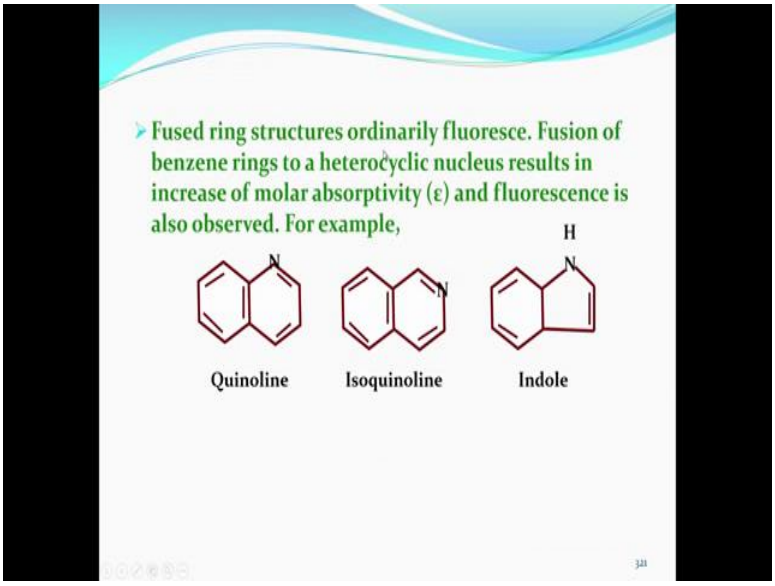
Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

Dr. J R Mudakavi
Department of Chemical Engineering
Indian Institute of Science, Bangalore

Lecture- 22
Florescence Spectrophotometry – II

There are other structural information that we can derive from our knowledge of chemistry and electronic structure and molecular structure, which substances are more likely to fluoresce.

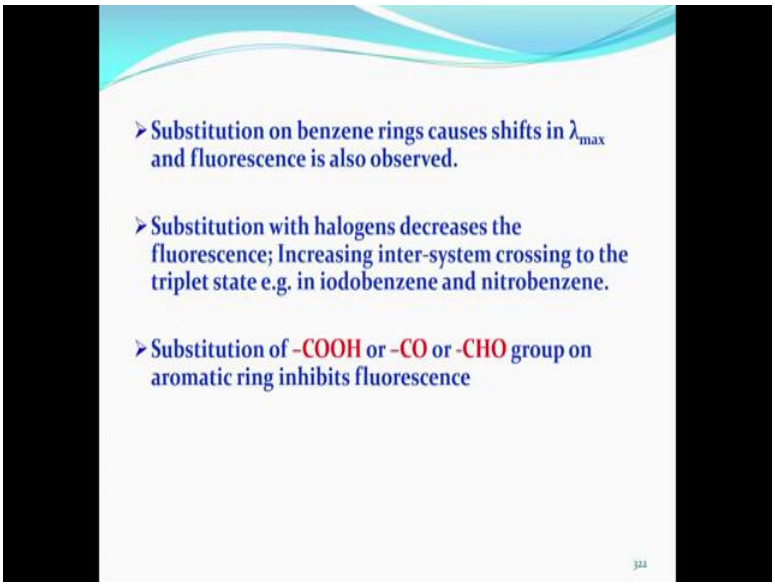
(Refer Slide Time: 00:35)



This is another structure that we can think of, this is basically a fused to ring structure two aromatic substances rings are fused here, and here it is one benzene ring and one pyrrole ring. So, fused ring structures now ordinarily fluoresce. Fusion of benzene rings to hetero cyclic nucleus results in increase of molar absorptivity. Normally, what we should expect is from molar absorptivity if it is high fluorescence also may be observed and it will be of high intensity only. For example, you can look at the structure of quinoline, see there are two fused rings and all the bonds are conjugated there is one nitrogen here.

So, in iso quinoline this structure is different slightly different, but again you can see that there are conjugated double bonds. Here they should have been one more bond here in the in between opposite to this bond or in conjugation with this bond. So, in the fused ring structures, all these kind of structures will fluoresce and many more structures with 3 rings, 4 rings, 5 rings definitely they will be having a probability of fluorescing.

(Refer Slide Time: 02:24)

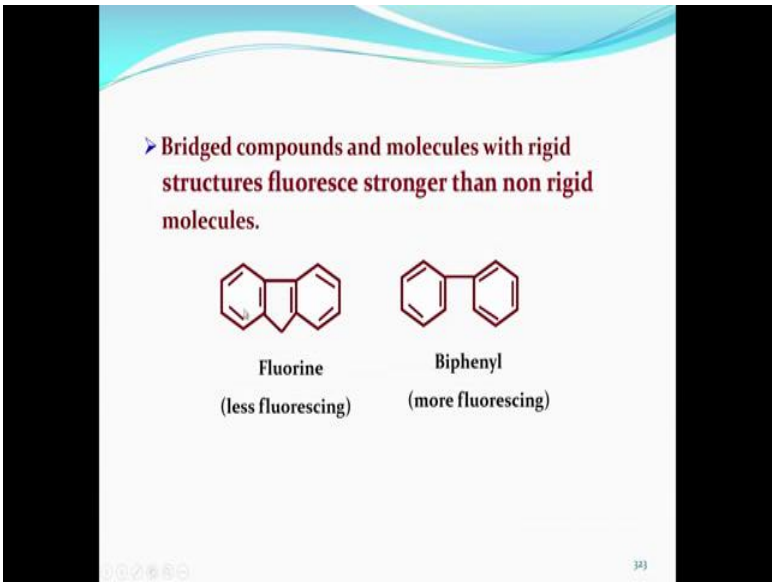


Then substitution on the benzene rings, these results in the shifting of the lambda max and sometimes fluorescence is observed. So, if you have a lot of benzene rings in a given molecule, and if there is substitution occurring then fluorescence can be observed. Another possibility is substitution with halogens, normally halogens are having large number of electrons and number of orbitals, and inter systems and conversion will be possible. So, the chances of fluorescence decreasing with fluorescence with halogens are quite large and increasing inter-system crossings also to the triplet state.

Now, these are the very simple examples of iodobenzene and nitro benzene and in such cases fluorescence always decreases. Sometimes the we classify organic cum groups as a substitution of COOH functional groups that acid, this is an acid group, this is a carbonyl group, this is an aldehyde group and all these things on the aromatic ring enables the fluorescence. So, the because these are all electron with drawing groups; if there are

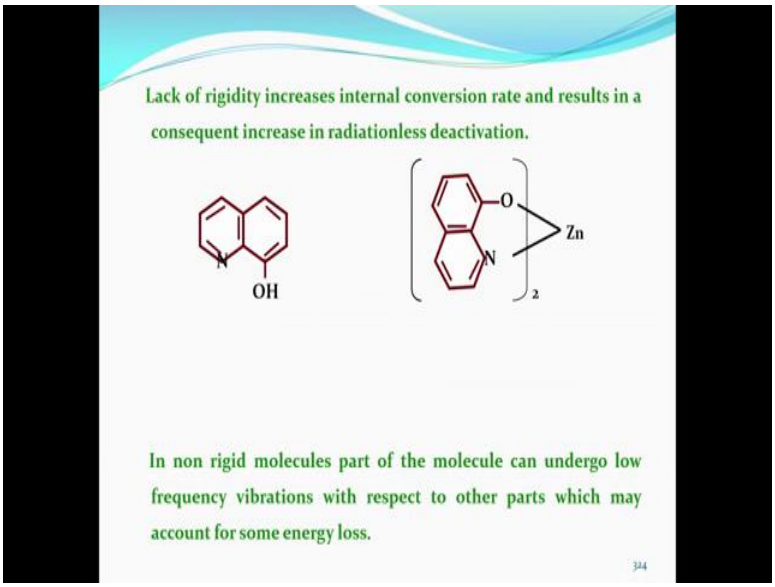
electron releasing groups NH 2, OH etcetera, the fluorescence will be possibility of fluorescence will be much higher.

(Refer Slide Time: 03:58)



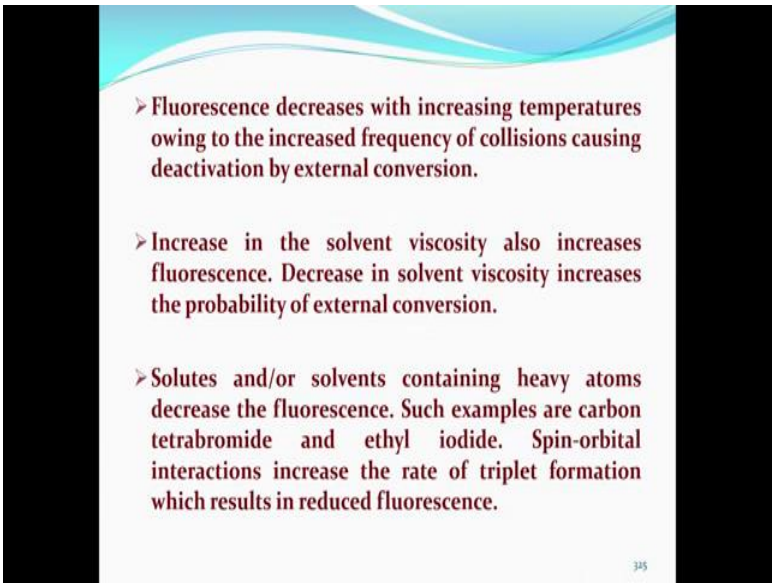
Now, bridged compounds, these are known as bridged compounds and then this not fluorine there is some other compound I will tell you what it is later. So, bridged compounds and molecules with rigid structures fluoresce stronger than the non-rigid molecules. So, this is a biphenyl structure very well known this fluorescence. And here there is less fluorescence because the rings are all fused here in this case.

(Refer Slide Time: 04:40)



So, lack of rigidity is another problem. Lack of rigidity increases internal conversion rate and that result in a consequence increase in the radiation less deactivation that is solvent-solvent interaction, molecule solvent interaction, and then molecule-molecule interaction also there are three types of interactions where the collision will takes place and the fluorescence decreases. And for example, there is one more possibility that there is a nitrogen here there is oxygen here OH group is here if I add a little bit of zinc to this it forms a complex. So, once it forms the complex again it gets bridged and bridge compound do not fluoresce as much as non-bridge compounds. So, in non-rigid molecules part of the molecule can undergo frequency vibrations and with respect to other parts which may account for some energy loss not all.

(Refer Slide Time: 05:49)

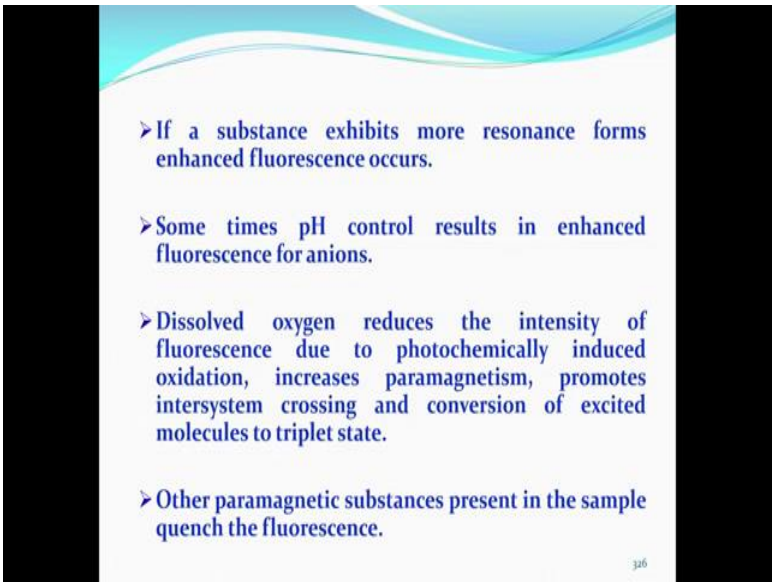


So, other conclusions that we can derive from fluorescence is fluorescence normally decreases with increasing temperatures owing to the increased frequency of collisions causing deactivation by external conversion. This is a very simple phenomena, because as the temperature increases, molecules will start moving faster and faster again the kinetic energy. And once the kinetic energy of the all the molecules is increased to very high level then what happens is the collisions will increase and the fluorescence intensity will come down. Sometimes solvent viscosity this I have already referred that use of polyvinyl alcohol Thermax and other not Thermax, use of polyvinyl alcohol, glycerol, and dextrose, maltose etcetera they increase the viscosity.

Viscosity increase means there will be less movement of the molecules. So, decrease in solvent viscosity increases the probability of external conversion, this is a very logical conclusion we expect from increasing the viscosity. So, normally what we would like is the viscosity of the substance should be as less as possible in fluorescence measurements. Solutes and or solvents both containing heavy atoms decrease this fluorescence this I have already shown you in the previous slide with zinc. So, such examples are carbon tetra bromide and other examples ethyl iodide the bromide is a heavy atom and iodide is heavy atom. So, if I had a little bit of ethyl tetra bromide or carbon tetra bromide, carbon tetra chloride, ethyl iodide etcetera the fluorescence will reduce because spin orbital

interactions increase the rate of triplet formation which results in reduced fluorescence.

(Refer Slide Time: 08:01)



So, if a substance exhibits more resonance forms is very simple. Basically whenever there is a resonance, we expect the molecules to stay in different forms which are in resonance with each other that means, the electronic structure will be alternating between different structures without undergoing any change. So, the more resonance forms means the lower average energy of the molecules. So, this should normally increase in the fluorescence, so that is what we are telling number of double bonds, conjugated double bonds, if there are more conjugated double bonds the fluorescence will increase that is exactly what we are writing here.

And sometimes pH, pH is a very important parameters in most of the fluorescence methods you increase the pH sometimes it will increase in some type of chemicals and you decrease the pH sometimes fluorescence also will get destroyed. So, the pH can act either way depending upon the structure of the molecule rather than the general conclusion. And the basic idea is in colored molecules, there is more resonance; in non colored, leuco type of dyes the fluorescence will dye down. So, such substances are mainly organic dyes, they could be fluorescing in acidic medium as well as in fluorescence medium. So, those substances which fluoresce in acidic medium will not

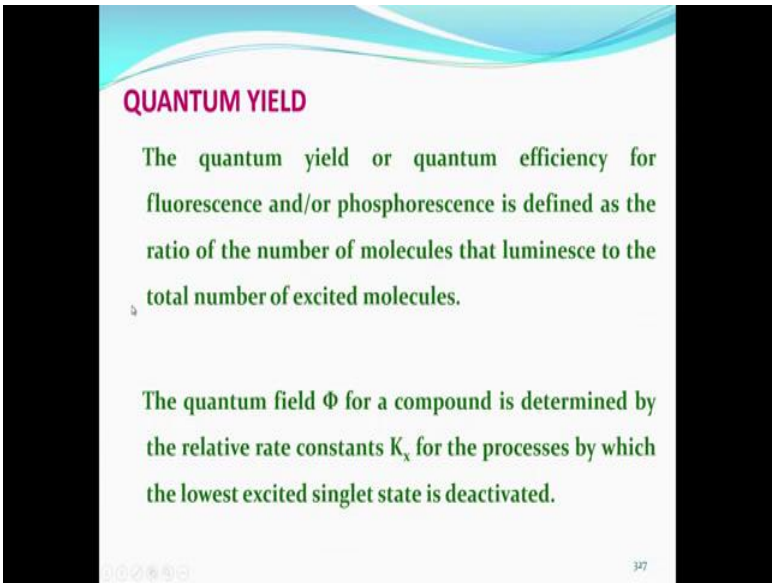
fluoresce in the basic medium and they and vice versa also basic medium substances which are having highly resonating structures will get protonated the functional groups will get protonated and the resonance structures will become less, the energy requirement will be high. So, fluorescence will decrease.

Now, dissolved oxygen, another parameter; so dissolved oxygen what happens it reduces the in10sity of fluorescence due to photo chemically induced oxidation normally oxygen is a very corrosive substance whether it is in the air or anywhere else pure oxygen is a corrosive substance, so that is why god has created our environment air also diluted with about 80 percent nitrogen for us to breathe. If we were to breathe only pure oxygen all the time, most of our internal body parts could have been oxidized and destroyed in no time and none of us would have survived.

So, dissolved oxygen what it does basically is it can photo chemically induce oxidation in chemical systems I am not talking about the body systems. In chemical systems, if there is dissolved oxygen it increases paramagnetism, it promotes the inter system crossing and conversion of excited molecules all these processes will result in the reduction of fluorescence. Basically oxygen itself in aqueous even if you have a system where there is no oxygen, but if it is an aqueous solution, you keep it open to the atmosphere, it will keep on dissolving oxygen from the air. So, it is very important in most of these spectrophotometric procedures that as well as fluorescence procedures, the oxygen the system should be kept covered and there should not be much interaction with oxygen also.

Similarly other paramagnetic substances also will reduce the fluorescence reduction of fluorescence is normally referred to as quenching the fluorescence. So, what we understand from the discussion so far is fluorescence is natural phenomena, it occurs in substances which are having resonance structures. And these resonance structures and fluorescence intensity is associated with pH, dissolved oxygen and several other paramagnetic substances and others bridge compounds etcetera. And the fluorescence procedure must take care of all these parameters which adversely affect the fluorescence that is important for us to know.

(Refer Slide Time: 13:20)

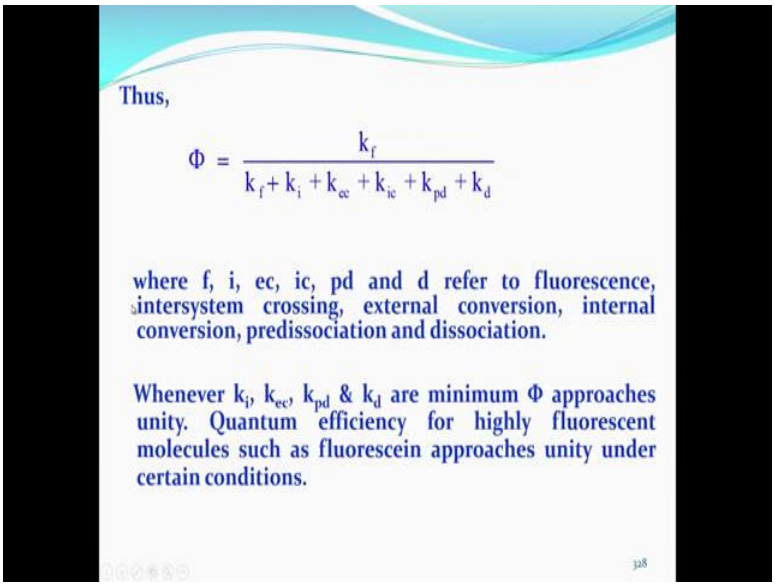


So, now we come to the practical aspects. The fluorescence how do you quantify fluorescence. The quantum yield is a term defined for fluorescence substances, and or you can call it even quantum efficiency for fluorescence or phosphorescence that is defined as the ratio of number of molecules that luminous to the total number of excited molecules. All the excited molecules need not result in florescence as we have seen so far with a long discussion. So, the incidence of electromagnetic radiation on a molecule results in the number of molecules going to next higher energy level and then populating there. From there the electrons have to come down to the lowest energy state and then cross over to ground state.

So, during this ground state crossover we have seen earlier that there will be number of molecules which are in the excited state, but all of them do not result in fluorescence lot of material molecules will lose fluorescence by other means which you have already discussed so far. So, the total number of molecules in the excited state to that of the total number of the substances, which cause luminous, luminescence that ratio is known as quantum yield or quantum efficiency. This is a very standard term denoted by phi for a compound that is determined by relative rate constants for the processes by which the lowest excited singlet state is deactivated. So, basically what we do if it is a theoretical term defined to differentiate between the two phenomenons, but it also differentiates the

excited singlet state and the triplet state or deactivation either way.

(Refer Slide Time: 16:07)



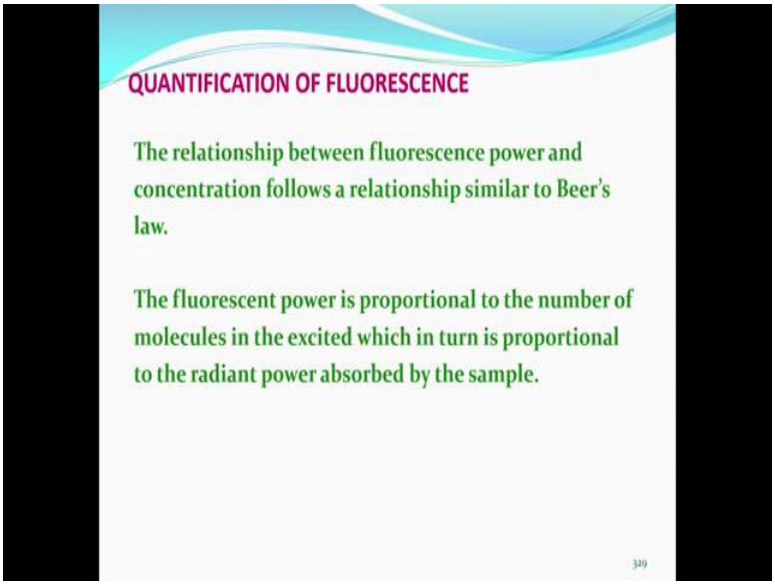
So, how do we define fluorescence in florescence the quantum efficiency phi we define it as number of parameters that cause the deactivation. So, in this equation, you can see that phi is kf divided by a lot of things like k f, k i, k ec, k ic, k pd and kd. What do these things mean is f refers to fluorescence and i refers to intersystem crossing, ec is electro external conversion, and internal conversion, and pd refers to pre dissociation, and kd refers to dissociation constant, all these terms are sort of equilibrium constants. So, it is the ratio of the equilibrium ratio of the equilibrium constant of all this to the excite number of excited molecules.

Whenever the other things at the bottom in the at the denominator that is k i, k ec, k ic, k pd and kd are kept minimum phi approaches unity, when all these are the denominator systems, they are kept minimum. That means, we can almost equate them to 0, k f by k f should be 1, because k i, k ec all these things we can neglect with respect to kf. So, in pure fluorescence fluorescing systems are ideal systems the maximum conversion rate you can get is 1 or 100 percent whatever way you want to refer to that. So, it approaches unity whenever the other things are kept minimum.

So, quantum efficiency for highly fluorescent molecules, such as fluorescing approaches unity under certain circumstances. So, this fluorescing I have shown you earlier this is fluorescing there is a spelling error, but this I want you to remember that there was an error there is an error and you can check up the good structure proper structure in your text books. And the relative quantum field for a compound is determined by the relative rate constants by which the lowest excited singlet state is deactivated. So, this we have seen already and fluorescence approaches unity.

So, if the fluorescing approaches unity then what we mean is you take a compound of fluorescing use it as a standard to compare other fluorescing systems that is the beauty of fluorescing, because it approaches unity. That means, the inter system crossing is less, and then external conversion is less internal conversion is less, pre dissociation is less, dissociation is less, so why not we take it as a standard for fluorescence. Similar same thing is to even with quinoline that also can be used as a standard for fluorescence measurements.

(Refer Slide Time: 19:46)

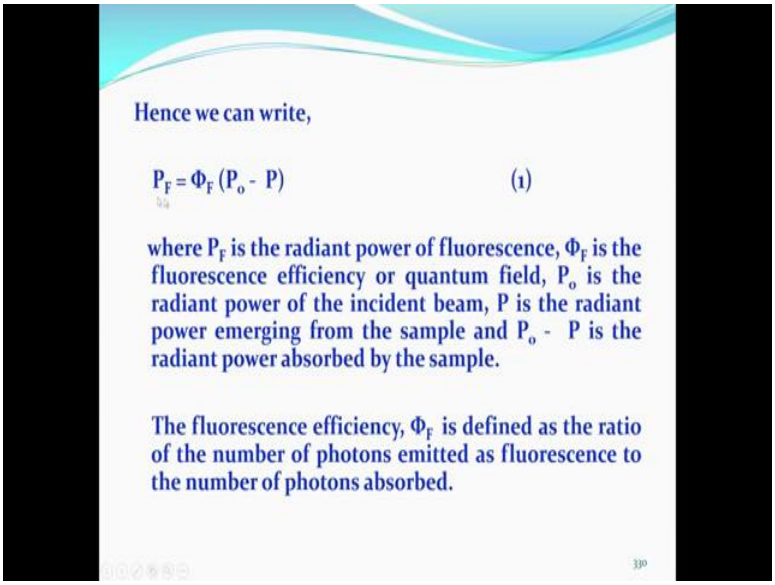


So, how do we quantify the fluorescence I was explaining to you, you should treat fluorescence or quinoline as a quinoline sulphate also you can try all these things are having fluorescence efficiency as unity. So, the relationship of fluorescence with

structure can be quantified by comparing with fluorescence or quinoline sulphate. So, this is an expression which will be using quiet over and over, and this relationship between fluorescence and concentration is similar to Beer-Lamberts law. So, what does Beer-Lambert law say it says concentration is proportional to observance? And when fluorescence intensity is unity near unity the concentration should be linear to we replace theta or absorbance by fluorescence, all other derivations essentially remain the same except for accounting the fluorescence efficiency that we will see now.

So, the fluorescent power just like what we did in absorption spectrophotometry is proportional to the number of molecules in the excited state which in turn is proportional to the radiant power of the observed radiant power absorbed by this sample this is understood very easily, there should not be any confusion regarding this.

(Refer Slide Time: 21:30)



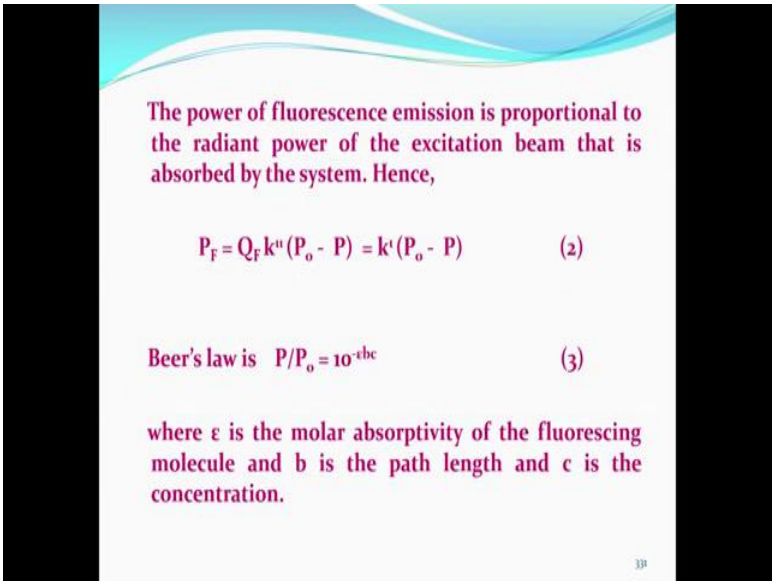
So, we can write the total fluorescence P F radiant power of fluorescence must be a function of fluorescence efficiency that is phi F and related to the incident and that emitted light that is P naught by P. So, the energy difference P naught minus P is a function of Q F; if the Q F is 1, it will be one, but if Q F is very less the total fluorescence of the substance will be quite low. So, here P naught is the radiant power of the incident beam, and P is the in the radiant power of the emerging beam from the

sample after it passes through the sample. And P naught minus P is the radiant power absorbed by the sample, it is not emitted.

Now, the fluorescence efficiency is defined as the ratio of number of photons emitted as fluorescence to the number of photons absorbed, this is also we can define. Earlier we had defined that we defined fluorescence efficiency as number of total excited molecules to that of number of molecules resulting in fluorescence that is by subtracting by removing the inter system crossings etcetera the other deactivation methods. Similarly, I can define in an instrumental analysis that the fluorescence efficiency is the ratio of the number of photons emitted as the fluorescence, because it is a emission where we are measuring not the absorbance.

So, to the number of photons absorbed that ratio also I can take it as fluorescence efficiency. So, both of them should be essentially same if we consider the structure of the light that is involving photons.

(Refer Slide Time: 23:50)

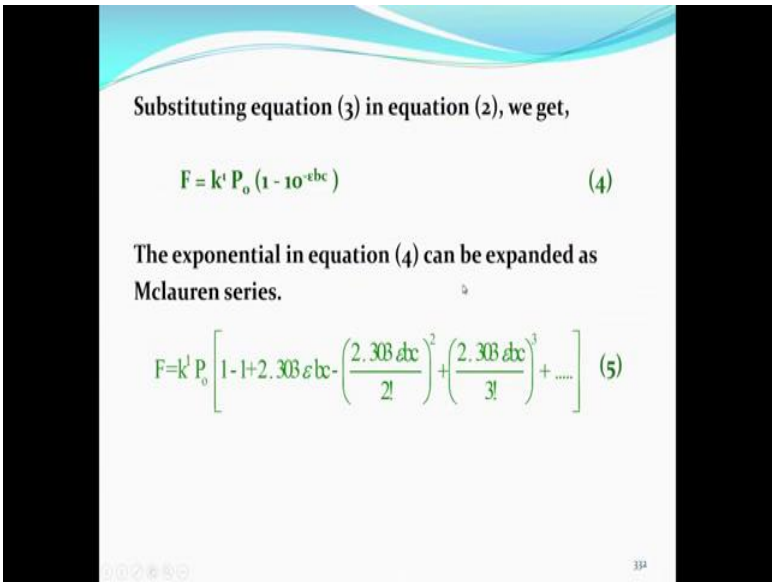


So, I can write an expression something like this that is the power of fluorescence emission is proportional to the radiant power of excitation beam that is absorbed by the system. So, I can write this equation P F is a function of quantum efficiency Q F. And

then another proportionality constant this we are introducing here and P naught minus P that is equal to k dash I just put a simple constant because phi F is a constant fluorescence efficiency is a constant and k 2 is a constant. So, combining this I have put it as k 1 and P naught minus P remains the same.

Now, what does Beer-Lambert law say P by P naught is equal to 10 to the power of minus epsilon b c or a b c where epsilon is the molar absorptivity of the fluorescing molecule, and b is the path length, and c is the concentration this is what we have delivered in Beer-Lambert law.

(Refer Slide Time: 25:09)



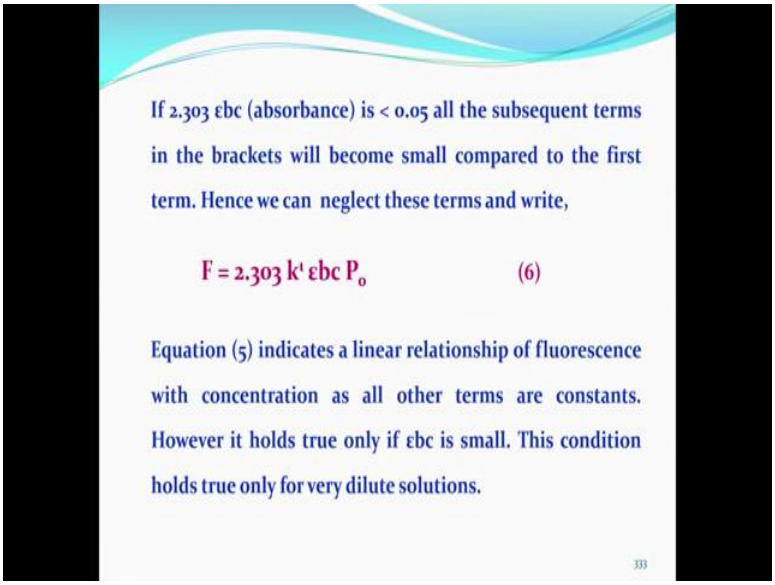
So, what I want to do the substituting the equation 3 in equation 2. So, I write fluorescence intensity f is fluorescence intensity now k dash into P 0 into minus into bracket 1 minus 10 to the power of epsilon bc. This is a very simple derivation resulting from the previous equation and the definition of absorbance. See, P by P naught is equal to 10 to the power of minus epsilon bc. So, if I take out P naught from this i end up with an expression something like this P naught into bracket 1 minus 10 to the power of minus epsilon bc, because the P naught P, P is P naught into 10 to the power of minus epsilon bc. So, P naught is there in the second term also I replace this 10 raise to P naught into 10 raise to minus bc, P naught I take it out of the bracket and I end up with an expression

something like this P naught into 1 to the power of minus 10 to the power of minus epsilon bc.

Now mathematically I can treat this as an exponential equation and expand it. There is an simple expression process for this that is known as Maclaurin series, you can look up your first year engineering mathematics or any mathematics book, you can see that 1 minus e to the power of x can be expanded something like this. Now I put 10 to the power of minus bc as x, and then if i put it as x, I will be writing 1 minus 1 plus 2.303 into x minus 2.303 x square by 2 factorial and then x cube by 3 factorial like that.

So, I am going to put this 10 to the power of e epsilon bc in the same equation and expand it according to Maclaurin series this k 1 is here and then P naught is here this I am expanding 1 is here. And expansion of this is minus 1 plus 2 into 3.302 bc divided by factorial 1. I have not taken the first factorial 1 is 1 only and 2.303 epsilon bc is factorial to factorial 3 like that it is an unending series.

(Refer Slide Time: 27:55)

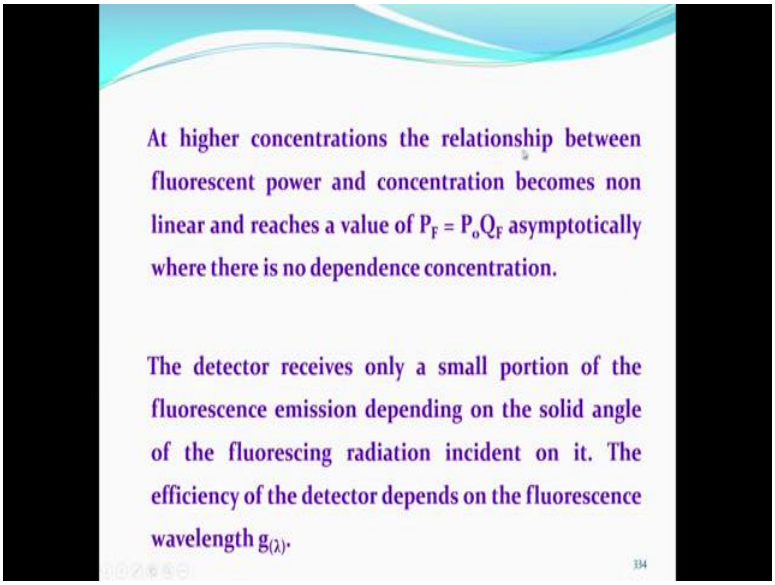


So, in this equation 2.303 into epsilon bc absorbance, if it is less than 0.05 all the subsequent terms in this bracket, that is two point this one square terms and cube terms in a if it is a less than 0.05 you can square it, it will become 0.0025. If you can make it

cube 0.000125 like that it becomes all they become negligible. This one and one will cancel and what will be left with is 2.303 into k dash into epsilon bc into P naught. So, this equation again this is constant 2.303, it is constant. Epsilon is constant if I keep the P naught is constant and b is path length. If that is all constant I have only the term fluorescence, I can relate it with a constant term and concentration.

So, if this is a constant it can be evaluated by plotting fluorescence versus concentration and determining the slope of the curve provided it follows a linear response. So, equation 5 indicates, equation 6 also indicates a linear relationship of fluorescence with concentration as all other terms are constants. However, it holds true only if epsilon bc is very small this condition holds to only for dilute solutions. So, you should never forget the fact that the dilution fluorescence is a phenomena which follows linear response only in dilute solution not in concentrated solutions.

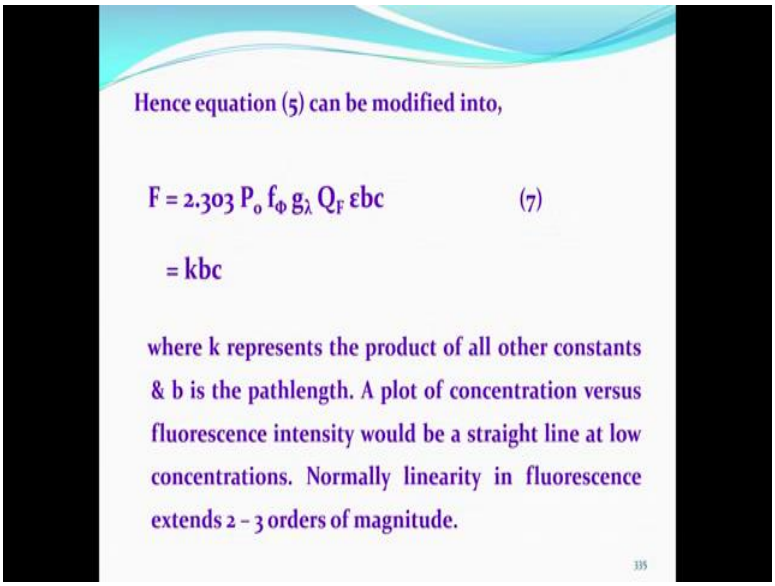
(Refer Slide Time: 29:53)



So, at higher concentration what happens, the relationship between fluorescence power and concentration becomes non-linear and reaches a value of P naught into Q F you can asymptotically where there is no dependence on concentration. For example, if you were here, if the concentration is very high then fluorescence will become constant. So, we can neglect the terms and P F would be P naught into Q F asymptotically where there is no

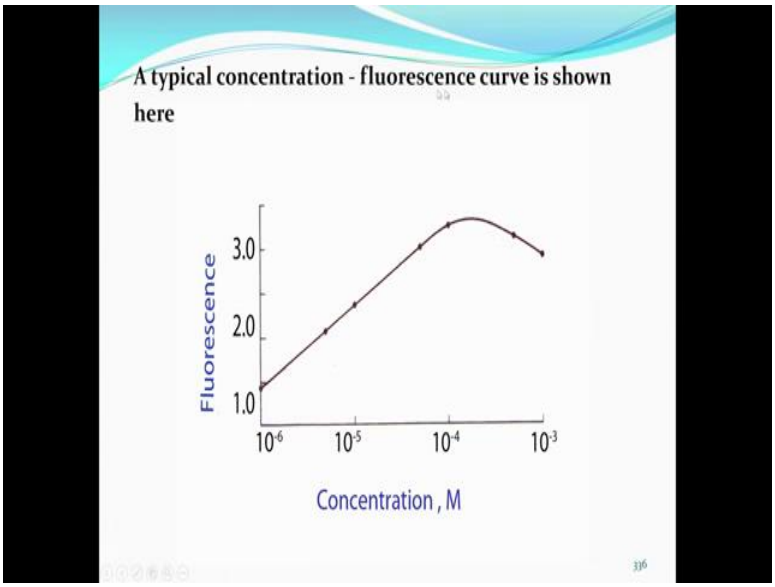
dependence upon concentration, this is a mathematical derivation of the fluorescence phenomena. So, actually what happens in the in the actual practice the detector receives a very small portion of the fluorescence emission depending upon the solid angle of the fluorescence radiation incident on it. The efficiency of the detector depends upon the fluorescence wavelength that is g lambda.

(Refer Slide Time: 31:09)



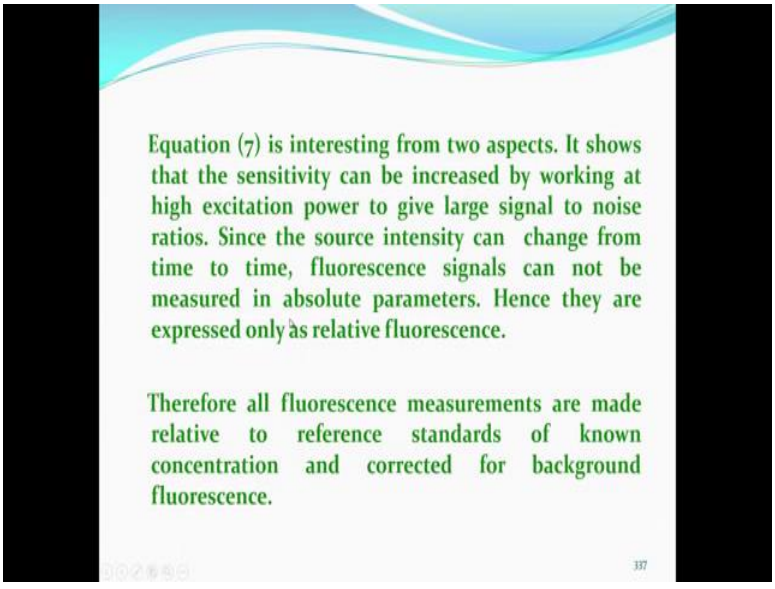
So, I can modify this equation 2.303 Q F into epsilon bc, all other terms are constant k into bc, k represents the product of all other constants, and b is the path length a plot of concentration versus fluorescence should result in a straight line. This we have already seen. And linearity in fluorescence extends from 2 to 3 orders that is 10 raise to minus 1 to 100 to 1000 concentration.

(Refer Slide Time: 31:46)



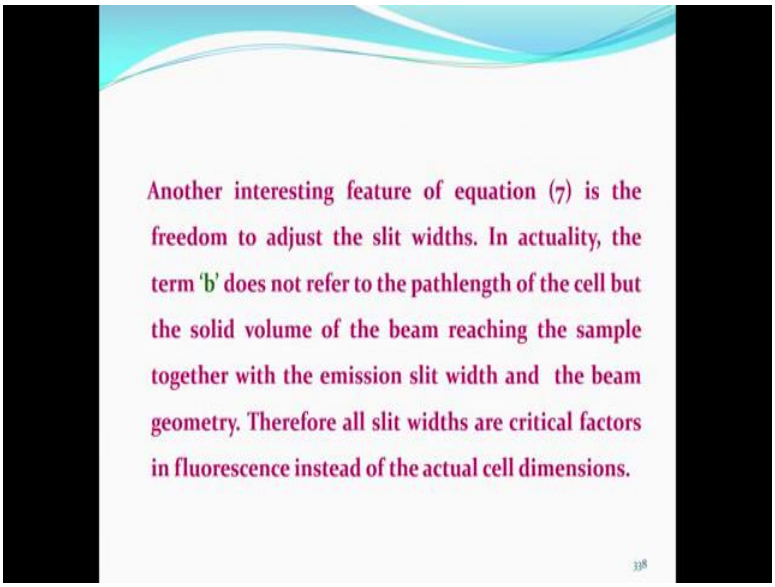
So, a typical concentration fluorescence curve is shown here. You can see that at very low concentration. There is a standard gradually increase almost linearity, but at higher concentrations 10 raise to minus 6, minus 5, minus 4, this is two orders minus 6 to minus 4. And the moment it becomes minus 3, fluorescence intensity will start decreasing. So, it is obvious that we should operate only in the region where there is only linear response.

(Refer Slide Time: 32:19)



So, this equation is interesting from two aspects, it shows that the sensitivity can be increased by working at very high excitation power to give large signal to noise ratios. Since, source intensity we can change from time to time fluorescence signals cannot be measured in absolute terms, so we need a time there. Therefore, all fluorescence measurements are made relative to reference standards of known concentration and corrected for background fluorescence.

(Refer Slide Time: 32:51)



So, we will continue our discussion about this fluorescence essentially to how we can adopt a fluorescence system into a Beer-Lamberts environment, and see how we can determine different concentration of these substances, parameters, water molecules etcetera in our next class.

Thank you.