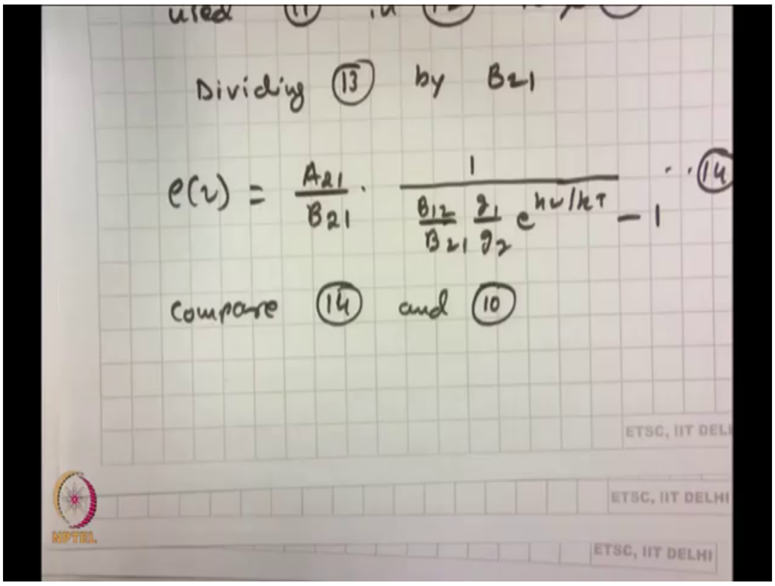
$$\rho(\nu) = \frac{A_{21}}{B_{21}} \cdot \frac{1}{\frac{B_{12}}{B_{21}} \frac{g_1}{g_2} e^{h\nu/kT} - 1} \quad .. ⑭$$

ETSC, IIT DELHI

So, here so what I have done is, we have used 11 in 12 to get 13 ok. Now tell me you look at number 10 by yourself and look at equation number 13, what extra thing do we need to do to get a fair comparison between these two equations? So, look at the last factor of equation number 10, what is the last factor? 1 over e to the power h nu by k T minus 1 ok.

So that means, since it is minus 1 I should be I should need to get rid of B 2 1 by putting a 1 out there. So, what do I do? I divide throughout by what? B 2 1. So that means, dividing 13 by B 2 1 we get rho nu is equal to A 2 1 over B 2 1 divided by B 2 1 times 1 by; times 1 by what should I be having here. Now I should be having B 1 2 over B 2 1 right, then g 1 g 2 e to the power h nu by k T minus 1 this is equation number 14 for me see is this ok? I have just put in 1 by dividing throughout by B 2 1 ok.

(Refer Slide Time: 36:07)



Dividing ⑬ by B_{21}

$$\rho(\nu) = \frac{A_{21}}{B_{21}} \cdot \frac{1}{\frac{B_{12}}{B_{21}} \frac{g_1}{g_2} e^{h\nu/kT} - 1} \quad \cdots ⑭$$

compare ⑭ and ⑩

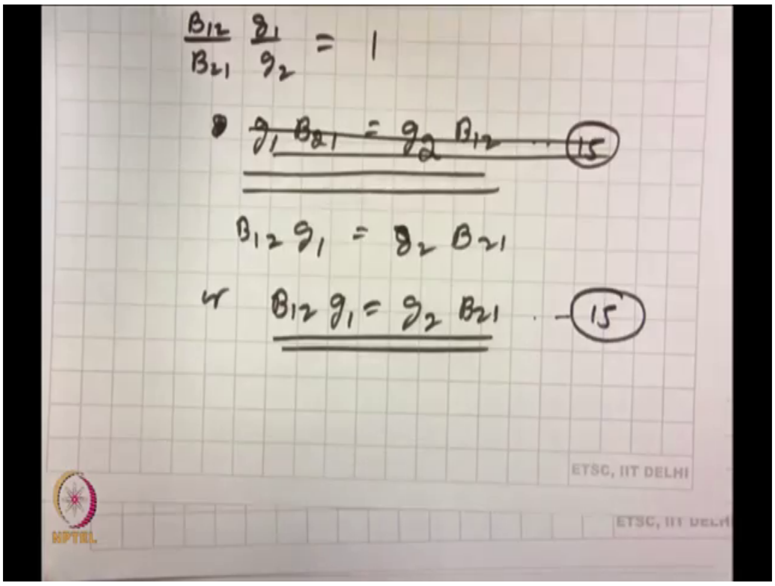
Now, what you do is compare 14 and 10; compare 14 and 10. If you compare 14 and 10 if you compare 14 and 10 what would you have? For example, in 14 or 10 has two factors, the first factor is 8 by h nu cube over C cube which should be equal to what? A 2 1 over B 2 1, the next factor is for 10 is 1 by e to the power h nu by k T minus 1 ok. We already have minus

1 we already have e to the power h nu by k T, but what is the coefficient it turns out with e to the power h nu by k T?

Student: 1.

1, so then comparing this what can I write?

(Refer Slide Time: 36:56)



$$\frac{B_{12}}{B_{21}}\frac{g_1}{g_2} = 1$$

~~$g_1 B_{21} = g_2 B_{12}$ ⋯ ⑮~~

$$B_{12} g_1 = g_2 B_{21}$$

or $\underline{\underline{B_{12} g_1 = g_2 B_{21}}}$. — ⑮

ETSC, IIT DELHI

So, I can write so comparing 14 and 10 the first thing is B 1 2 by B 2 1 g 1 over g 2 should be equal to 1 or I can write g 1 B 2 1 is equal to g 2 B 1 2, that is one equation for me. This would be number 15. Now let us look at the much the other fundamental one the other.

Student: (Refer Time: 37:25).

Where?

Student: g 1 into (Refer Time: 37:26). g 1 into B 1 2 (Refer Time: 37:29).

g 1 into B 1 2.

Student: B 1 2.

Why? It was g 1 by g 2 right? Tell me if I am wrong.

Student: (Refer Time: 37:45). g 1 into B 1 2.

It is g 1 into B 2 1 is not it or no why?

Student: B 1 2 (Refer Time: 37:52).

No, B 1 2 over B 2 1 times g 1 over g 2 is equal to 1 is not it so then B 1 2 times g 2.

Student: First one is right second one is (Refer Time: 38:05).

Right [FL].

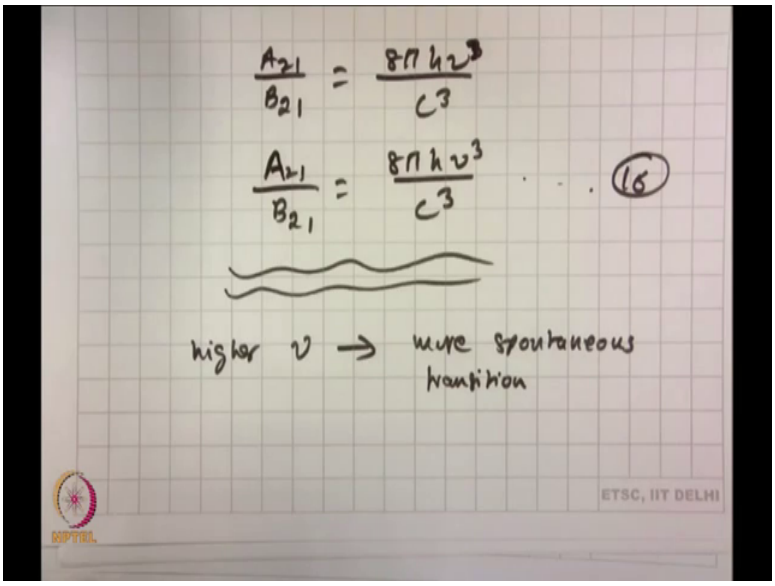
Student: Sir g 1 into B 1 2.

My bad [FL] B 1 2 times g 1 is equal to g 2 times B 2 1 this is what your saying right.

Student: Yes sir right (Refer Time: 38:30).

B 1 2 times g 1 is equal to g 2 times B 2 1 right.

(Refer Slide Time: 38:46)



$$\frac{A_{21}}{B_{21}} = \frac{8\pi h\nu^3}{c^3}$$

$$\frac{A_{21}}{B_{21}} = \frac{8\pi h\nu^3}{c^3} \quad \ldots \text{⑯}$$

higher ν → more spontaneous transition

So, that is one and the next thing is yes from the further comparison we have A 2 1 by what was the other one? By B 2 1 is equal to 8 pi h nu cubed over C cubed. This is nu cubed over C cubed, let me write it again A 2 1 over B 2 1 is equal to 8 pi h nu cubed over C cubed. This would be 16.

Now look at the importance of this, here what you are looking at is the ratio of two rate constants A is the spontaneous rate constant B is the stimulated one; 8 pi h over C cube is a constant everything is constant. What is the variable? Nu cube; that means, the higher is your nu. The higher is your nu which one would be more spontaneous and stimulated.

Student: Spontaneous (Refer Time: 39:47).

The higher is a nu the more would be your spontaneous transition right. So that means, higher nu leads to more spontaneous transition ok. Now pause a little bit and think about this, lasers it is light amplification by stimulated emission or radiation I have to have stimulated emission. What is the competing process along with what is the competing process with stimulated emission?

Student: Spontaneous (Refer Time: 40:30).

Spontaneous emission right, I would not; I would not like to have too much of spontaneous emission when I am talking about lasers. I will tell you why try to understand this. In lasers see if you are stimulating something that means, if you if you have a certain electromagnetic field with a certain phase coming in and that radiation is stimulating the downward transition of a molecule from the exited to the wrong side.

What will happen is the way where it will interact it will keep the same phase information ok. The phase information would be the same right, this leads to something known as coherence in light. So what is what do you mean by coherence? Coherence means everything kind of is moving along in a same direction maintaining the same phase relationship, because if they move out of phase what will happen?

They would destructively interfere with each other. So that means, you will always have to maintain the same phase ok; that is where it comes from. To give you a very simple example a real life if you would ever see soldiers marching on a bridge you would see they always lift the legs together in coherence that is in coherence. That means, they are not running out of phase and it really looks beautiful when they do the drill, because each and every one is marching.

Simultaneously synchronously actually that is the word by lifting that respective leg or being the other respective leg down simultaneously. So, if one is lift lifting the right leg everyone else lifts the right leg, that is a coherence. That means, if you are hitting if you are hitting or stimulating radiation as a certain phase then the ones which it stimulates to come down will also be coming out in the same phase. Now this give rise to something known as coherence, which it is a called a coherent property of light.

But think about spontaneous in spontaneous there is no nothing right. In spontaneous because a spontaneous is nothing the photons or the molecules can come down in any way maintaining any phase relationship. That means, the light coming out can have any phase relationship. So, that is called non coherent radiation.

Lasers is always about coherent radiant's, because you need a very focused light where high intensity beam right. So, laser radiation is always coherent right. So that means, to have a coherent laser radiation, I would have to have the least amount of spontaneous radiation possible and the highest amount of stimulated radiation possible. Now you look this up and you come back and tell me, but I will give you something to think about. It is very hard; it is very hard to find lasers in the blue specially in the UV can you tell me why?

Student: Hard (Refer Time: 43:13) see more the spontaneous (Refer Time: 43:15).

Right, so think about this. Whatever lasers you see; whatever lasers you see you know there are dark layers and all these things nowadays it would be mostly like 400 up and so. The moment you come to the UV what will happen is the nu is very high, now because the nu is very high what is happening?

Student: (Refer Time: 43:34).

The ratio is increasing there is a spontaneous emission is becoming more; if the spontaneous emission is becoming more than the efficiency of the laser is going down at anything. That is why it is very hard to get lasers in the blue in the UV and if you are going to get one it becomes really expensive. Just because of this simple principle remember.

Because this is a fundamental nature of your light you cannot do anything with it. The only thing you can do is devise some method to counter it, but there is no other way you can do it;

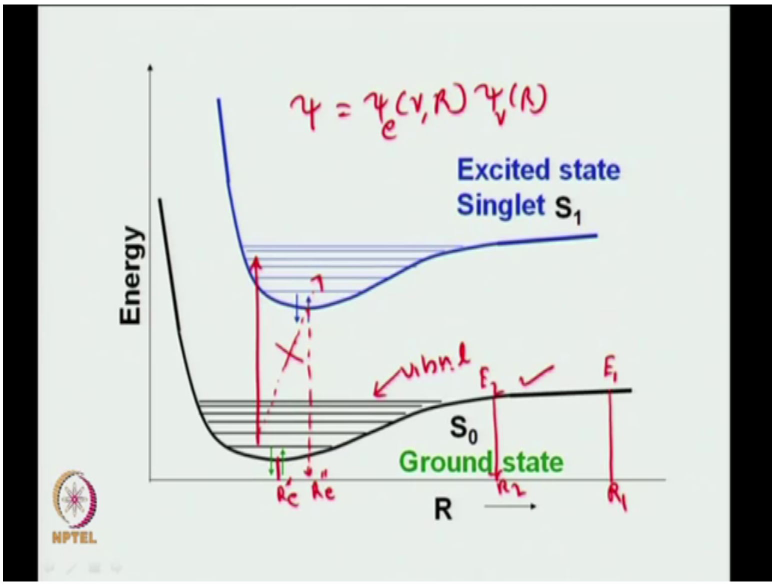
because this is what you have just derived without any principle right. It just based on equilibrium so please remember this.

The amount of stimulated or the amount of spontaneous emission you have or the ratio of the stimulated and spontaneous emission is governed by the nu cubed, that is why you go higher up you go visible and all these things where the nu becomes lower and lower. What will happen is you will see more and more lasers coming up ok, that is typically why it is.

Again take it from me this is a very fundamental thing you ought to know, it is a very fundamental thing and that is why Einstein's A and B coefficients are so important in spectroscopy absorption and fluorescence right. Einstein's A and B coefficients specially the ratio of A over B, because it tells you exactly in which region you are not going to get a very efficient laser system. Because your stimulated emission is going to decrease drastically rather your spontaneous emission increases like anything ok. Will you keep that in mind? Good.

So then these Einstein's A and B coefficients also have also other meanings, I will come to those when we deal with those things later as and when we deal with those things right. Now before ending this class, I will just introduce something with which I can carry on in the next class.

(Refer Slide Time: 45:39)



So, let us go back to the slides, let us talk about a transition let us talk about transition. Now what do you see here? What do you see here on the y axis you have energy potential energy on the x axis you have R. What is R?

Student: Distance (Refer Time: 45:56).

No, reaction it is not the reaction coordinator.

Student: (Refer Time: 46:00).

Well, it is a reaction coordinator, but it is a bond distance it is an inter nuclear distance right. Where have you seen this before? What feature of quantum mechanics does this come across?

Student: Planck quantum principle.

Planck quantum principle excellent, it comes across in Planck quantum principle right. So, what are you here you have one of the very simple pictures. What do you have is you have two states one is, the ground state which is S 0. What does S stand for?

Student: Singlet.

S it stands for singlet you can see the electrons are paired right, then in the excited state you also have another singlet which is S 1. So, 0 is the ground state 1 is the excited state right. And what you will be doing is, if the if you would be sending in enough radiation, then the molecule or radiation of the current frequency of the correct frequency the molecule had absorb and then it would it would make a transition to the upper state ok.

Now before going deeper, see there has to be something which determines this right; which determines the intensity of your transition the probability of your transition and all these things. Now that is why Planck's quantum is so important.

Then look at this why did I draw a vertical arrow? I could have drawn an arrow which was like this. What is the difference between me drawing a vertical and me drawing a slanted arrow what happens?

Student: Actually so it does not (Refer Time: 47:32).

Not tell me what happens I know that, tell me what is the difference between a vertical transition and a sloping transition. Just refine what you said and tell me what do you mean by vertical transition, what is not changing when you make a vertical transition?

Student: (Refer Time: 47:48).

R is not changing right that is what you said. But I just want to know the answer R is not changing. If I do a sloping transition what happens?

Student: (Refer Time: 47:58).

Because your R is changing.

Student: (Refer Time: 48:01).

Right, now Planck quantum said that each and every transition has to be what vertical and why does it have to be vertical? Because as you said nuclear much more massive than electrons right. That is why this is a vertical transition you cannot have a transition which goes like this. You cannot have a transition which goes like this right. Second, you look at the two states S 0 and S 1 right. What are these levels what are these levels, you know these levels what are these levels?

Student: (Refer Time: 48:32).

Ok, very good. So, these are vibration states right. So that means, in S 0 you are looking at an electronic state along with vibration energy levels, in S 1 you are looking at electronic state along with vibration energy levels right. Now you remember your psi your total wave function is or should be having electronic component, vibration component which is the nuclear and there is one more what is that?

Student: Rotation.

Rotation, then in between the vibration energy levels you will also be having what rotation ok. But we will not worry about rotation that much here, two principles which you are going to worry about one is electronic and one is vibration. Second guys how do you think or tell me if you would be drawing this curve ok, if you would be drawing this curve how would you draw this curve?

This is actually a Born Oppenheimer approximation. This curve actually tells you what is Born Oppenheimer approximation is. Tell me how would you draw this curve, what is Born Oppenheimer approximation say? It says that essentially these two motions are independent of each other, the electron moves much faster than the nucleus.

Do you remember in Born Oppenheimer approximation you said something that the electronic wave function has a parametric dependence on the nuclear coordinates? Do you remember that word parametric dependence? See what happens is it is very simple, the nuclear also vibrating right ok. Whether it is in the same state or whether go to a different state, the inter nuclear distance can change. See for example in these two curves, the ground equilibrium distance for this one was R e say prime for the excited state, it is R e double prime.

Now has the equilibrium internuclear distance changed or not? It has changed right, because you can see the upper state is shifted slightly to the right as compared to the lower state and because the minimum occurs at what? The equilibrium bond distance. What you can say is that once you have excited the molecule what has happened? The bond length has increased is not it? The bond length has increased.

Tell me a typical situation, suppose I give you a molecule which is ethane it has a sigma bond and a pi bond ok, it is a certain bond length. Now you hit it with the radiation where what happens one of the pi electrons goes to into the water orbital from the bonding to the anti bonding molecular orbital. If it goes to the anti bonding molecular orbital what happens to the bond order now? Initially it was 2, now it is now it is 1 right.

Now it is 1 because one of the electrons already is the anti bonding orbital. So, what does happened? The bond order has changed decreased. So, what will happen to the inter nuclear distance?

Student: Increase.

Increase, so this is possibly one of the situations right, when the inter nuclear increases right it is one of the cases. Now what does the parametric dependence come from? So look at this, because the nuclei cannot move as fast of the electrons. But remember, even when you are making the transition the molecule is still vibrating it is only that you have decoupled these two, that means it is called a separation of variables in that sense which you do 2 D and 3 D harmonic oscillators or particular in a boxes.

So, parameter dependence means is what do you do is you take a certain R ok, you take a certain R and solve the Schrödinger equation at that R. So, you will be getting since you are solving for psi electronic you will be getting e electronic. That means, energy of the electronic state at that value of R. Now again you change R. So, for example, what you are starting is you look at this R this R. So, this is say R 1 R1 at R 1 what does happened? At R 1, there is almost no attractive force between these two that is why it is tailed off. Now what you are doing is you are keeping the nuclei at fixed at R 1 and now you are solving what is shown in your equation. So, you get e electronic right then you plot E 1 out here [FL].

Now what you do is you change R 1, so you go to say R 2 once you go to R 2 again you solve for e. So, then see you get E 2 and you go on doing that. Once you go on doing that what will you get right, see because you cannot take these two things together; the thing that helps you is the electrons are moving much faster than the nucleus. So, if you are assuming that the nuclear nuclei has changed the electrons are bound to follow that.

So, what you are saying is that at I fix the nuclei positions at different R and then I solve for the electronic energy e electronic and then I plot that. Once you plot that this is the curve you get and this is your Born-Oppenheimer approximation this plot tells you the Born-Oppenheimer approximation this is how you get it ok. That is a signature of this plot.

So, Born-Oppenheimer approximation, that is how you would plot it and this is called a parametric dependence. That is why you will see; that is why you will see if you are looking at psi total it is given as psi electronic then it says r comma R and psi nuclear or I can write vibration R. So, this is your psi I will come to this in the next class, but just realize the importance of this.

The psi electronic how many coordinates does it depend upon, what are the two coordinates? One is r small r and the other one is big R. What is small r? Small r is a distance between the electrons is interelectron distance right or it is the distance of the electron from the nucleus ok. So, it is a position of the electron. What is big R? Big R is a distance between the?

Student: Nucleus.

Nuclei. So that means, your electronic wave function is not only dependent upon the position of the electrons, but what is it also dependent upon? The position of the nuclei and when you draw a plot like this this is what you get out of it ok. This is essentially what you Born-Oppenheimer approximation is, psi vibration essentially just depends upon the distance between the nuclei ok. So, remember each and every plot like this is actually a picture of a Born-Oppenheimer approximation that is how you get it.

So, this is where we start with some of the very essentials of spectroscopy. And next class we will go into Planck quantum factor or Planck quantum overlap that is what determines, the intensity of the transition all those things.