Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture - 23
Fluorescence spectrophotometry – III

Good evening. I think we had started quantification of fluorescence in the last class.

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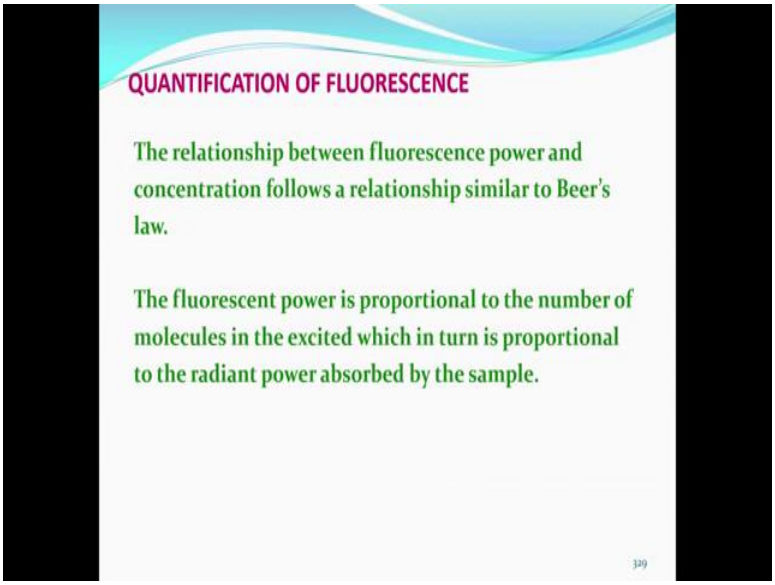

I had indicated to you that if we take a look at the relationship between flourescence power and the number of molecules which can be excited. Then we can derive a relationship similar to beer lambert law. This we had seen earlier in our last class.

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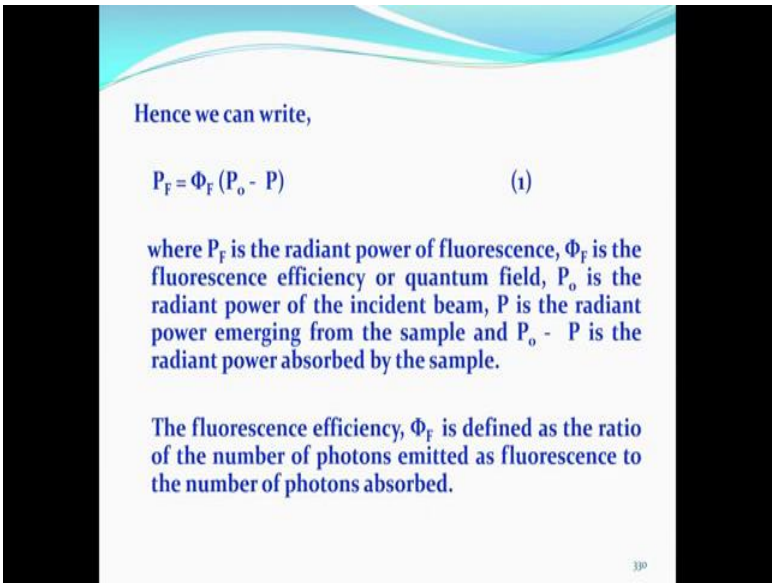

And there what we had done was, we define the radiant power of the fluorescence as a fluorescence eff function of fluorescence efficiency or quantum field, and the difference between the amount of radiation that is absorbed or that is absorbed. So, the fluorescence efficiency we described it as the ratio of the number of photons emitted as fluorescence to the number of photons absorbed.

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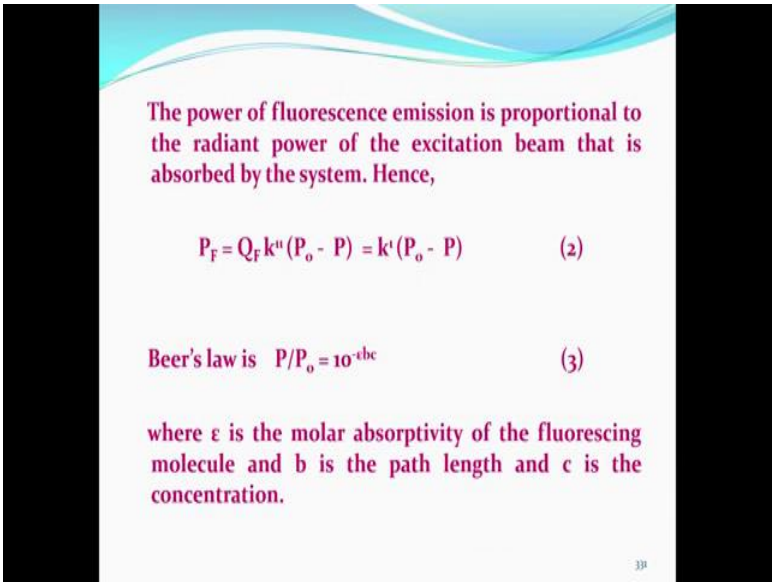

So; obviously, we can write an expression something like this that is P F is equal to Q F and then second constant k 2 and then P naught minus p. So, put all this together Q F and k 2 we can write k dash or k 1 into P naught minus p. Beers law is something similar to this P by P naught is equal to 10 to the power of minus epsilon bc, where epsilon is the molar absorptivity. Since both the quantities are similar what we derived was we substitute equation 3 in equation 2, that is P naught minus P in that equation.

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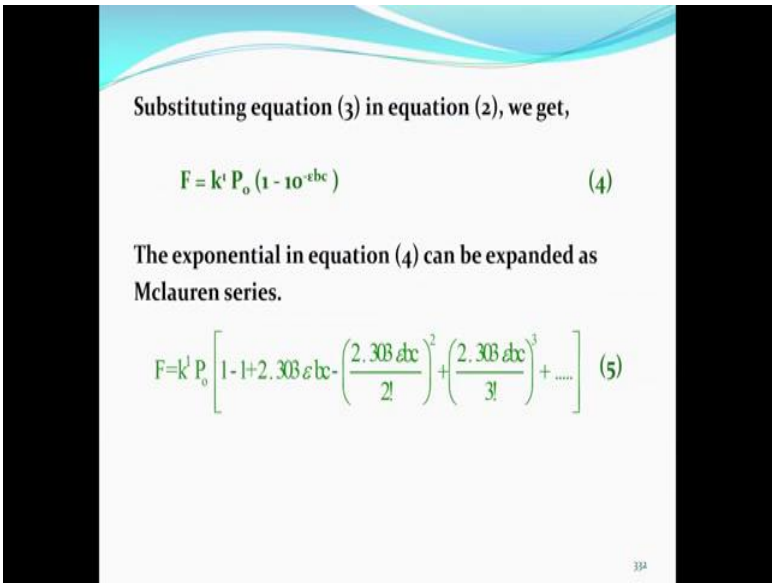

So, what we wrote is fluorescence is a function of P naught into the power of 1 minus 10 to the power of minus epsilon bc this is absorbance basically.

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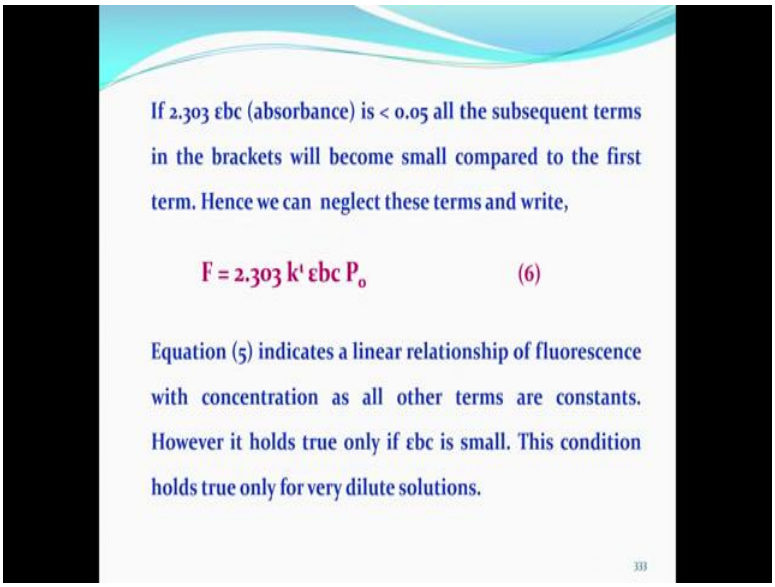

So, the exponential can be expanded in that bracket something like this. And then we have neglected the longer terms and then retain the first term that is 2.303 into the power of epsilon bc, and once epsilon bc square etcetera epsilon being the very small we neglect that and this one.

And one will cancel what remains is 2.303 epsilon bc. All square terms will go this indicates the here, again fluorescence is a function of P naught. This is a constant 2.303 is a constant k dash is constant, molar extension coefficient is constant and b is path length is constant. We can keep it P naught is the initial radiation that is constant. So, F is proportional to c that is what we can write.

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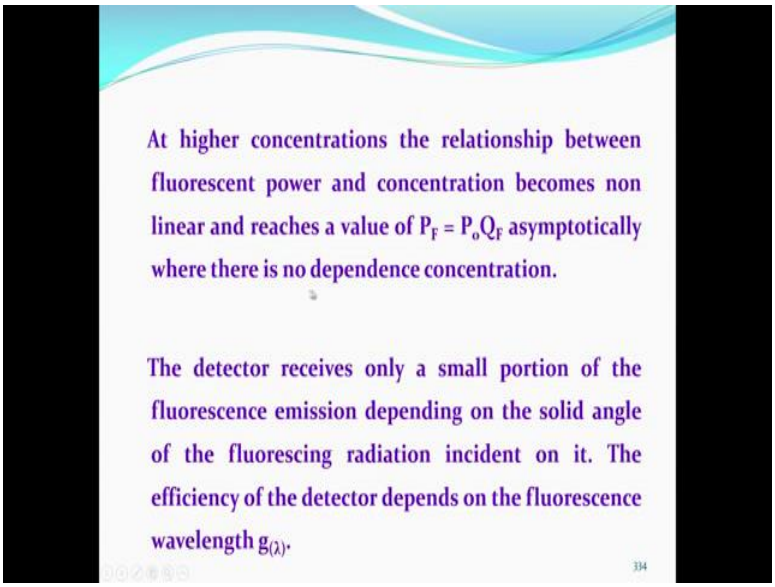

And at higher concentrations what happens. At higher concentration the equation becomes definitely non-linear and reaches a value of P naught minus P naught Q F; so that means, all these terms will can be replaced as P naught P F is equal to P naught and Q F and replaced as Q F asymptotically, where there is no dependence on concentration. See this is very important difference between absorbance and fluorescence. What we are saying is at higher concentration fluorescence is so high that it is easy to be a function of the concentration.

Therefore, essentially fluorescence is a function which is concentration dependent only at lower concentration at higher concentrations. It reaches a saturation value and once the saturation value is reached you cannot go related to concentration why this happens. So, it is a very interesting function because if you. So, if you imagine we have an incoming radiation and here is the fluorescing material in this q 8, what happens is the fluorescence fluorescing molecules will be concentrating only in this area.

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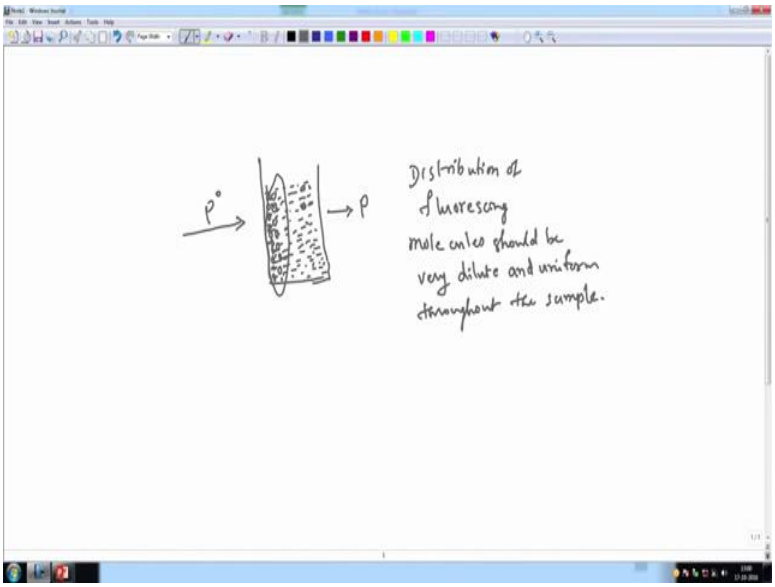

So, most of the radiation is absorbed by a layer of molecules present here. And then the molecules present in this side will be started off incoming radiation. So, what happens is maximum fluorescence is obtained only in the left region. Therefore, if you take a look after that whatever is the concentration of the fluorescing material on the outside the circle what I have drawn here, will become useless because they will not contribute to the fluorescence at all. So, it is important to have the distribution of the molecules distribution of fluorescing molecule, should be very dilute and uniform. Throughout the sample if this is not. So, then we have the problem of fluorescence concentrating only a near the at near the junction where the P naught and the q 8 molecules are located and the pre molecules located behind it are stared.

So, this is what I was trying to tell you. Then we have derive a relation something like this. And this is how it asymptotically richest as concentration becomes more F will become P naught, will be equal to; however, this holds true only if epsilon bc is small otherwise it is it holds to only for dilute solutions. At higher concentrations what happens is the relationship between florescent power and concentration becomes non-linear and reaches a value of P naught into Q F, asymptotically.

So, there is no dependence on concentration. Moreover, the detector being at a

perpendicular position to the incoming radiation, it receives only a very small portion of the fluorescence emission, depending upon the solid angle. So, we are not going to measure the total fluorescence come a when the radiation is coming from one side, we are measuring the fluorescence starting which is facing near the incoming radiation. And total fluorescence we are not measuring because you are not placing the detector on the other side of the q 8. We are placing a detector at the bottom; that means, the radiation will come like this and then since fluorescence occurs in all direction I can place the detector at the bottom. So, that only the fluorescing radiation comes through, but the absorbing radiation is automatically cut off. This is one of the fundamental principles of fluorescence that means; essentially we are measuring a small solid angle through which fluorescence is coming through not the total fluorescence.

Suppose, I keep the aperture very small, a small amount of fluorescence will reach the detector. Suppose I make the aperture slightly bigger more amount till reach the detector more amount of radiation that is fluorescing radiation. So, the fluorescing radiation is actually dependent not upon the sample per say, but it is also dependent upon the solid angle through which we are measuring. This is a great difference between fluorescence and absorbance. So, if I keep the measuring angle bigger I for the same concentration I can get a higher absorb higher fluorescence reading. So, the there is no limit, there is no lower limit through which I can say this is a fluorescence procedure is applicable to only this concentration.

And I cannot say this is the absorbance reading. This is the epsilon value for the fluorescing molecule. Why? Because I can open the detector angle solid angle collect more fluorescence, I can always say linearity changes this is a very important aspect of florescence. So, the efficiency of the detector again is the deciding factor of how much fluorescing molecule can be detected.

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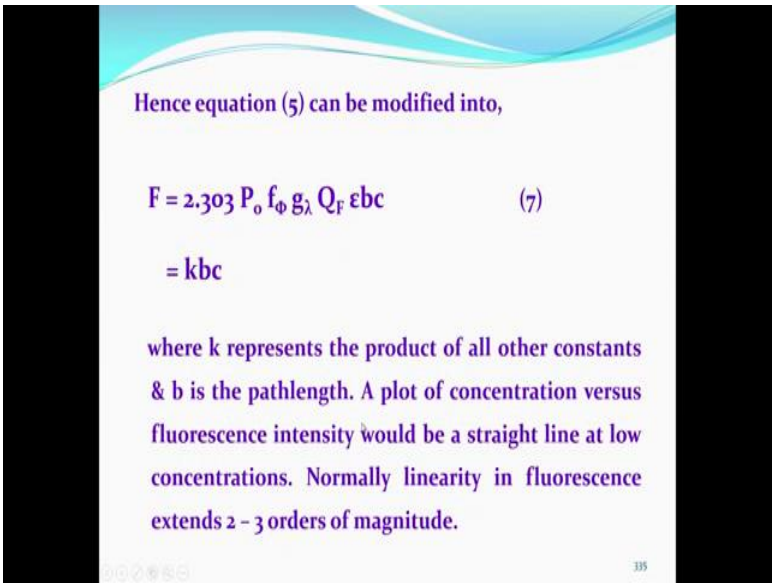

So, it depends upon the fluorescing wavelength also. And the equation 5 can be modified into this formula. That is 2.303 P naught into P naught is incident radiation F phi is the efficiency and g lambda is depends upon the wavelength weight factor, and Q F is the quantum efficiency and again epsilon bc will remain as such. So, again all these things can be, again all these things can be converted into another constant except c. So, this k represents the product of all these numbers, and b is the path length that will remain constant.

So, a plot of fluorescence consent fluorescence verses concentration should be a straight line. So, this is something similar to beer lamberts law now. So, normally linearity in fluorescence occurs 2 to 3 orders of magnitude that is because I can always have extended the solid angle through which I can collect the fluorescence.

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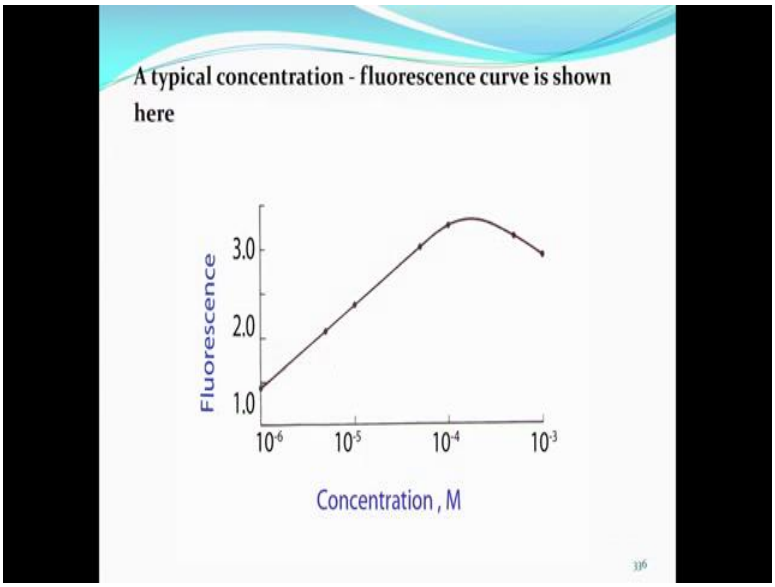

So, in absorbance in absorbance, when I make a calibration curve like this and then I get a calibration I get a linear relationship between 1 ppm 2 ppm 3 ppm 5 ppm, but of the same order. Maybe one order more 1 ppm 10 ppm then above 10 ppm beer lamberts law will not hold good. So, if it is up to linearities up to 150 ppm then I can measure in tens 10 ppm 20 ppm like that 10 50 75 like that, but in fluorescence I can measure 3 or 4 orders of differences.

For example, in the figure what I am showing you in the next slide will show you that this linearity, this extends from 10 raise to minus 6 molar and 10 raise to minus 5 molar 10 raise to minus 4 molar and maybe up to 10 raise to 2 into 10 raise to minus 4 or something like that, but definitely 10 raise not 10 raise to minus 3 molar; that means, the linearity varies from 10 raise to minus 6 to 10 raise to minus 4, that is 1000 times. This is 10 100 times this is 10 and then 10 raise to minus 4 is 100 and then sometimes if you are lucky it may go up to 10 raise to minus three, but rarely the fluorescence will be linear in the higher regions.

But still itself is a great advance for the detection of materials especially fluorescing materials. Because they cons the application of this principle would permit you to work at higher ranges and different ranges also.

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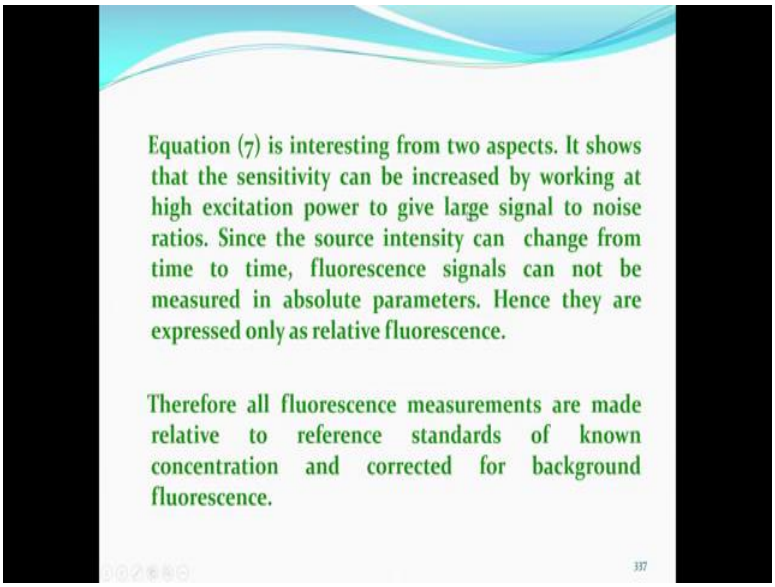

So, this equation that equation 7 know that what we have this (Refer Time: 14:39) F is equal to kbc or all these put together. That equation is interesting from 2 aspects. One is it shows that sensitivity can be increased by working at high excitation power. Again we go back to the equation. See F is proportional to P naught also, and higher the incident radiation more would be the florescence. And F phi this is the florescence efficiency. So, more high input power more is the florescence efficiency. So, that is one aspect and the second aspect is it gives a large signal to noise ratio.

Since, the square of intensity can change from time to time source intensity can change from time to time. Florescence signals cannot be measured in absolute parameters. This I have already told you taking an example of solid angle. So, in solid angle what happens is at for the same concentration, I can get a higher reading by opening the slit more slit of the detector more or lower reading by opening the by reducing the slit for the detection. Therefore, they are all fluorescence data are expressed as a relative fluorescence.

Therefore, all fluorescence measurements normally are made relative to some reference standard of known concentration, and corrected for background fluorescence and also for the fixed slit width. If you remember this, you will be mastering the fluorescence methods. So, what all you have to control, you have to control the incoming intensity that

should be as high as possible. And number 2 the solid angle can be increased much more and then the concentration also can be changed to different molar ranges.

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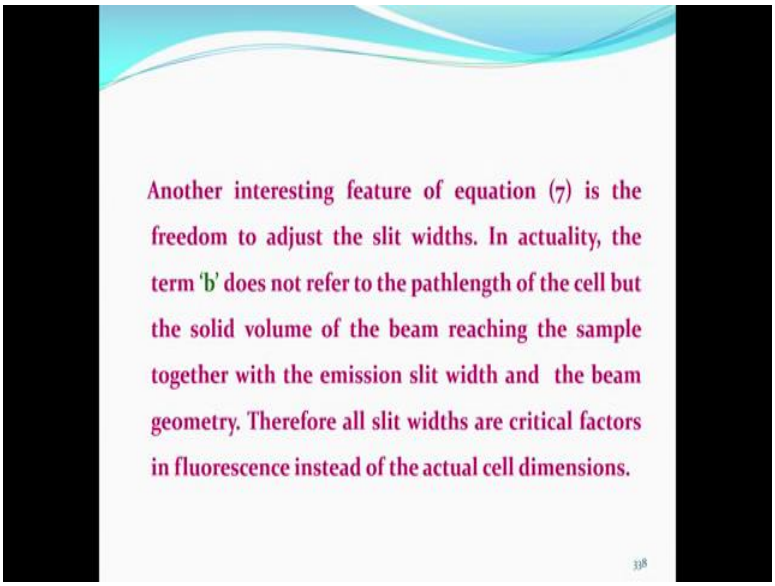

So, another interesting feature of equation 7 is the freedom to adjust the slit width. Therefore, there is no absolute fluorescence for any of these things nowadays even though now a day you can there are couple of instruments where you can where you can present the absolute fluorescence, but it is not actually absolute fluorescence at all. There will be some amount of you cannot measure 100 percent, there will be some amount of difference and in actuality the term b does not refer to the path length of the cell at all, but it is the solid volume of the beam that reaches the sample together with the emission slit width.

And the beam geometry therefore, all slit widths are critical factors in florescence instead of the actual cell dimensions. So, this is a very important aspect.

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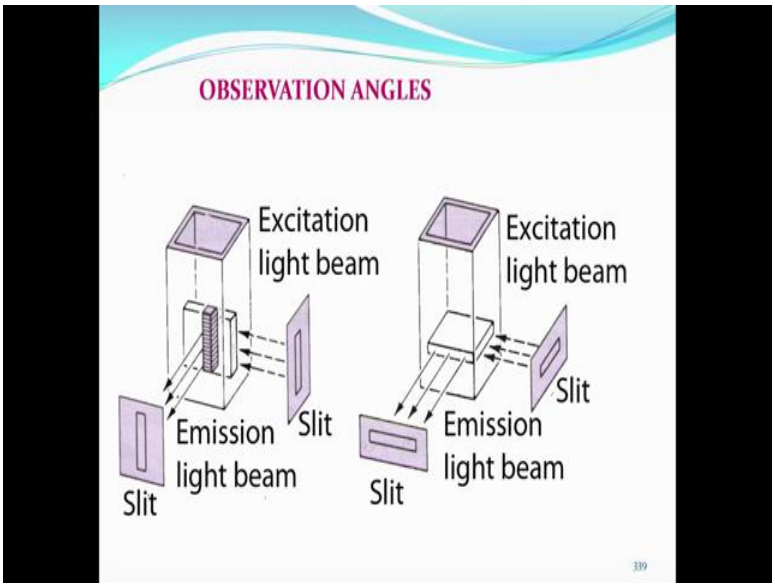

So, if we I show you the figure for fluorescence you can imagine how it is coming out like this.

Now, I have a slit here my sample is here, and another slit is here. So, my radiation is coming through this slit, into the cell this is the solid angle, I place a detector perpendicular to this I do not keep a detector across that. So, emission light is always perpendicular to the incoming radiation. So, this is known as excitation light beam this is known as emission light beam and again I need a slit width. So, this excitation light beam slit should be as large as possible.

And here the slit also should be variable. So, the same thing same geometry I can show you in different format, but the essentiality is that the collection of the fluorescence intensity is at right angles to this incoming radiation. So, these are the different observation angles. I can place a detector at the bottom also like I was telling you, but if the analyte is too concentrated the fluorescence verses concentration reaches a maximum and falls of.

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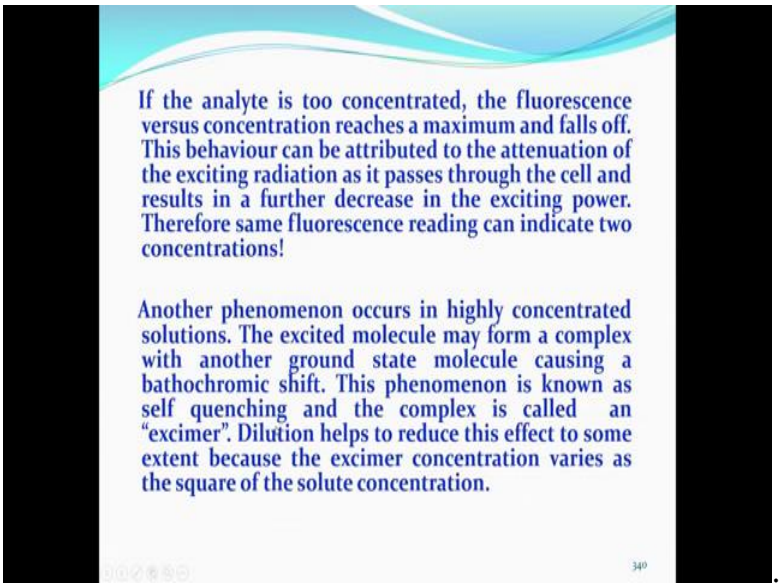

So, this I have shown you earlier. Here it reaches a maximum and false off because afterwards there is no enough radiation to reach the molecules. So, it this behavior can be attributed to the attenuation of the exciting radiation as I have already told you. And this results in a further decrease in the excitation power. Therefore, same fluorescence reading can indicate 2 different concentrations you should have understand this aspect.

Suppose I measure a fluorescence intensity of somewhere here. There is also another one another concentration which is on the straight line range. So, this we do not know what is the concentration here, when I measure an unknown I did not know whether I am in this range or in the linear range. So, for any fluorescence there could be 2 concentration ranges indicated as a solution. So, one has to be a little careful, which region we are really operating. So, other phenomena occur in highly concentrated solution that is excited molecule may form a complex with another ground molecule that causes a bathochromic shift. This phenomenon is known as self-quenching and the complex is called an excimer. So, dilution sometimes it helps to reduce this effect to some extent because the excimer concentration varies as the square of the solute concentration.

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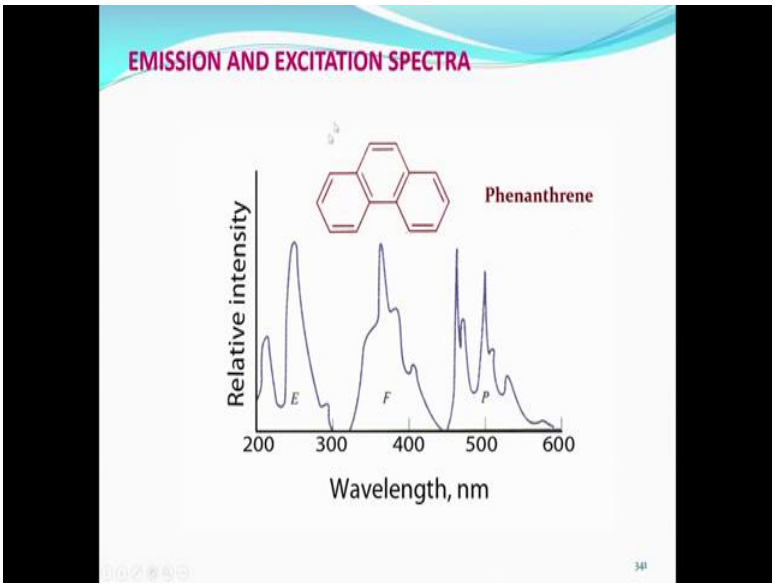

So, here I am showing you the excitation and emission spectra of the phenanthrene compound. And this is a aromatic 3 fused aromatic rings fused at ortho positions. Ortho and para positions and the excitation spectrum and plotted only relative intensity verses wavelength. Here you can see that the excitation wavelength is somewhere near 250 or something like that. There is one excitation wavelength here somewhere around 220, but 250 seems to be a slightly better, more sensitive. And the emission spectrum starts not from 300 or immediately there about, but from about 350 325 330 or something.

And the excitation emission excitation spectrum has got a maximum somewhere around 350 and then you can see that there is one more on the around 460 or something another at 500 then there are other small peaks which are this thing therefore, what is important in this case to note is both the excitation and emission are approximately similar in shape. We can see e and F and P is of course, there is a slight difference, but e and F they are approximately similar only.

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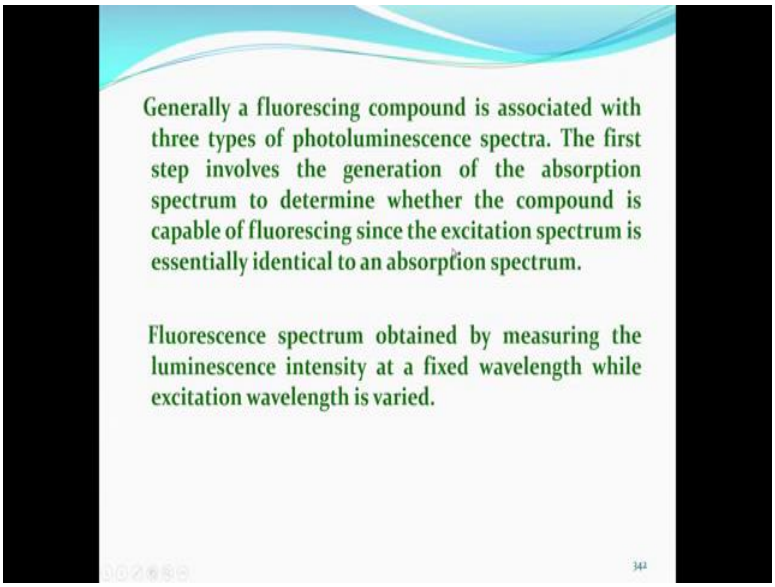

So, a fluorescing compound is associated with 3 types of photoluminescence spectra. The first involves the generation of absorption spectrum. First you should material should be having capability to absorb the radiation; that means, it must have an absorption spectrum. And then if there is an absorption spectrum the compound can be a fluorescing compound or it need not be but.

If there is no absorption spectrum the compound need not be fluorescing at all that follows. So, the second point is if the compound is capable of absorbing then excitation spectrum is essentially identical to an absorption spectrum also. So, there will be some similarity and emission also will be somewhat similar, but at a different wavelength. So, florescence spectrum obtained by measuring the luminescence intensity at a fixed wavelength. You can choose the excitation wavelength for different measurements concentrations. See for example, in the previous slide I had shown you that excite while excitation remains the same at 250, I can choose either 350 or 425 or something like that 460 and somewhere around find I can choose any of these 3 for my florescence measurement, but excitation remains the same that you cannot change.

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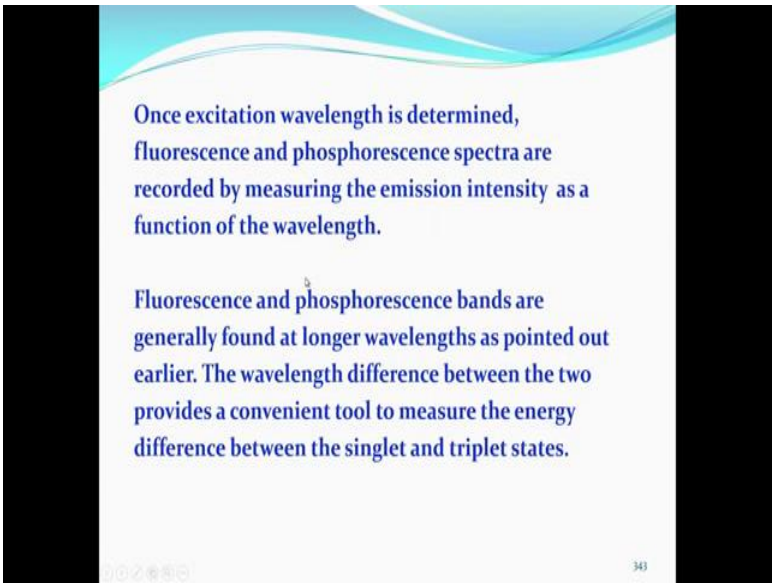

So, once excitation wavelength is fixed, then I can determine the emission florescence or phosphorescence spectra by recording the emission intensity as a function of wavelength. So, whenever you want to optimize a florescence method what you should do is you must record the florescence highest where the excitation, wavelength is fixed and using that excitation wavelength fixed excitation wavelength you should measure the emission wavelength. So, again there are different factors which can be increased, which can be inc texted to optimize a procedure.

So, normally what we say is florescence and phosphorescence bands are generally found at longer wavelengths. This I have already told you. The wavelength of difference between the 2 provides a convenient tool to measure the energy difference between the singlet and triplet states. This is also somewhat understood by now because phosphorescence would be through the triplet states.

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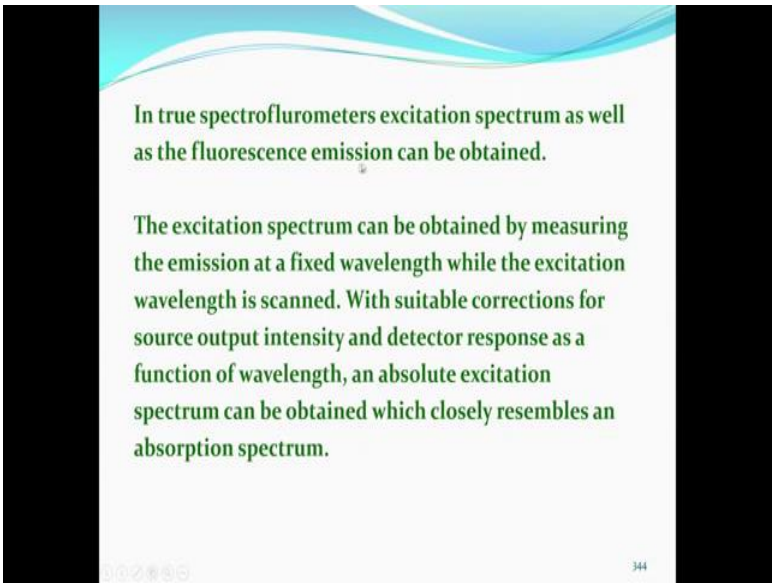

In true fluro spectroflurometrs excitation spectrum as well as florescence emission can be obtained. So, both these can be obtained in a good spectraphotometer. Spectraflurometer we call it. The excitation spectrum can be obtained by measuring the emission at some fixed wavelength it can be arbitrary, but wave whenever you get maximum emission, the corresponding excitation is the gets automatically fixed. So, that wavelength will be the excitation wavelength, and when we are scanning excitation and once the correction is made for excitation along with source output intensity and detector response as a function of wavelength, absolute excitation spectrum also can be obtained which closely resembles an absorption spectrum.

So, this I have point I have already conveyed to you. Then what we do we take the fix the excitation wavelength and then scan the emission wavelength.

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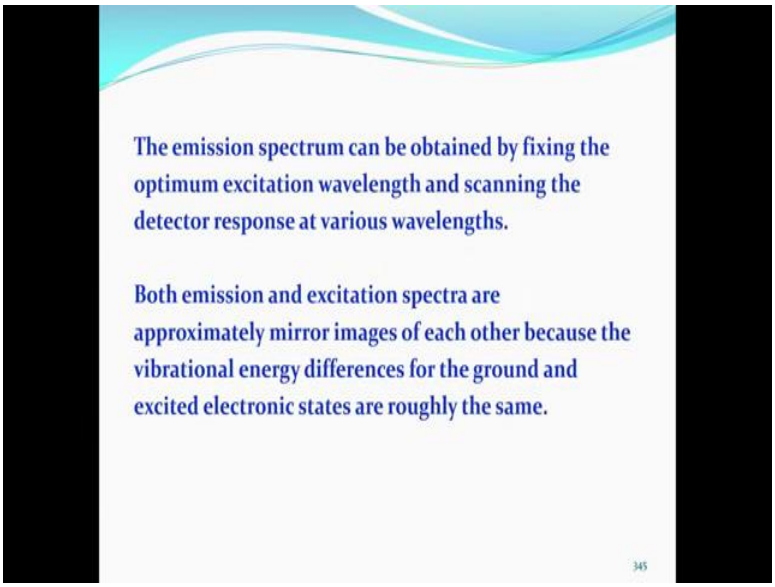

Now, that can be obtained by fixing the optimum excitation wavelength and scan the detector response at higher wavelengths not lower wavelengths. So, once both these are fixed excitation and emission spectra are approximately mirror images of each other. This point I have already conveyed to you. So, the vibrational energy difference for the ground and excited states are essentially the same. That is why the spectra also look approximately similar.

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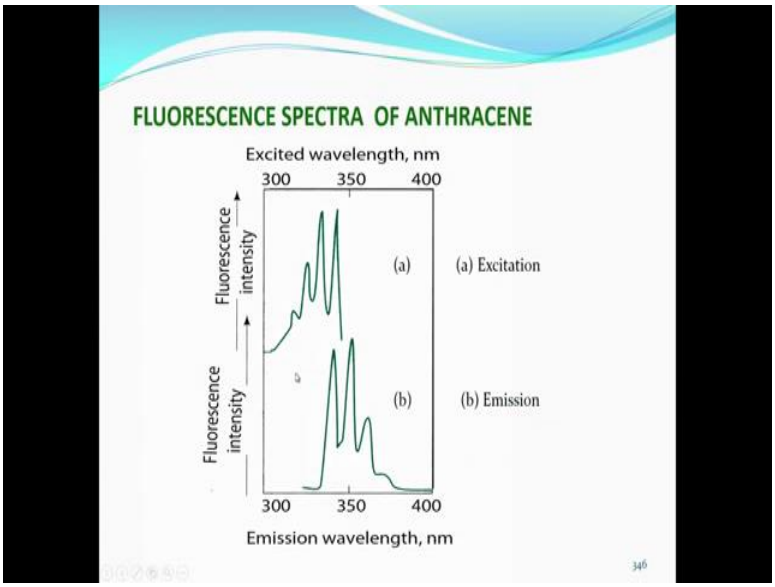

See for example, here in this figure you can see that the florescence intensity is plotted against emission wavelength. So, a is excitation wavelength see here. This figure is essentially similar to as if this figure is pushed to the right almost see these 2 peaks correspond to these 2 and these 2 have vanished here, and then there is one more appearing here. So, still you can say that aw both of them are approximately similar only. This proves the point what I was trying to teach you earlier and now having learned so much about the florescence.

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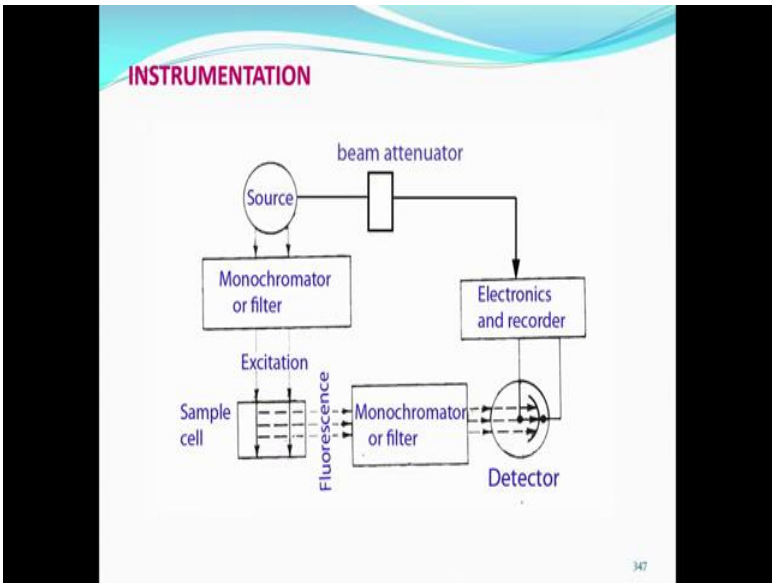

We can look at the instrumentation in a fairly quick manner. What we need basically is a source of light and then what I need is a monochromator or a filter and then this one I need to choose the excitation wavelength. So, then I have a sample cell here, and the job of this monochromator is to provide the wavelengths suitable for excitation to the sample and then I place a monochromator on at right angles to the incoming excitation beam and then I put a detector. And then I connect it to a electronic recorder etcetera. And then I can also have some sort of a beam attenuator to account for differences in the intensity of the source during measurement.

So, that is our part of the electronics, but if it is there in place then what I can do, is what I can say is the whatever changes happen in the source are automatically corrected during measurements, or throughout the experiment. So, basically the instrumentation is a very simple this thing. And then it needs 2 monochromators and sample cell and beam attenuator and computer and other things as depending upon this thing, but we also need a detector. The most of the things what we have learned earlier with respect to instrumentation are all there in this system also. So, there is no need for us to go into details of this except for some small minor changes.

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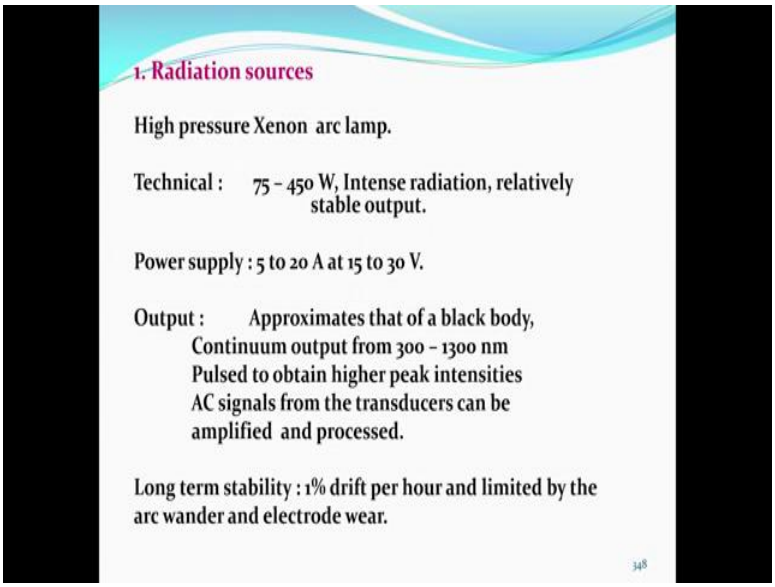

So, now let us look at the radiation sources. So, what are the radiation sources for this? We can choose a high pressure xenon arc lamp. These are nothing very special if you are going to a marriage or somewhere you would have seen that there is a guy who shoots the video picture he uses this high pressure xenon are xenon lamp. And nowadays most of the cars also whenever they are travelling on a highways etcetera are fitted with xenon lamps. And this specific technical specification includes about 75 to 450 watts is required this gives very intense radiation and gives a relatively stable output.

Higher the weightage the output will not be stable. So, the power supply should be between 5 to 20 angstrom at about 15 to 30 volts. So, this output approximates that of a black body contain and continuum output from 300 to 1300 nanometers; that means, using a xenon arc lamp you can have an excitation wavelength from 300 to 1300 nanometers and emission also. So, it is normally it is pulse to obtain higher peak intensity ac signals from the transducers can be amplified and processed. Therefore, we convert from dc to ac. So, there is approximately about one percent drift per hour and limited by the arc wander and electrode wear. Normally arc instead of arc you can use xenon lamps also.

Nowadays there are lots of xenon lamps which are which give you steady output, but

their life will be slightly lower because the temperature of the xenon radiation, xenon radiation source could become very high. Therefore, it is essential in the instrumentation for the xenon lamp to be covered with a cooling tower cooling water circulation.

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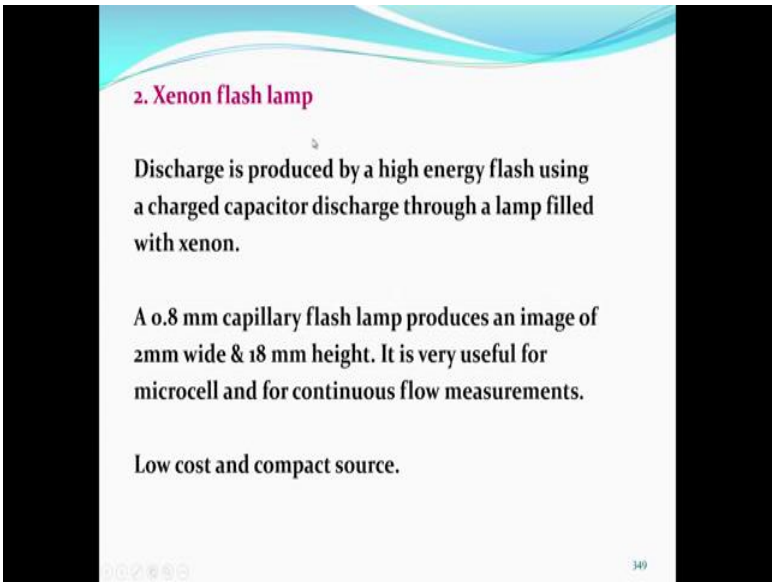

So, we can also choose a xenon flash lamp. And essentially it is a charged capacitor discharge through a lamp filled with xenon. And we can have an image about 2 mm wide and 18 mm height. It is very useful for microcell and continuous flow measurement. This advantage of this is it is the flow cost and compact source.

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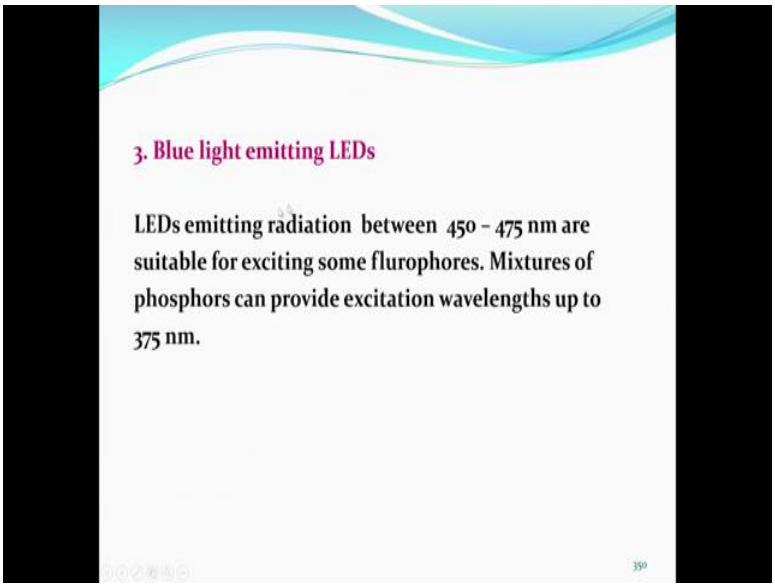

You can also have blue light emitting leds as a source. And they are not much in use as a routine instrument, but they are they are around. You can use hydrogen lamp also for a excitation, but again the hydrogen lamp could also cause decomposition of the sample.

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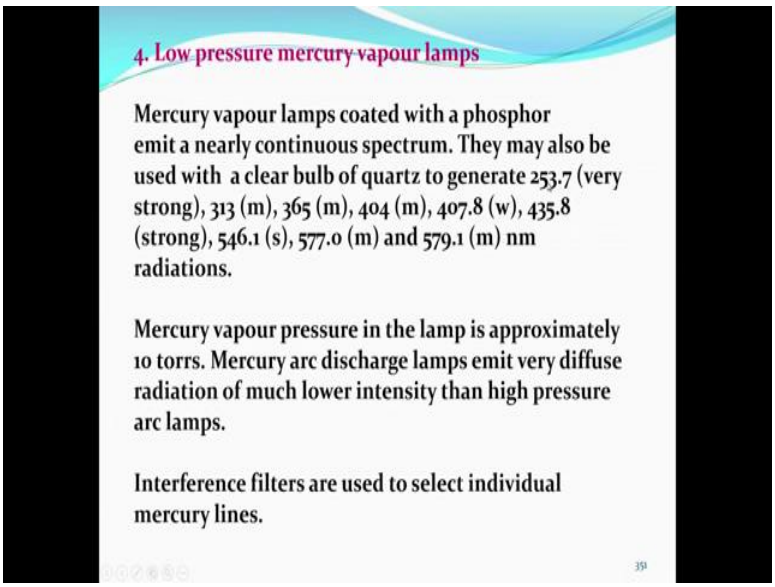

So, low pressure mercury vapor lamp is another one this is not so, good as a xenon lamp.

Mercury vapor lamp you must have seen all over they are coated with a phosphor that emit a nearly continuum spectrum, but they may also be used with a clear bulb of quarts. If they can encase in a quartz bulb you can generate a very intense light corresponding to 53.7 nanometer and 313 nanometers, and 404 nanometers 407.8. And this is a these are all I have indicated the strengths of the lines and the excitation lines. For example, 253.7 is very strong 313 is medium 365 and 404 is they are medium strength and 407 is a fairly weak, but 435.8 is a stronger radiation, it also gives you another 3 radiations that is 546 577 579.1 nanometers. So, what does this tell you?

Basically, mercury vapor lamps give a continuous spectrum, but in the continuum spectrum you will see very strong lights corresponding to these wavelengths, that is 253.7 313 365 404 407.8 435 like that you can see. The point to be noted here is that most of these lines can be separated from each other by using simple filters. So, the I can use mercury vapor lamp as a low end substitute low end spectra flory meter which filters instead of monochromators. If I use a xenon lamp I have to use a monochromator to get plus or minus 0.2 nanometer accuracy. Where as in this case in mercury vapor lamps I can go for filters. So, filters are cheaper compare to monochromator lenses monocrometer mirrors and all those things are avoided if I use a filter. So, the other details are for you to remember is mercury vapor pressure in a lamp is a approximately 10 torrs and arc discharge lamp emit very diffused radiation.

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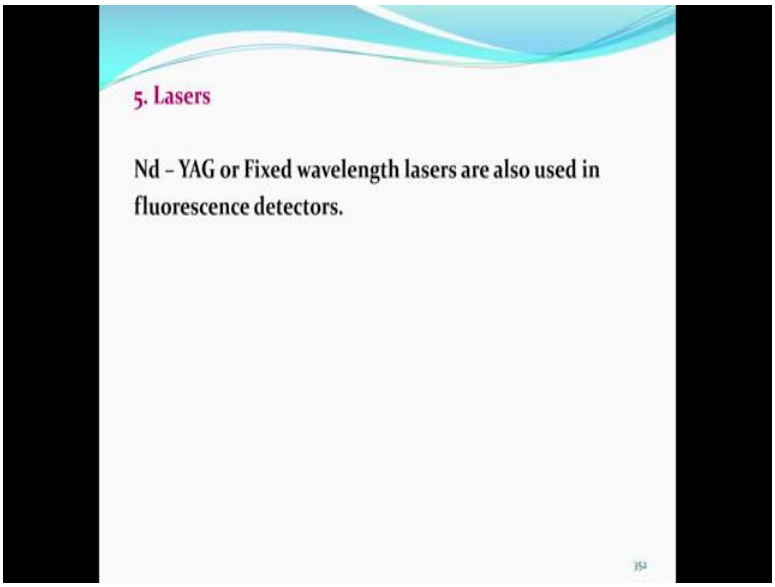

So, it is not. So, good, but high pressure arc lamps are better. And interference we need to use interference filters that are used to select individual mercury lines of 253 313 etcetera all these things. So, many filters are available if it is used mercury vapor lamp.

Now, that is another part third part is if you can use a laser. Lasers are also used in the yag or any other fixed wavelength lasers can be used if the wavelength of the excitation matches the florescence excitation wavelength, otherwise they are very difficult, but nowadays you also get tunable lasers, with tunable lasers again it should be possible for us to get very high intensity, but again compared to xenon arc the mercury filter will be less costly, but tunable lasers would be of very high cost. So, again there is a need for justification of the lamps depending upon your work.

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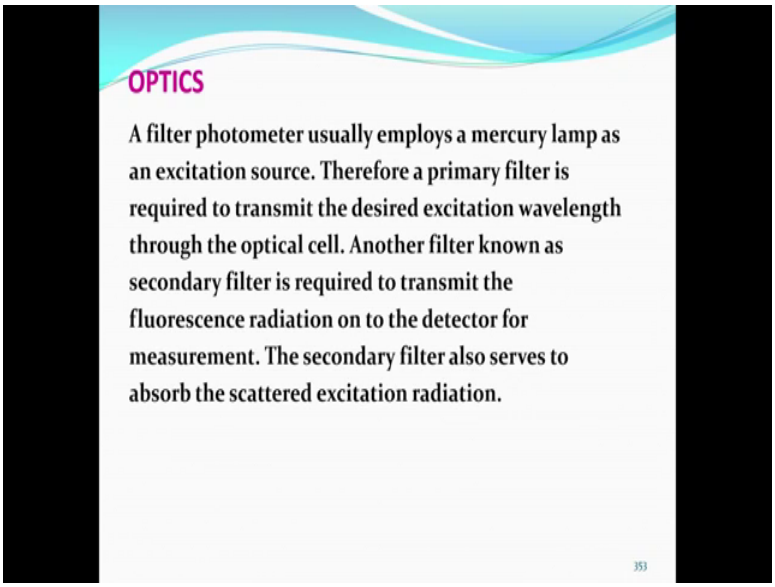

So, if we talk about optics, what we can say is a normal filter photometer usually employs a mercury lamp as an excitation source. Therefore, primary filter is required like an interference filter. And then through the optical cell another filter known as secondary filter is required to transmit the florescence radiation on to the detector. So, the secondary filter also serves through absorb these scattered excitation radiation belonging to other wavelengths. So, that permit is only the emission wavelength.

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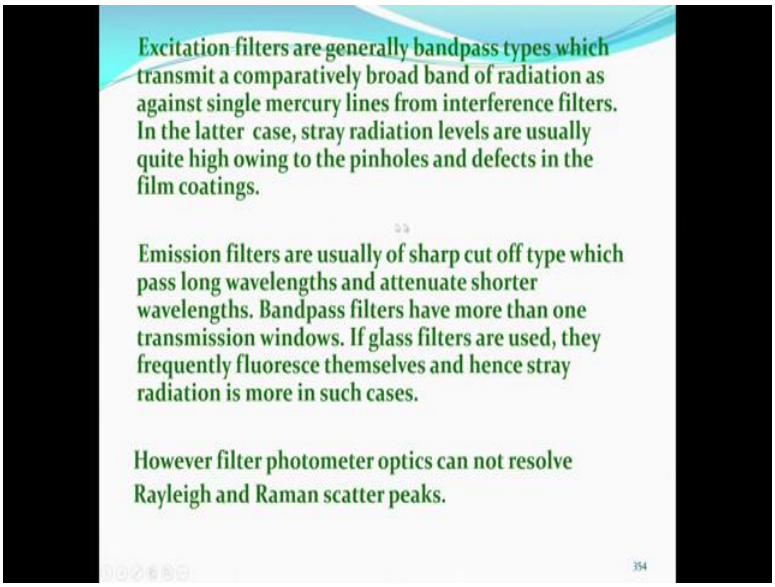

So, excitation filters are normally band pass filters. That transmits a comparatively broad band or radiation as against single mercury lines. So, emission filters are usually of cut off type. So, with which pass long wavelengths and attain a shorter wavelength. So, band pass filters have more than one transmission windows. This if you look at the discussion what we had during optics you will get all this once again that is why I am not going in to details of this. So if glass filters are used they frequently fluoresce themselves that itself can be a little bit of (Refer Time: 39:23) and stray radiation is more whenever you use glass filters. So, filter photometer optics cannot resolve rally and Raman's scatter optics that is understood.

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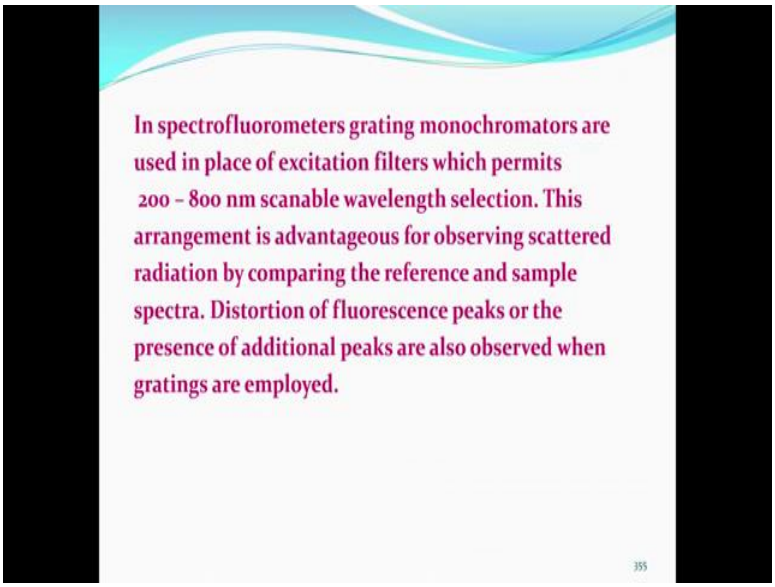

So, if you want to resolve rally and Raman's scatter then you need to go for xenon arc or in the yag laser. So, grating monochromator they are used in place of excitation filters that permit is 200 800 nanometer. That is where you get maximum florescence for most of the organic compounds, and then some time distortion also occurs whenever gratings are employed these things we will have to be very careful whenever we are using the this thing.

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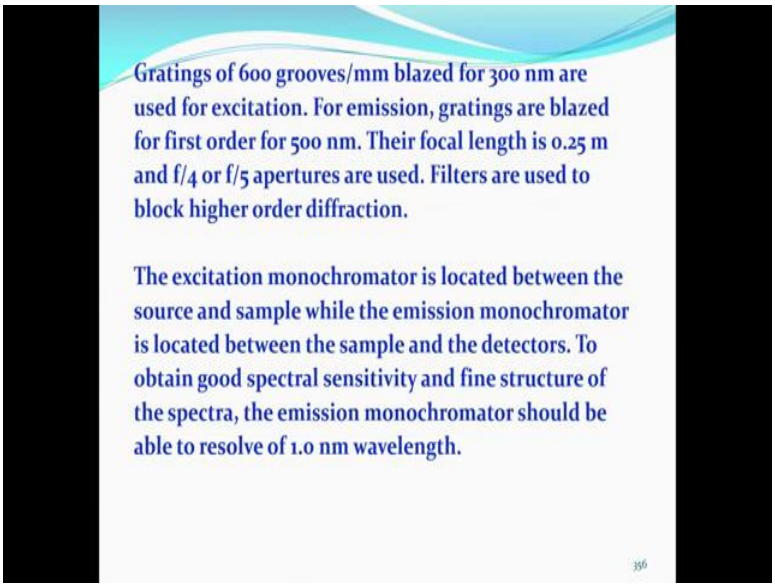

So, gratings normally of 600 grooves per mm blazed for 300 nanometer are used for excitation. Focal length etcetera are all matter of details that corresponds to 0.254 meter and F by 4 and F by 5 apertures are used. Filters are used to block higher radiation and excitation and emission should be able to resolve normally of about 1.0 nanometer wavelength that is the ultimate.

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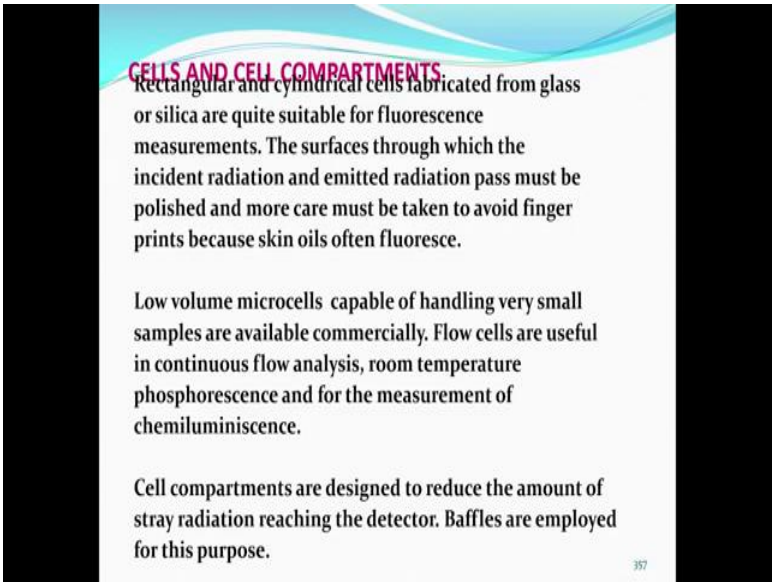

So, we will talk about the cells and compartments. We will discuss about this after a small gap. So, thank you very much. We will continue our discussion on the instrumentation of florescence shortly.