Modern Instrumental Methods of Analysis

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Module No. # 02

Lecture No. # 07

Ultraviolet and Visible Spectrophotometry – 3 Theoretical Aspects

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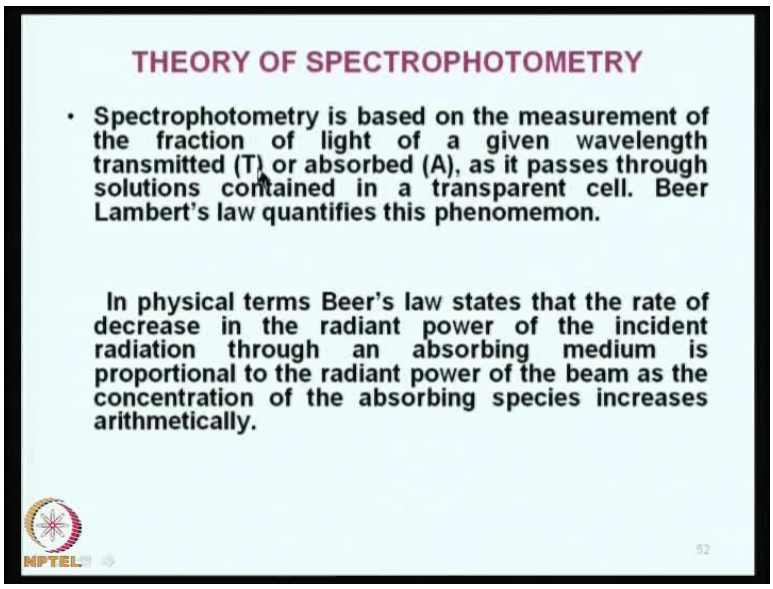

Welcome to the seventh lecture on the theory of spectrophotometry. We are still continuing our discussion on the spectrophotometry. And today, we will examine the quantitative aspects of spectrophotometry.

In the last class, I had shown you the slide, that is, spectrophotometry is based on the measurement of the fraction of light of a given wavelength transmitted or absorbed, as it passes through solutions contained in a transparent cell Beer Lambert's law quantifies this phenomenon.

So, what is Beer Lambert's law? In physical terms, we can say that Beer Lambert's law states that the rate of decrease in the radiant power of the incident radiation as it passes through an absorbing medium is proportional to the radiant power of the beam as the

concentration of the absorbing species increases arithmetically, that means, if you take a substance and increase its concentration, the radiant power would become less as it passes through the solutions which are more concentrated.

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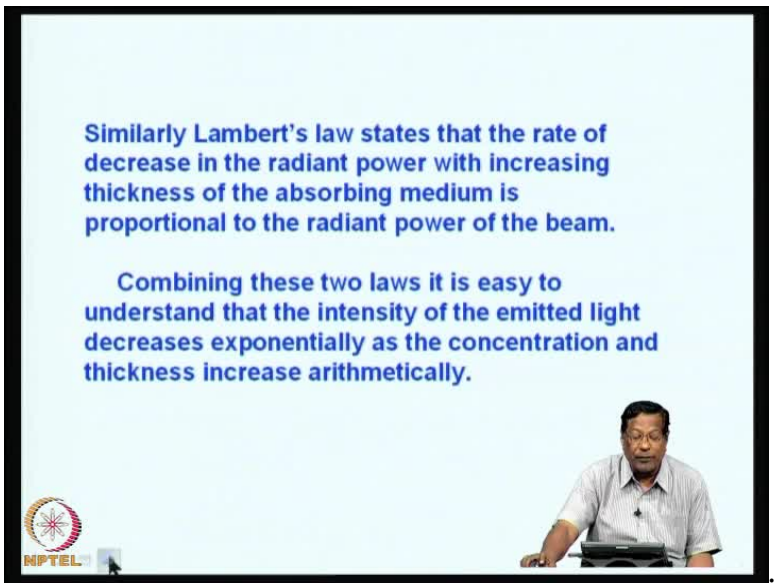

Then similarly, Lambert's law relates see in the same way to the thickness of the absorbing medium, that is, if you take the absorbing medium in 1 centimeter cell and 2 centimeter cells, the absorption should be naturally much more the in the 2 centimeter cell than in 1 centimeter cell, because the path is doubled.

So, how it is correlated? It is states that the rate of decrease in the radiant power with increasing thickness of the absorbing medium is proportional to the radiant power of the beam.

So, we have two laws which are very similar to each other- one relates to the decrease in the radiant power with increasing concentration; another is related to the decrease in the radiant power same phenomenon, but with increasing path length.

Now, we can combine these two laws and if you combining these two laws, it is easy to understand that the intensity of the emitted light decreases exponentially as the concentration and thickness increase arithmetically.

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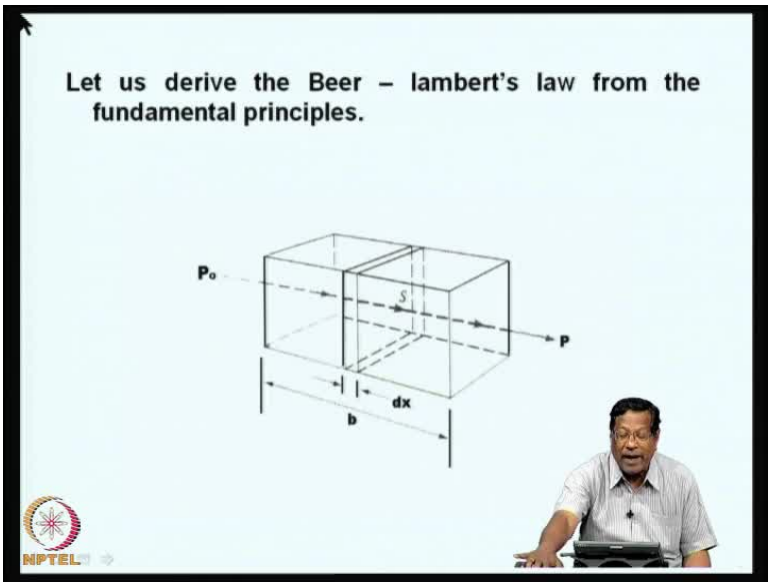

So, it is very simple; let us try to derive this Beer Lambert's law from the fundamental principle. Here what I want you to do is, here I want you to take a think of a box which contains an absorbing solution, incident radiation is P 0 as it passes through the solution, part of it is absorbed depending upon the concentration of the absorbing species as well as the thickness of the transparent absorbing medium and what comes out is P that is radiant power.

So, we have two parameters: one is incident power that is P naught and another is P and the length is given by b. Now, I am going to consider a small portion of this absorbing species of thickness dx and concentration S.

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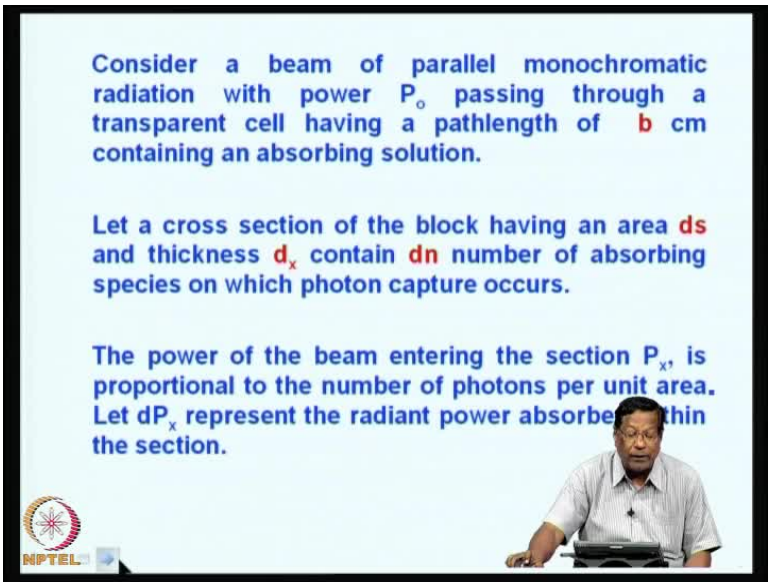

So, if we take a look at it, here you have to consider a beam of parallel monochromatic radiation with power have power P naught passing through a transparent cell having a path length of b containing an absorbing medium.

Let a cross section of the block having an area ds and thickness d x contain dn number of absorbing species on which photon capture occurs, that is, the beam P naught is going to pass through dn number of molecules in a section containing thickness d x. The power of the beam entering the section P x must be proportional to the number of photons per unit area. Let dP x represent the radiant power absorbed within the section.

Now, what we are considering d x is there, dn is there, ds is there and then, we have dP x is the radiant power that is absorbed.

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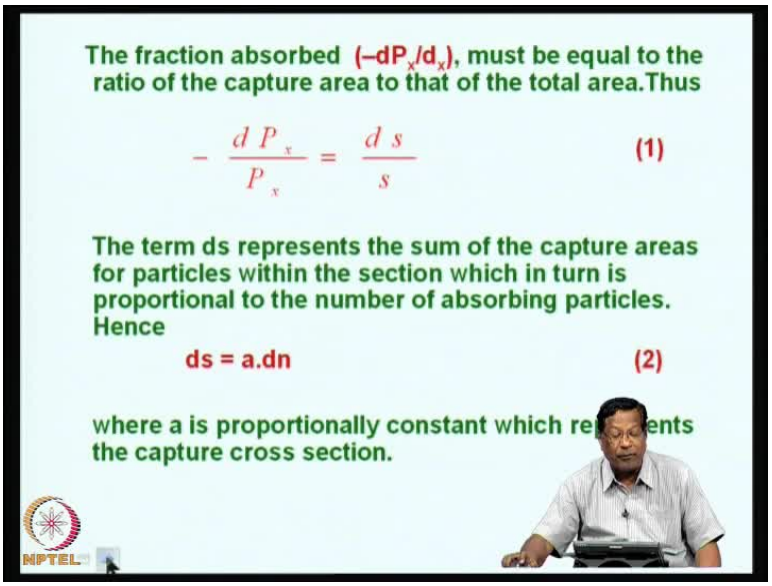

Now, the fraction absorbed is minus dP x by d x, this is negative sign comes because the concentration is the fraction, that is, radiant power is decreasing; so it must be equal to the ratio of the capture area so that to that of the total area. Therefore, we can write minus d P x by P x is equal to d s by s. The term ds represents the sum of the capture areas for particles within the section which is turn is proportional to the number of absorbing species or particles.

So, we can write ds is the function of absorbing particles and then, number of absorbing particles and the area, the both of them can be combined to write an equation like this, where a is proportionally constant which represents the capture cross section that is area.

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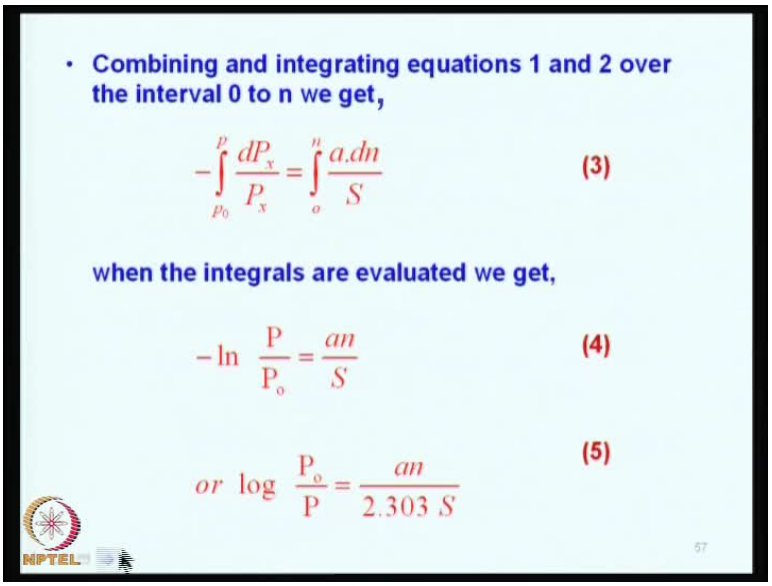

Now, you can integrate this and over a range of P naught to P, the power decrease is from P naught to P so you are integrating in this area and then, the numbers of molecules are also in this area 0 to n.

So, combining and integrating equations 1 and 2 that is the see previous equations over the interval 0 to, n we get integral dP x by P x is equal to 0 to n integral a of a into dn divided by S. So, when these integrals are evaluated, it is a very simple mathematical operation as college mathematics. So, the equation works at to minus ln P by P naught is equal to an divided by S, where the all these notations you know already.

So, log of P naught by P, that is, we are going to convert to logarithms from natural logarithms to the base tile; so we all other things will remain same and this 2.303 will be incorporated in the equation.

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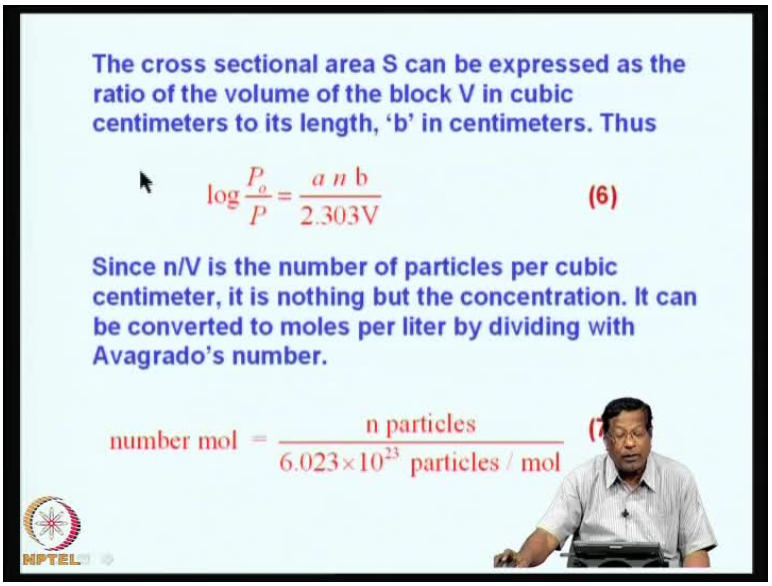

Now, what is the equation? The cross sectional area S can be expressed as the ratio of the volume of the block V in cubic centimeters to its length and length would be in centimeter. Thus log of P naught by P should be equal to a into n number of molecules into b, that is, path length divided by 2.303 into V, that is, we are incorporating a concept of volume.

Now, n by V is the number of particles per cubic centimeter which is nothing but the concentration. So, what happens, it can be converted to moles per liter by dividing with Avagrado's number very simple operation; number moles is equal to number of particles divided by 6.023 into 10 raise 23 particles per mol.

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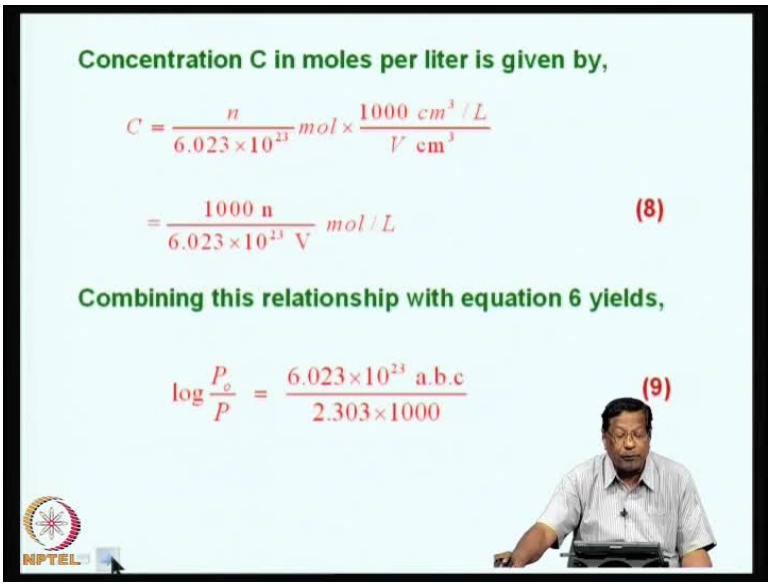

So, now, look at this equation, the concentration C in moles per liter is given by this figure n by 6.023 into 10 raise 23 moles and 1000 centimeter cube per liter divided by total volume of the sample.

So, that gives you 1000 n divided by 6.023, all other things will remain the same and this will be n by V, but the units are going to change now to moles per liter. So, combining this equation with the previous sixth equation what we you get log of P naught by P is equal to 6.023 into 10 raise 23 into a into b into c divided by 2.303 into 1000, these are all basically in substitutions happening in the original integral.

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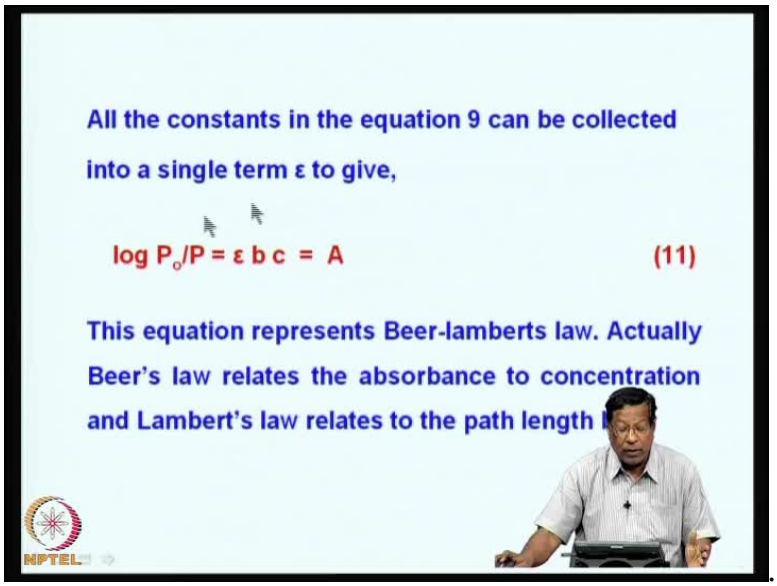

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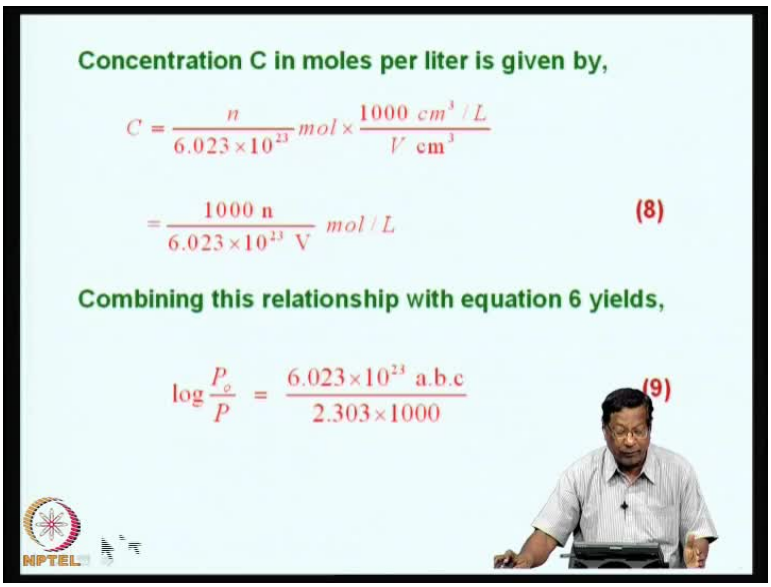

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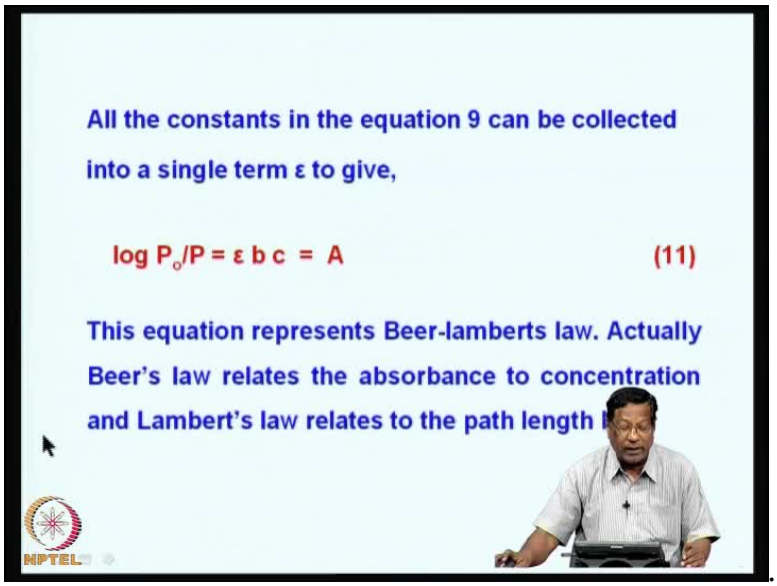

Now, all the constants in the previous equation, that is, 6.023 into 10 raise 23 is one constant, 2.303 is another constant, 1000 is another constant and b is a constant and all these things you can convert it into a simple number, that is, log of P naught by P is equal to epsilon b into c that is equal to A that is absorbance.

Now, we are going to designate A as absorbance, instead of log of P naught by P, because it is going to be slightly cumbersome, if you keep on writing log of P naught by P.

So, our basic equation works out to A is equal to epsilon b c; this equation represents Beer Lambert's law as it is known popularly. Actually Beer Lambert's law state relates the absorbance to concentration Beer's law that is, and Lambert's law relates to the path length.

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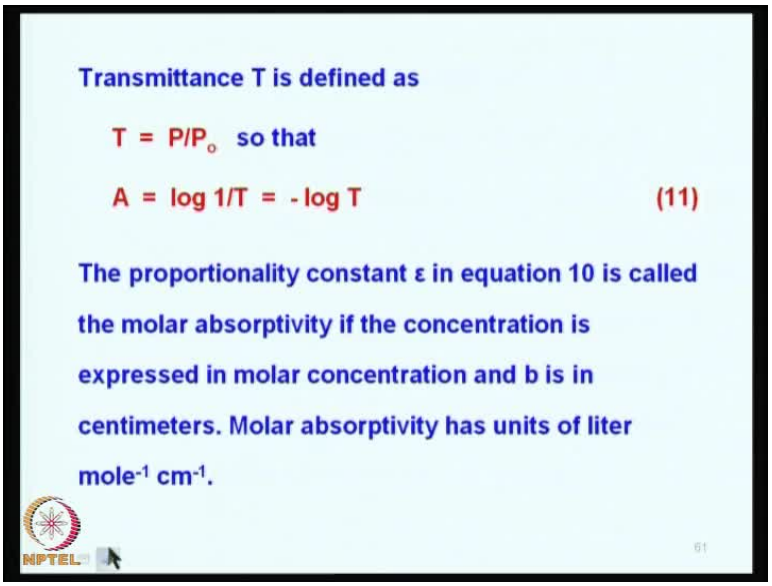

So, if we measure the transmittance, then it should be T is equal to P by P naught that is the amount of radiation that is transmitted so that absorbance should be A, an inverse function of log of 1 by T that is negative log of transmittance.

The proportionality constant in this equation, that is, epsilon in equation ten is called the molar absorptivity if the concentration is expressed in molar concentration and b is in centimeters. Molar absorptivity has units of liters moles inverse and centimeters inverse.

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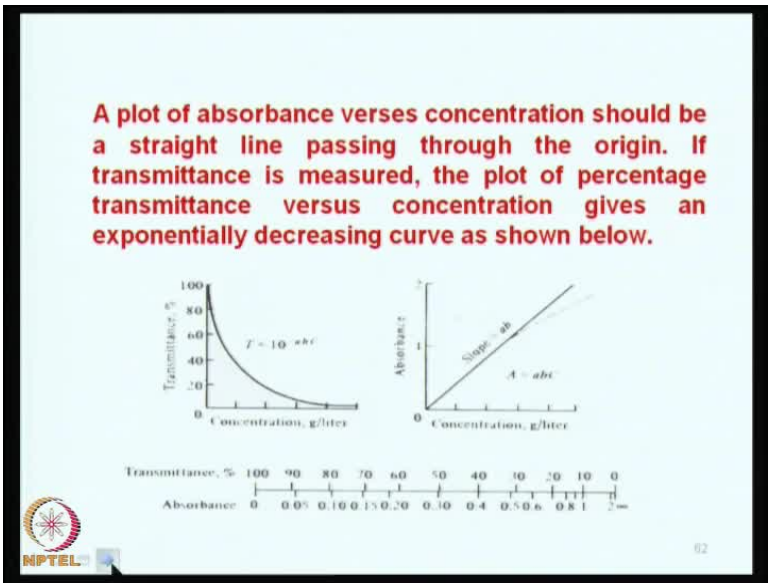

So, this entity we are going to discuss quiet a law further. And if you plot absorbance verses concentration, what type of figure you should be getting? We should be getting a linear curve, that is, absorbance versus concentration in grams per liter and it will be a straight line curve with a slope of a into b, because a is a constant of proportionality; b is a constant. So, A is equal to a b c, that is, the concentration is linearly related to the absorbance so long as a path line it remains the same.

Now, if you plot transmittance versus concentration, you should end up in a figure looking like this; the same figure instead of absorbance, I am going to plot transmittance. Now, in this transmittance you can see that it is plotted as a percentage, that is, 0 to 100 percent; if the substance is very less concentrated or if there is no absorbing species, all the radiation, that is, P naught will be transferred completely transmitted completely to the detected; so you should get 100 percent transmittance. As the concentration increases, transmittance keeps on decreasing and as the concentration increases to a critical value, it is going to reach 0 asymptotically.

Now, you can plot these two in the spectrophotometric instruments, you will see the scale analog scale of both transmittance and absorbance that is 100, 90, etcetera 0 and absorbance would be 0 for 100 percent transmittance and infinity for the 0 percent transmittance. So, this is on the log scale; so you would see that on the log scale, absorbance varies from 0, 2, 1 etcetera, but here the top scale is only arithmetic function.

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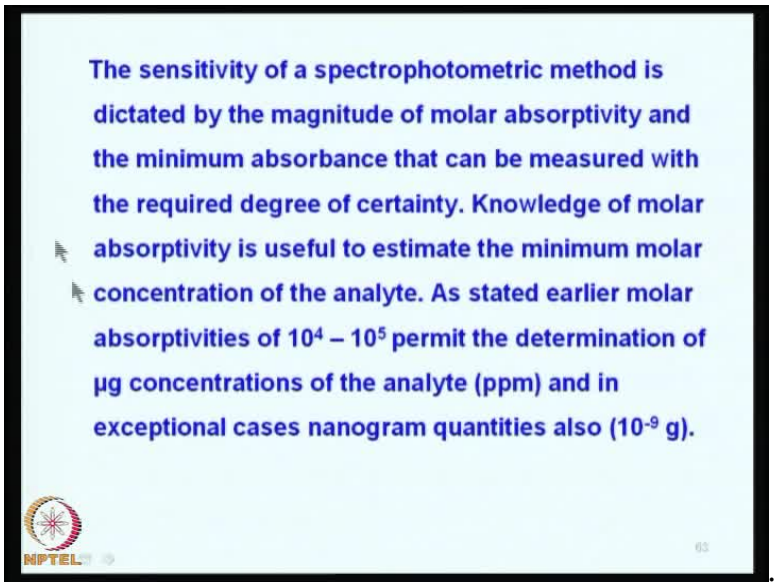

So, the sensitivity of a spectrophotometric method is dictated by the magnitude. So, the sensitivity of a spectrophotometric method is dictated by the magnitude of the molar absorptivity that is epsilon and the minimum absorbance that can be measured with the required degree of uncertainty.

So, as stated earlier, molar absorptivities are of the order of a 10 raise 4 to 10 raise 5, these things permit the determination of microgram concentrations of the analyte in ppm level, that is, in ppm and in exceptional cases nanogram quantities also.

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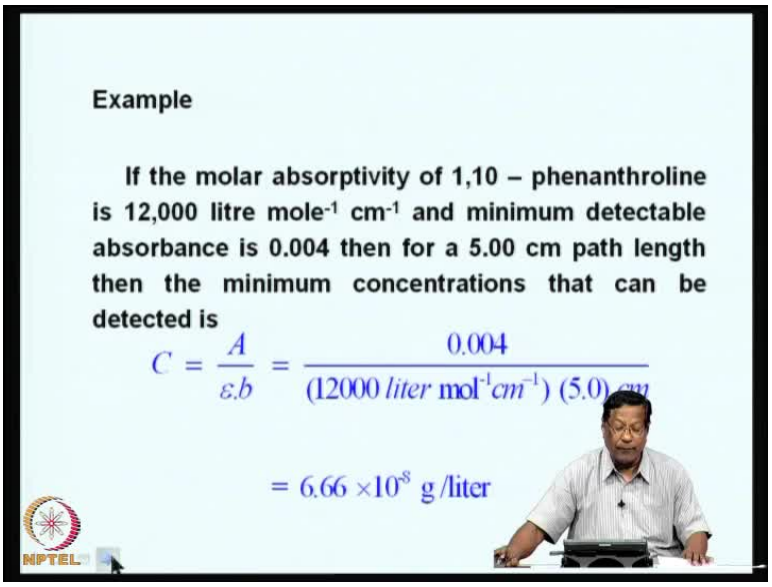

Now, let us take an example here that is the molar absorptivity of 1, 10 phenanthroline is a 12000 liter mole inverse centimeter inverse and minimum detectable absorbance is 0.004 that is the instrument limit.

Now, if a this is a we take a 5 centimeter path length that is the sample we take in a 5 centimeter cell, then the minimum concentrations that can be detected is A is equal to epsilon b c. So, C is a divide by epsilon b and 004 these are numbers, when we put them, we can determine 6.66 into 10 raise minus 8 grams per liter that is less than micro grams.

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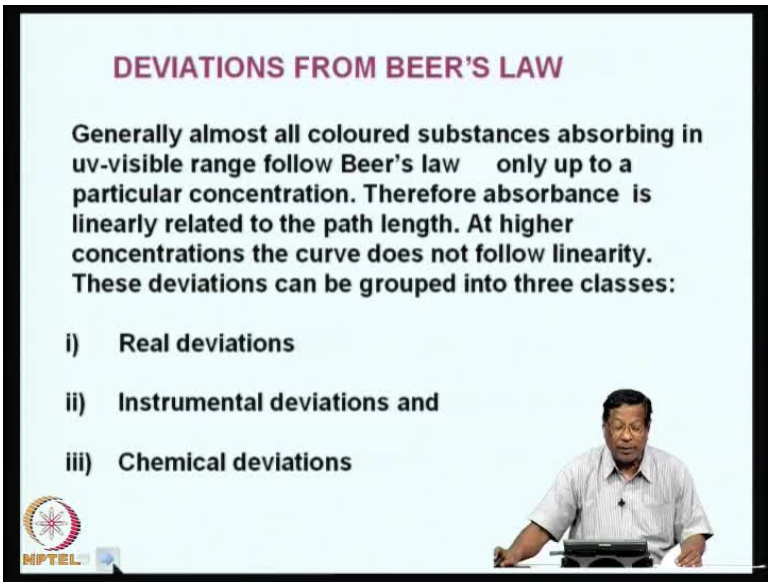

So, this is the power of atomic molecular absorption spectrophotometric. Generally, you remember that we have discussed that A is equal to epsilon b c should result in a linear curve and it is a not always that you will get linear curve; deviations from Beer's law are more rule than exceptions. So, generally almost all colored substance absorbing in uv-visible range follow Beer's Lamberts law or Beer's law only up to a particular concentration, that is, above the particular concentration, it will no more be linear with respective absorbance.

Therefore absorbance is linearly related to the path length only upto a particular concentration. At higher concentrations what happen the curve deeps towards the or x axis or y axis depending upon some of the experimentally conditions and the curve does not follow linearity.

So, these deviations we can group into three classes: one is real deviations, another is instrumental deviations; instrumental some can also cause some problems to make the response non-linear and then, there is another possibility, that is, chemical deviations. Sometimes different chemical reaction may take place producing different colors and other aspects of the chemical deviations. So, we will see later that is right after some time.

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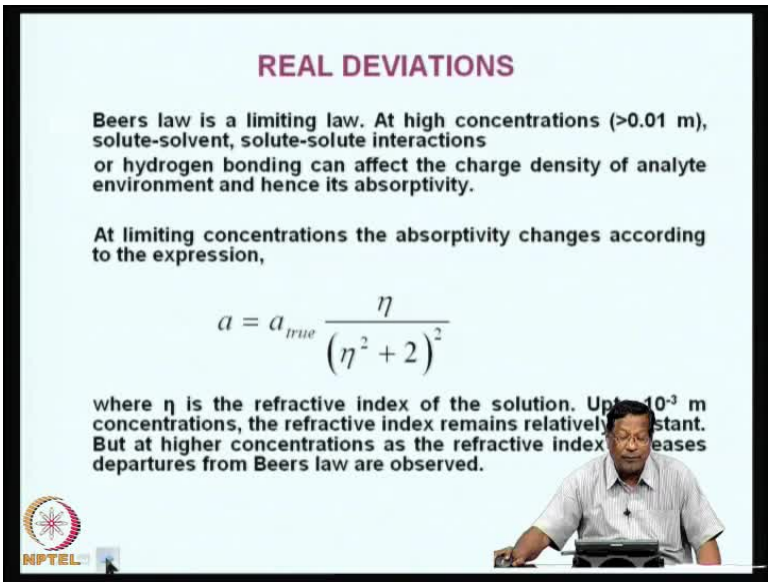

Now, what is a real deviation? Real deviations is basically what we want to tell you is Beer's Lamberts is a limiting law, that means, only at very low concentrations Beer's Lamberts law should theatrically hold good. At high concentrations, solute-solvent, solute-solute interactions or hydrogen bonding, etcetera can affect the charge density of analytic environment and hence its absorptivity.

So, at limiting concentrations, the absorptivity changes according to the expression is as shown here, that is a is equal to that is absorbance and absorbance is true absorbance multiplied by n by n square plus 2 whole square, there where n is the refractive index of the solution, that means, if taken concentration increases, n is also going to increase and we have to replace a is equal to epsilon b c in that, we have to replace the epsilon value corresponding to change in the refractive index. So, up to 10 raise minus 3 molar concentrations, the refractive index remains relatively constant. But at higher concentrations as the refractive index refractive index increases, obviously departures from the Beer's Lamberts law are observed. So, this is one reason.

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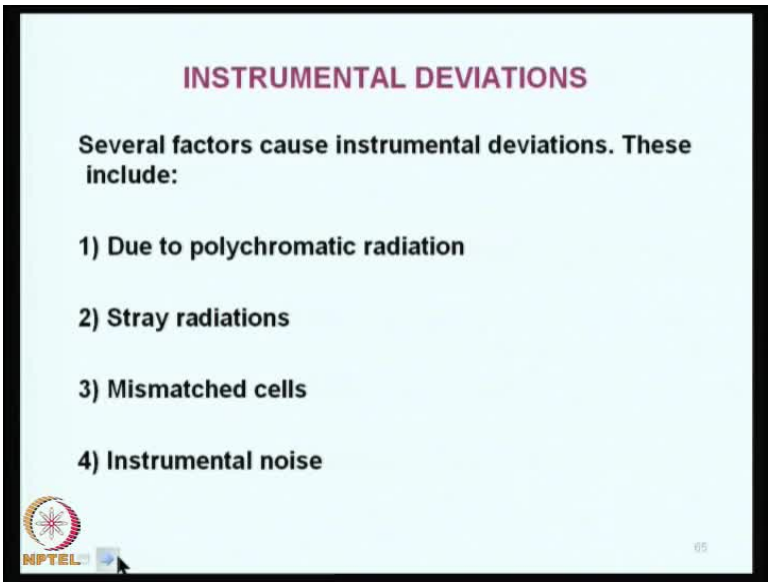

Another reason is instrumental deviations what happens, instrumental deviations include polychromatic radiation. Remember that we have been discussing the instrumental parameter, what Beer's Lamberts law says, if a b form of monochromatic radiation is pass through the substance, then in the decrease intensity is propositional to the concentration as well as path length.

Now, it is rarely that you will get a monochromatic radiation in any instrument. So, what you will end up is due to polychromatic radiation and these polychromatic radiations are nothing but the desired wave length plus some other unwanted wave lengths, what you are getting out of the instrument. So, this polychromatic radiation is not conducive for good Beer's Lamberts law, we should try to as near the monochromatic radiation that is required for the analysis.

Another think is stray radiation; stray radiation means white light, wherever you put the instrument in the lab or outside or in the field or somewhere else, whatever you will do instrument cannot act as a black body, that means, always there will be some amount of radiation coming from the sources which are not identify as coming from that of the intensity source.

So, the source all the radiations that come out from the other sources are known as radiations. So, to for good Beer's Lamberts law to follow, you need to have minimum stray radiation and the stray radiation approximate from 1 to 5 percent in a various

instruments and third one is mismatched cells, that means, you must follow measurements using two cells: one cell is the sample, another cell is the standard.

And when you make the measurements, the cells should be having exactly same optical properties; if they do not have optical same optical properties, the absorbance value going to change. So, it is always preferable to use matched cells and 100 percentages matching of the cells is not at all possible.

So, you should remember that; therefore, mismatched cells always constant source of instrumental deviations. Another thing is instrumental noise; this instrumental noise arises from various factors such as the source noise, electronics and line voltage, so many other things short noise and several other aspects which are going to increase the distortion in the absorption measurement. Because in the end, everything else is converted into electric signal and this electrical signal is subject to line voltage, frequency changes this and other things, etcetera.

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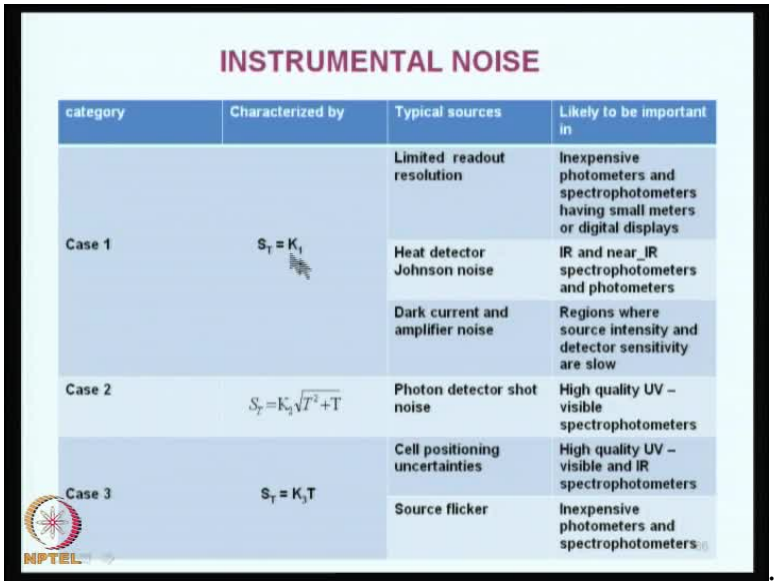

So, these are two instrumental and we can see I have listed here three cases, where the category one is nothing but S T is equal to K 1 that is limited readout resolution. If you do not have a very good scale to read, then you are going to make an error in the readout. And for example, if it is an analog meter, you will never know exactly where you are, inexpensive photometers and spectrophotometers having small meters or digital displays show these kinds of problem. And head the heat detector Johnson noise there in they are

in instrument infrared and near infrared. Sometimes dark current and amplifier noise also comes in to this and this is the going to be important in regions, where source intensity and detector intensity sensitivity are slow.

Now, second case is the noise can come from the photon detector, that is, when the light falls on the photon the on the photon detector, suddenly there will be shot noise and this is very critical in high quality UV visible spectrophotometers and this has to be minimized.

Third case is cell positioning uncertainties. We have to put the cells at exactly at the same positions, if this position is slightly changed, again the total amount of path length, etcetera concentration exposed to the radiation, etcetera is going to change and this is going to cause errors in the high quality UV visible and IR spectrophotometers. And source flicker, sometimes there is the instrument the source itself if it starts flickering that means it does not give you continuous radiation of study intensity.

Sometime because of the line voltage, etcetera the source will also give more intensity, less intensity, etcetera and these changes in the intensity is again going to cause you different kinds of problems and this is more critical in inexpensive photometers and spectrophotometers.

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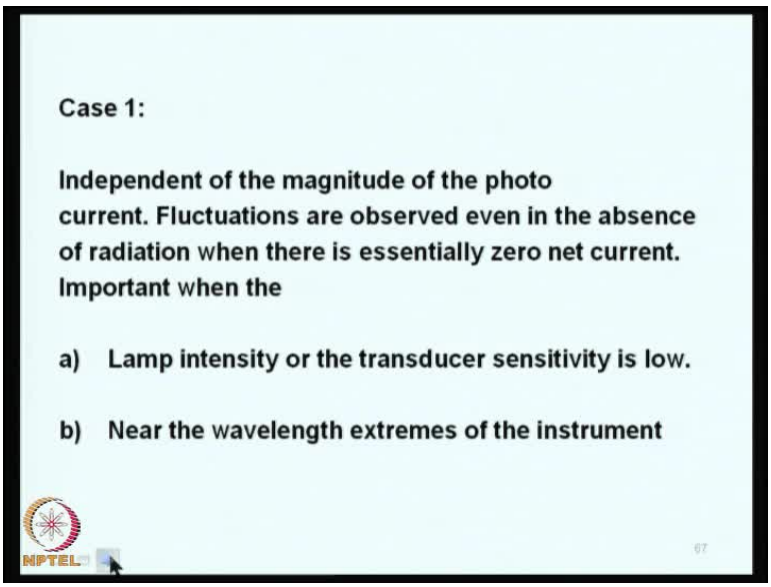

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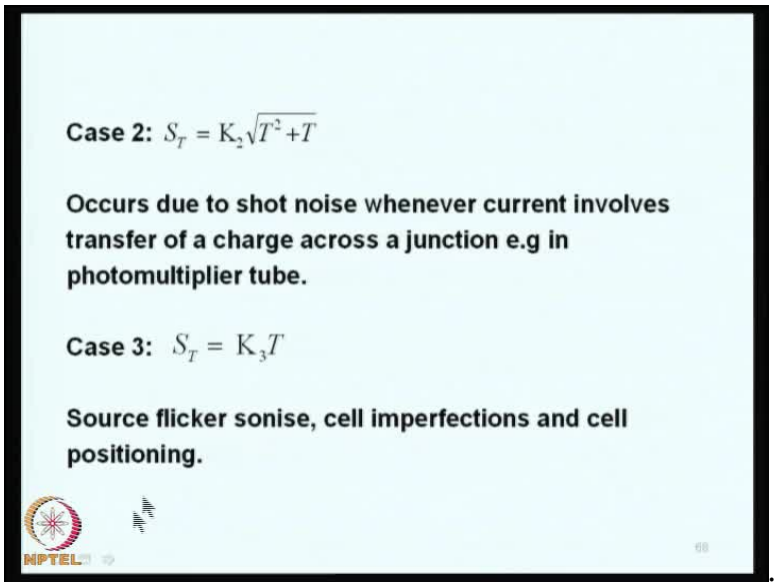

So, there are three cases of instrumental noise and we will discuss noise as slightly more later, but here fluctuations are observed even in the absence of radiation. So, when there is essentially 0 net current, this is important when the lamp intensity change is low. Near the wavelength extremes of the instrument that is one problem. In case two occurs due to shot noise as I have explained to earlier that is in the detector, that is, photomultiplier tube. In source 3, source flicker noise, cell imperfections as well as cell positioning are the causes.

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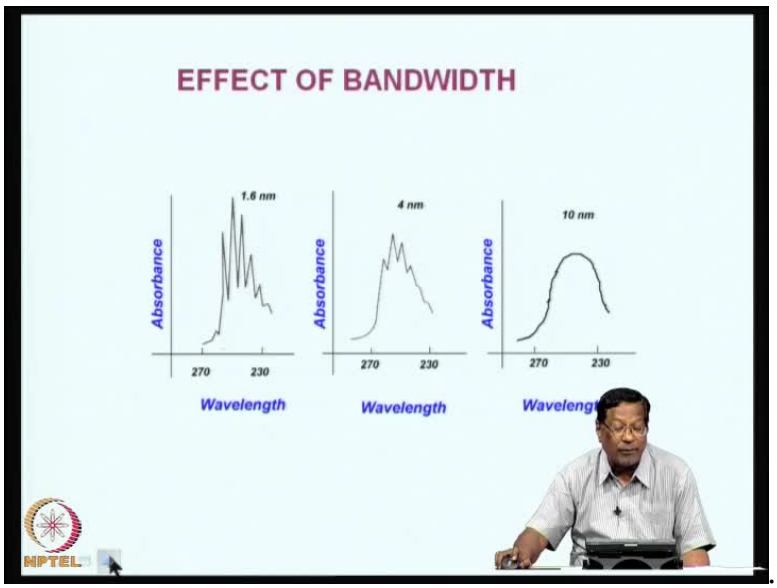

Now, you can see that effect of bandwidth I have put here, suppose the bandwidth is very less, then you can see it is 1.6 nanometer here; see this spectrum how sharp it is and then it keeps on increasing like this; suppose you increase this slit width to 4 nanometers, then what happens suddenly the fine structure is lost and you will see the peeks all the same, but the intensities are all gone bite.

So, suppose you increase to 10 nanometers, see this noise here, the wavelength a simple curve which has no specific characteristics of the observance peek at all.

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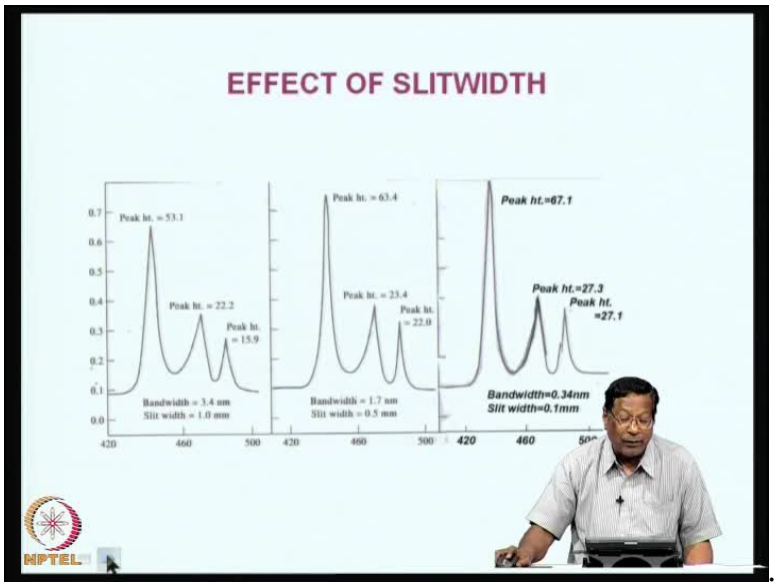

So, the for effective measurement your bandwidth should be as low as possible and this is one of the efforts that we keep on taking a look and I have put the same thing here in more detailed fashion, whether you can see here peek height is 53.1, here it is 63.4 and here it is 67.1; the bandwidth theory is 3.4 nanometer, 1.7 nanometer, another is 0.34 nanometer, you can see the 53 to 67 in just by changing the slit width.

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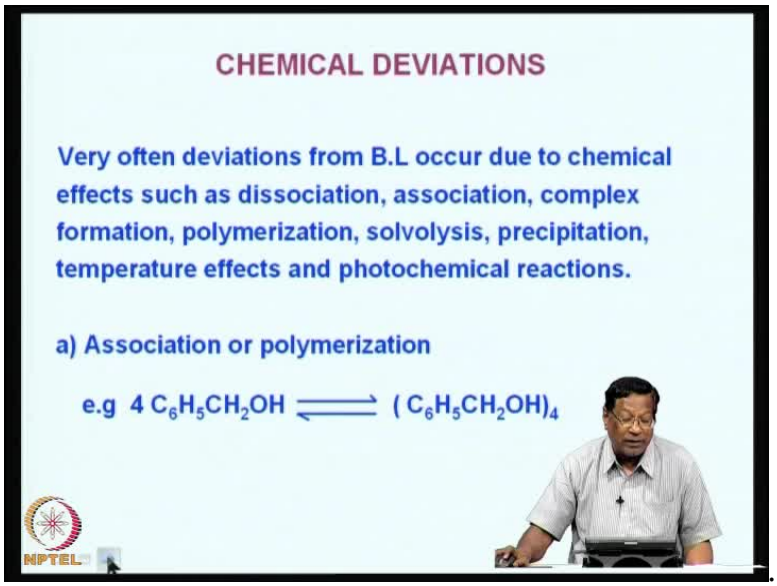

Now, let us discuss about chemical deviations. Now, very often deviations from Beers Lamberts law occur due to chemical effects such as dissociation, association and then, the p h a can change the concentration of the colors substance. Supposing you are doing the reaction at a higher temperature, then what happens, again the concentration of the absorbing spices will change. And sometimes a substance may polymerize, because you are going to prepare a standard solutions and as the concentration increases the intermolecular distance will decrease and polymerization may occur and this increase in polymerization also may have different absorption wave lengths or different lambda max.

Now, in the instrument, you will be measuring a one particular frequency; as it changes in the cell, you cannot do anything about it, but if polymerization occurs, lambda max will automatically change; it will shift somewhere and you will be measuring a absorbance at a low some other frequencies, where there is no lambda max at all, that means, the absorbance is going to decrease.

Now, similarly, the toll depends upon the concentrations and ph, etcetera. I have listed here some of the chemical deviations that occurs from be a Beer Lamberts law due to chemical effects such as dissociation, association, complex formation and also is possible, polymerization, solvolysis, precipitation and temperature effects and photochemical reactions.

Now, association or.. I have an one example here, this is benzyl alcohol 4 molecules can campaign to give you give a one single molecules of 4 units; this will have a lambda max of about 300, whereas this will have lambda max about 275 nanometer.

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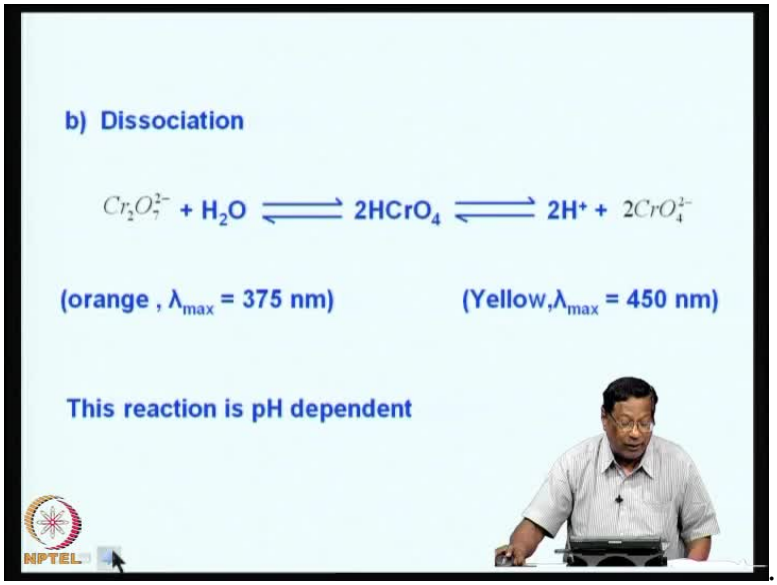

An example of dissociation, that is, potassium dichromate that is Cr 2 O 7 2 minus plus water that is aqueous solution we are taking and it forms chromic acid 2 molecules of chromic acid which can again dissociate in to 2H plus and 2CrO 4 mines chromatins.

Now, this dichromate in presence of water and acid, it forms monochromic acid; chromic acid which can again this dissociate into these things, whereas this one is orange and lambda max is 375 nanometers and this is yellow, which has got 450 nanometer. So, if you are measuring absorbance at 375 nanometers what species you are getting is only having a lambda max at 450 nanometer obviously, this will not follow Beer Lamberts law it all. This a Therefore this reaction is again pH dependent.

So, if you want to determine chromeo potassium dichromate in very low quantities, you have to be very sure at what pH you are going to determine; it has to be constant at least.

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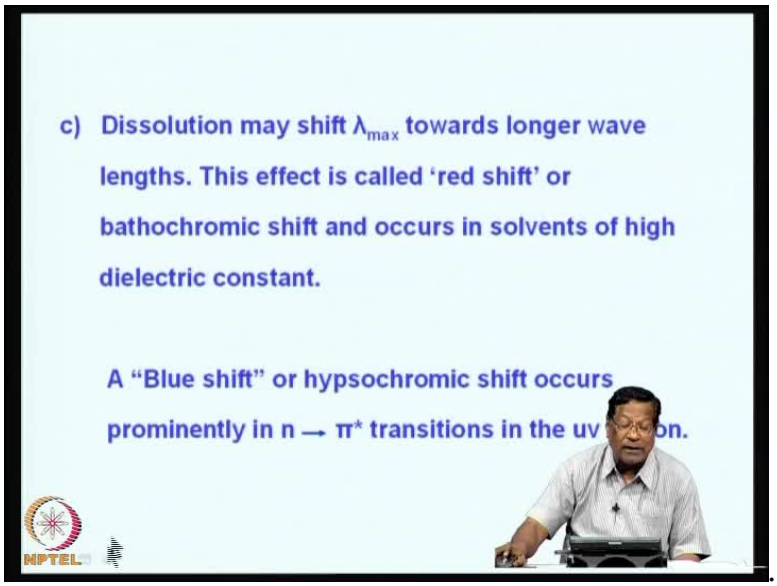

So, the dissolution may also shift some lambda max towards longer wave lengths. This effect is called red shift or bathochromic shift; this occurs in solvents of high dielectric constant. Sometimes it may go to shorter wave length that is blue shift or it is known as hypsochromic shift; this occurs prominently in n to pi start transitions in the uv region.

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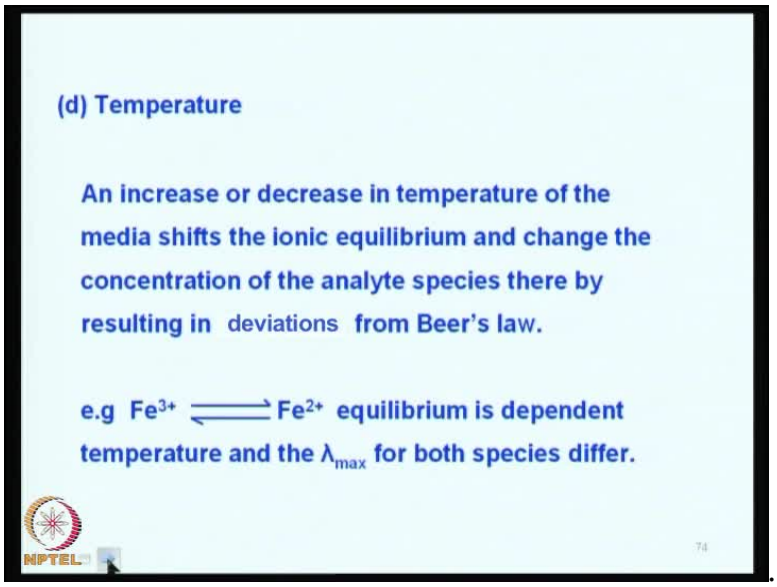

Now, let us look at temperature. An increase or decrease in the temperature of the media, shift the ionic medium ionic equilibrium and change the concentration of the analyte species there by resulting in derivation from beers law, that is, for example, Fe 3 plus

goes to Fe 2 plus and this is a temperature equilibrium dependent upon the temperature and as the temperature changes, the concentration of the ferric and ferrous will change and you will have lambda max different for each species.

So, obviously, whether you are following ferric iron or you are following ferrous iron, whatever you are following the console… because it is a temperature dependent reaction that will make sure that the reaction temperature remains constant.

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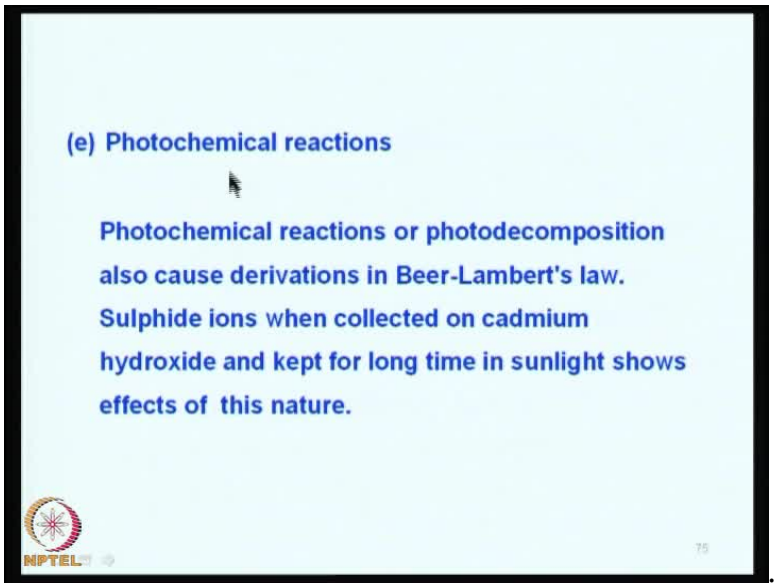

Sometimes, now look at another possibility. When the radiations falls on a substance, then photochemical reactions may occur that means compound may react to the light on coming and cause changes in the composition. So, photodecomposition also causes derivations in Beer Lamberts law. Sulfide ions when collected on cadmium hydroxide, so this is the very famous example. For example, you collect cadmium hydroxide and collect sulfadimethoxine capture for unit for sulphide irons and kept for long time in sunlight, it shows the effect of this nature.

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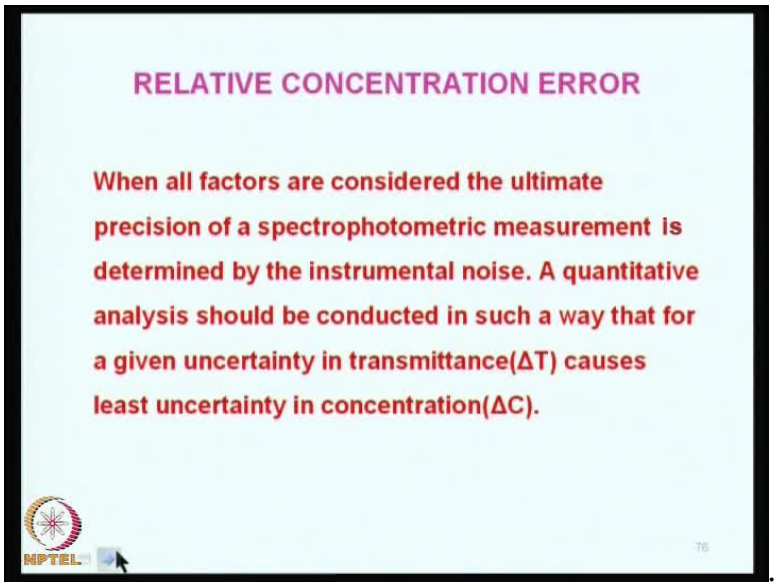

So, these kind of photochemical changes are also quite possible. So, when all these things are considered, we can make sure that we should be able to determine the absorbance under proper ideal conditions so that we should able to determined exactly which range beer lamberts law is operating.

Now, you have to make sure that the errors in the concentration are not exact are not exactly representing errors in the measurement, that means, a constant error in the concentration should result in the constant error in the absorbance or change, whether error occurs change is different.

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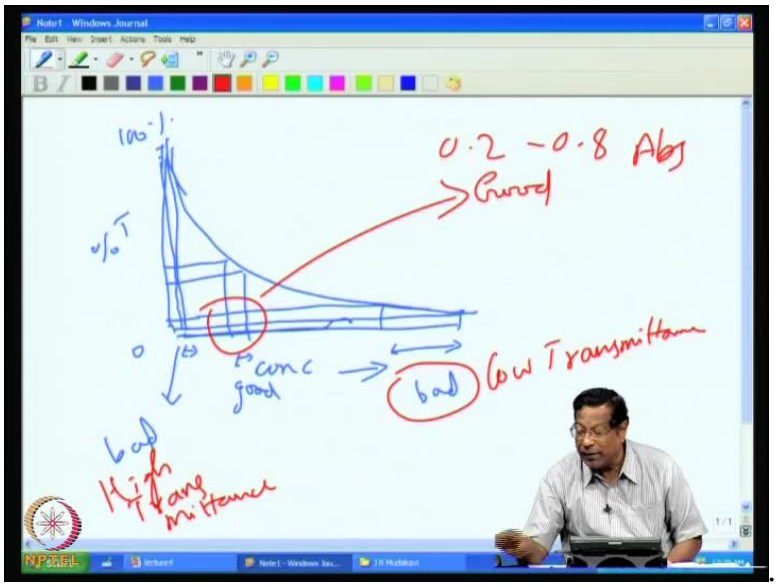

Now, suppose I hope you remember the concentration versus absorbance curve that is a percent transmittance; this is concentration; this is of course a straight line; if you remember, we had 100 percent transmittance here; 0 percent transmittance here; as the concentration keeps on increasing, you will see that we are having a curve like this, now you remember that this is a arithmetic scale.

So, the 1 percent change here is a same as 1 percent change here. Now, you look at it here, the 1 percent change that is here and the same 1 percent change to occur on the transmittance, you can see the change that occurs here is a much less for the same 1 percent in terms of concentration, whereas same 1 percent transmittance on this case, in this area represents change a large concentration. So, this is actually for good measurement, what you need, 1 percent change here should represent 1 percent change here; this is good; this is bad and this is also bad.

So, this 1 percent change we do not want and on the higher side absorbance and the lower side transmittance and on the higher side transmittance, we do not want this, because we should have this is at low transmittance; this is at high transmittance and this is where 1 percent change is going to give you a good 1 percent error change in the concentration.

So, this is good; so this is approximately somewhere between 0.2 to 0.8 absorbance and this is what I am going to tell you now.

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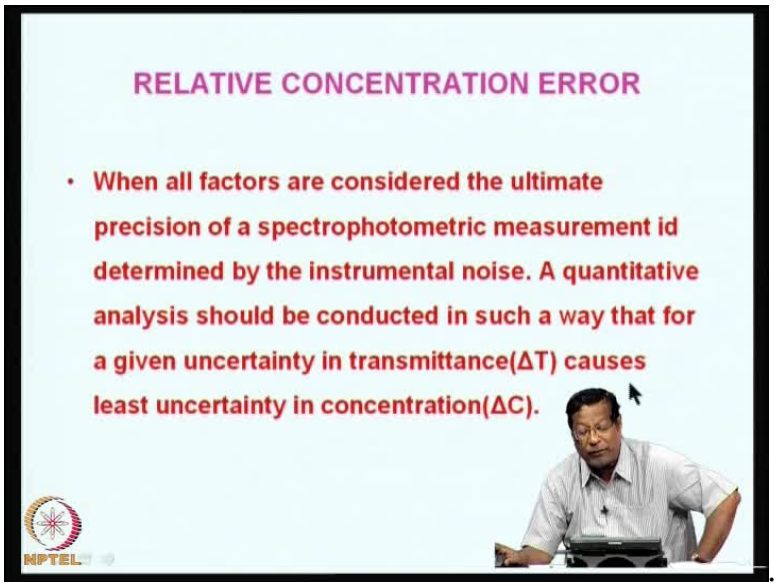

So, the relative concentration error at higher concentration is more relative concentration error in terms of a low concentration is also very high. So, when all these factors are considered, the ultimate precision of a spectrophotometric measurement is determined by the instrumental noise. A quantitative analysis should be conducted in such a way that for a given uncertainty in transmittance delta T causes least uncertainty in concentration.

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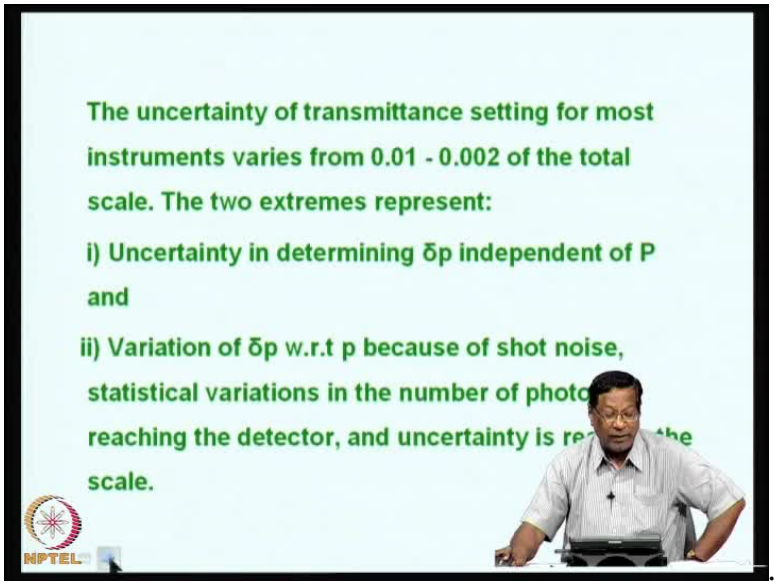

That means we can express the uncertainty of transmittance settings for most instruments varies from 0.01 to 0.002 of the total scale.

So, the two extremes represent uncertainty in determining delta P independent of P, that is, transmit radiant power and variation of delta P with respect to the incident radiation because of the shot noise, statistical variations in the number of photons that are reaching the detector, and uncertainty in reading the scale, etcetera have to be minimized.

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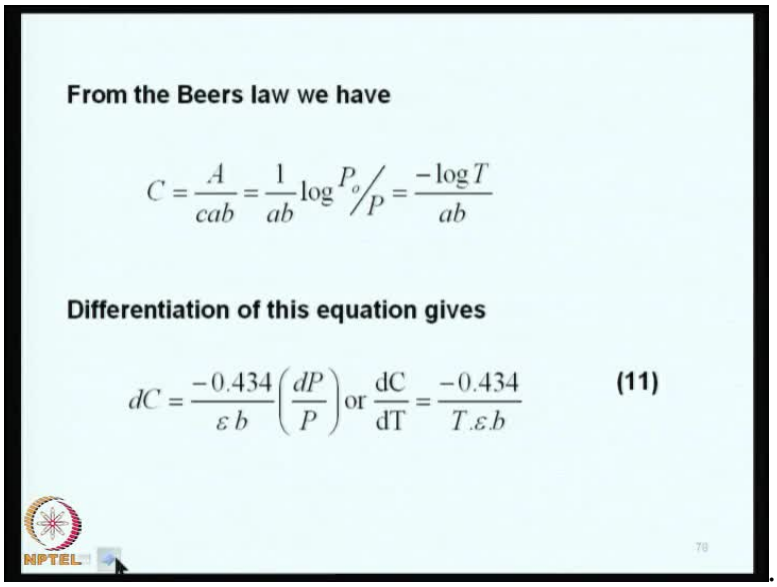

So, what you do? You write Beer Lamberts law in a slightly different fashion, that is, C is equal to A upon cab, A is absorbance and this this a is molar absorptivity, b is the path length, c is the concentration; this you can rewrite 1 over ab log of P naught P, we are replacing a by P naught P, which was nothing but negative log of transmittance divided by ab.

Now, differentiating this equation what you get is minus 0.434 by epsilon b into dP by P or dC by dT should be equal to minus 0.434 T into epsilon into b.

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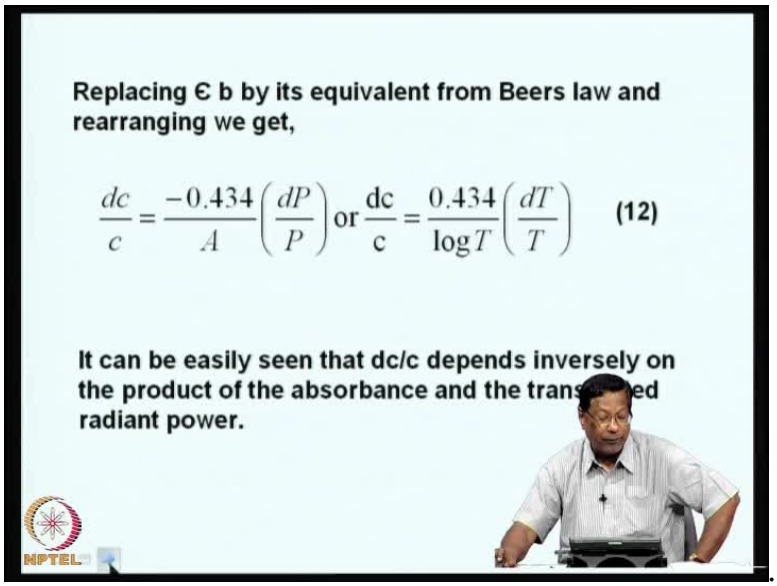

Now, we can replace epsilon by its equivalent term from the Beers Lamberts law and we can rearrange that equation what we get, dc by c is equal to minus 0.434 by A into dP by P or dc by c is equal to 0.434 by log T divided in log T multiplied by dT by T.

Now, when I am explaining, it my looks slightly complicated to you, but if you just write down the equations, you will see that these are not very complicated at all. Now, it can be easily seen here the dc by c depends inversely on the product of the absorbance and the transmittance radiant power.

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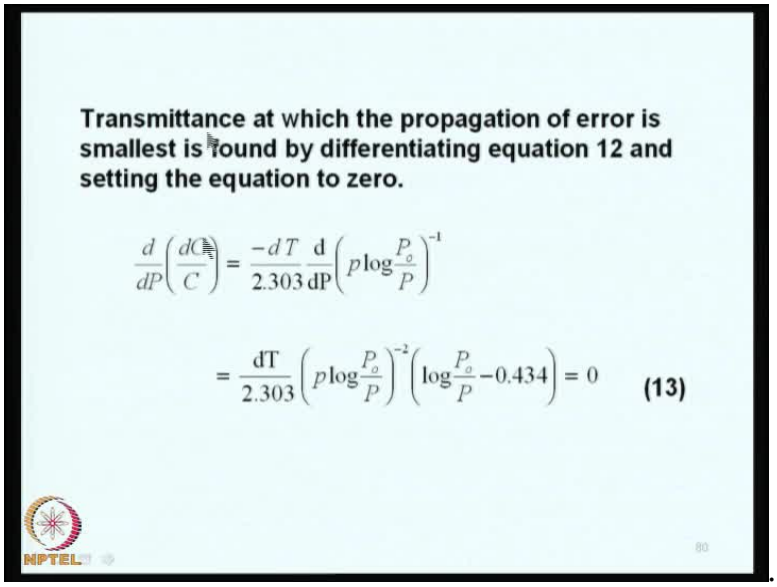

So, a transmittance at which the propagation of error is smallest is found by differentiating equation 12 again and set the equation to 0. Now, what happens d by dP is equal to dC by C minus dt, second this is second differential; so d by dP P log p log of P naught by P and this can be simplified to this second differential, set it to 0, that means, this is one part; this is the multiplication part that has to be that cannot be 0, because of all the these are all real constant; so the other part should become 0, that means, log of P naught by P should be equal to 0.434. So that is absorbance.

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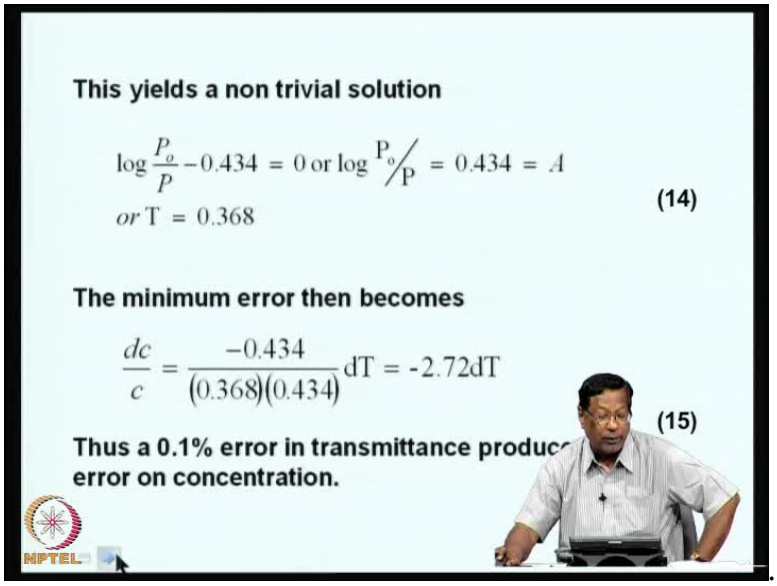

So, T is equal to corresponding transmittance for 0.434 is 0.368. So, the minimum error then becomes minus 0.434 divided by 0.368 into 434 dT that is minus 2.72 dt.

So, in terms of equation this is all very fine, but what it actually means for you as a practitioner of spectrophotometry is that, if 1 percent error in transmittance produces 0.27 percent error in unconcentration.

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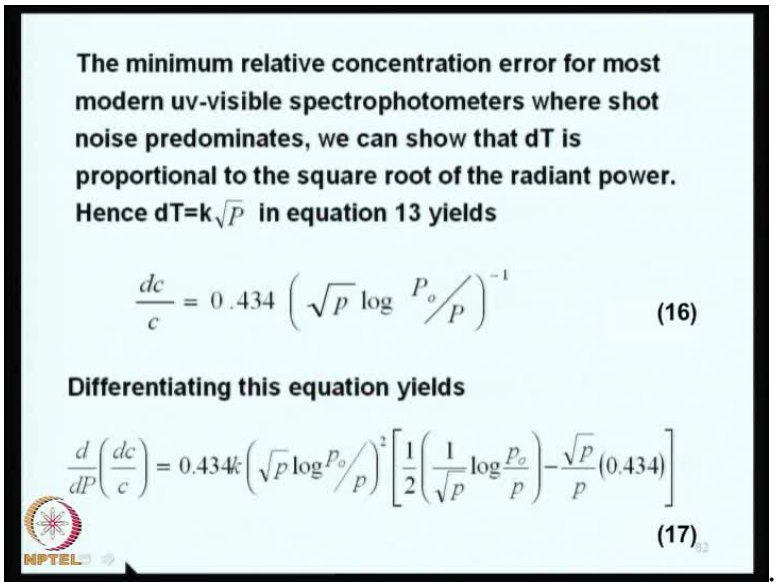

So, the in the error should be as less as possible. The minimum relative concentration error for most modern uv visible spectrophotometers where shot noise predominates, we can show that dT is proportional to the square root of the radiant power.

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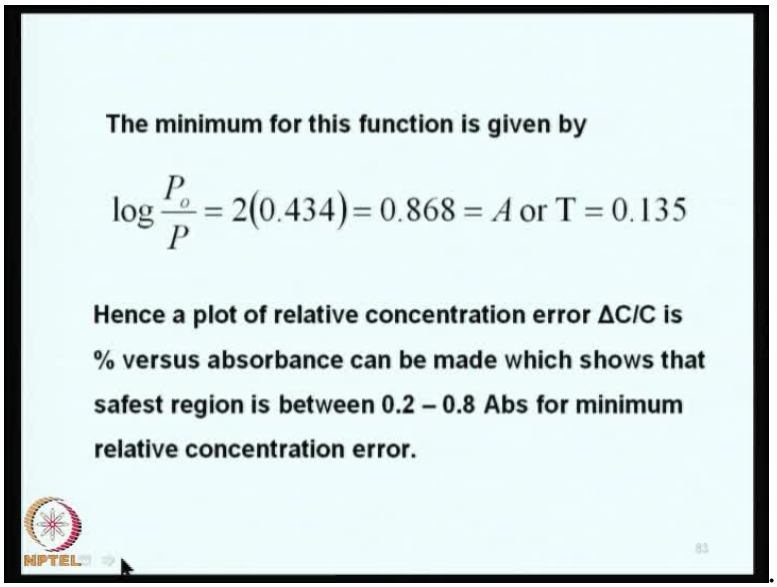

So, use inside these equations and you will get solve it in the same way differentiating equation and then second is d by dP and then do it second time differentiation. So, you will get 0.135, therefore a plot of relative concentration error dc by C versus absorbance

can be made which shows that the safest region for you to operate is 0.2 to 0.8 absorbance for minimum relative concentration error.

That is why if we say that absorbance is between 0.2 and 0.8, we say it is acceptable. Suppose sometimes an instrument manufacturer will come to you and you will say sir, I have an instrument which will measure up to 3 per up to absorbance 3 or absorbance 5, now how it is possible? The 100 percent transmittance if we take negative log, it will be only 2; now again sometimes the instrument will be seller will come to you and say yes, sir I got 3 absorbance, I got 5 absorbance. Now, the trick what they employ is, they convert the same transmittance scale into 1000 and negative log of 1000 would be 3.

So, if you electronically divide the same response into 10000, the absorbance value what you get would be 50 to 5. So, he will say yes sir I have done it. But if you know something about relative concentration error what is important is, you will know that absorbance measurements are absolutely reliable with minimum error between 0.8 and 0.8.

Suppose it is up to 5, it does not make sense; same thing if the absorbance is 0.001 or 0.0001, sir our instrument is so sensitive that it can detect up to 0.0005 absorbance, then we again we should always thing that yeah, but it is it to worth? It would not be worth it, because you should always be aiming between 0.2 and 0.8 absorbance.

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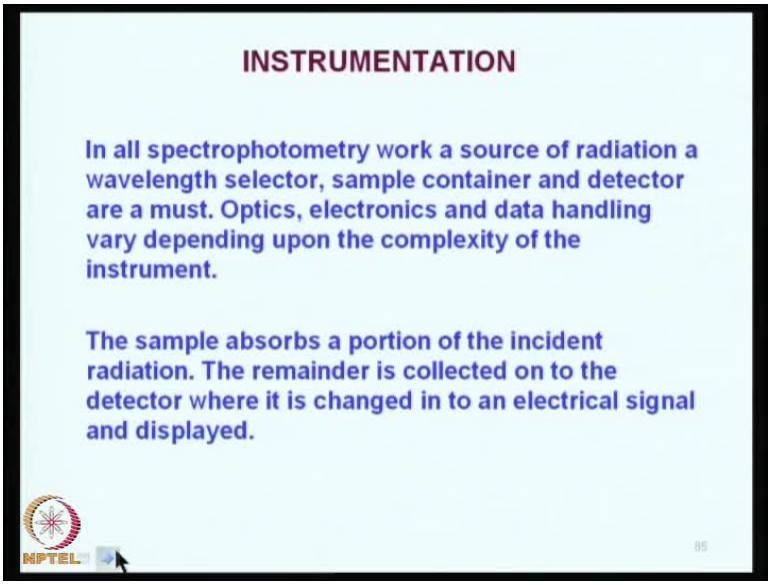

So, this is about the relative concentration error. We now will go into instrumentation aspects of the spectrophotometer. What things go to make good spectrophotometer? In all spectrophotometry work, you will instrument, you will find a source of radiation, a wavelength selector, a sample container and detector these are the must.

So, optics electronics and data handling they are all the there vary they all vary depending upon the complexity of the instrument. Now, the what is the job of the what is the job to be done? The sample absorbs a portion of the incident radiation and the remainder is collected on to the detector, where it is changed in to an electrical signal and displayed.

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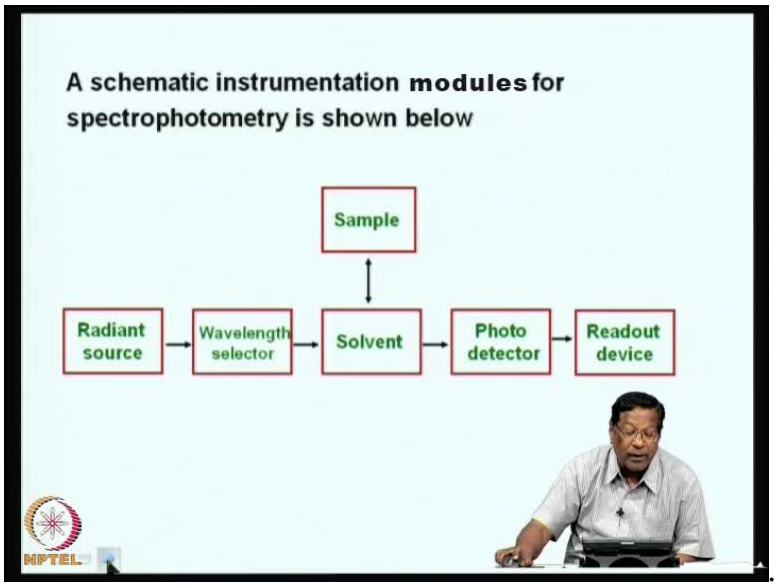

So that electrical signal must be measured. So, a schematic instrumentation module for spectrophotometry is shown below. So, here is sample and this is the radiant source, wavelength, solvent then photo detector, readout device. Basically this will give you the radiant power and then, we have a monochromator here; it will permit you to choose the correct wavelength in which this sample will absorb. So, you can either put the sample here or the solvent here, then pass it through that and whatever radiation is absorbed is absorbed; what it is not absorbed goes on to the photo detector and it reaches the readout device, very simple diagram.

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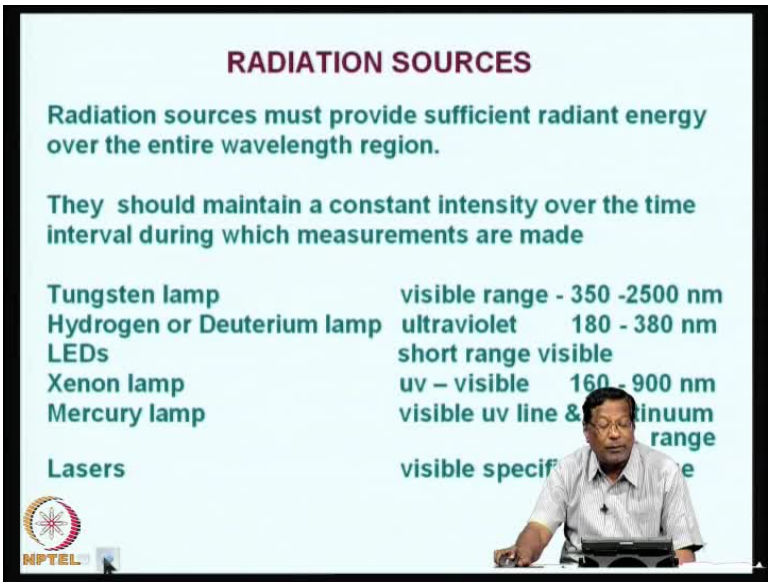

And now let us discuss a little about radiation sources. So, radiation sources must provide sufficient radiant energy over the entire wavelength region, because sometimes you may be measuring only at one wavelength, sometimes you may be measuring the whole lot, you would like to know the full spectrum at each wavelength what is the absorbance. So, they should maintain a constant intensity over the entire interval during which measurements are made.

Now, look at tungsten lamp, it is one of the most important source in spectrophotometry; these tungsten lamps are the same as what you see in your day today life, the same Edison's lamp and its visible range is 350 to 2500 nanometer range; it gives you the radiation; it does not give you the ultraviolet radiation. Another source is hydrogen or deuterium lamp, this provides you ultraviolet radiation between 180 to 380 nanometers. So, suppose you want to make 180 to 1000 nanometers, then you should have a hydrogen lamp or a and a deuterium lamp not or a.

So, sometimes LEDs are there; LEDs give you short range only in the visible range and xenon lamp is another one which is basically what you see in your video shooting, etcetera very high strength and very must as gives you lot of lights and it gives you radiation between in the uv as well as visible radiate visible range; the range is 160 to 900, it actually extends compared to 180 that is hydrogen or deuterium lamp.

Mercury lamps will give you some uv lines and continuum visible range. Lasers provide visible specific line range, etcetera, but what is important is these tungsten lamp and hydrogen lamps are the maximum used in almost all spectrophotometers, which cover uv and visible range. The hydrogen lamp is used as a reference lamp and mostly for ultraviolet, and tungsten lamp is use for visible range. In general what we do is when we use cover from 180 to 350 nanometer, hydrogen lamp is on and then, the moment you mount to change over to visible range, hydrogen lamp is put off and tungsten lamp will come on; so there used in tandem that is together.

Now, xenon lamp is also very useful for 160 to 900 degree centigrade, but again the xenon lamp gives you the problem of overheating; you must have seen in day today practice, the xenon lamps they are they become very hot.

So, in the instrument we cannot have very hot systems, because the temperature will is going to affect the measurements. Therefore xenon lamps, whenever you are you need to use you have to a cooling system, so that the xenon lamp does not become hot and the temperature should be like that.

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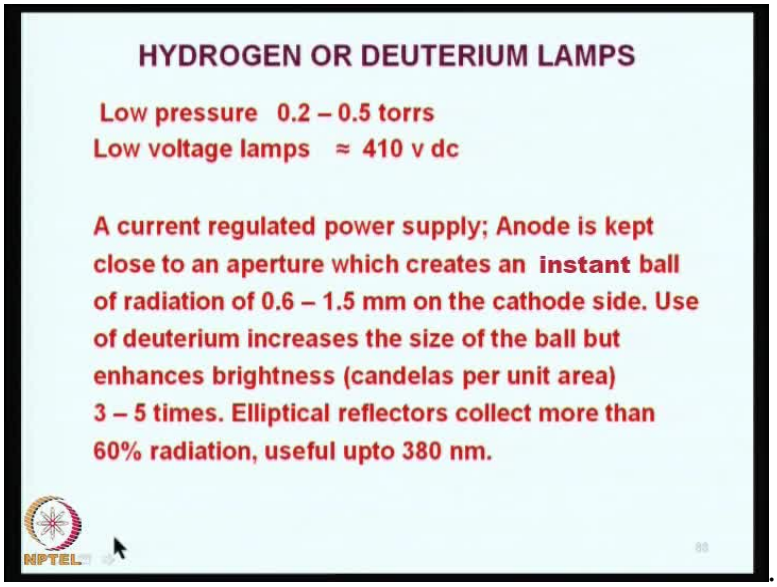

LEDs are for short range used for very small portable instruments. Now, this hydrogen lamp technical data is something like this, it gives you 0.2 to 5 torrs 0.5 torrs pressure; it works on low voltage lamps around 410 volt dc. A current regulated power supply is

required; anode is kept close to an aperture which creates an instant ball of radiation of 0.6 to 1.5 mm that is the slit that is what you need.

Use of deuterium increases the size of the radiation ball, and but enhances it bright its brightness which is major in candelas per unit area. So, it is approximately 3 to 5 times. Elliptical reflectors collect more than 60 percent, what it should be doing is, you should have an elliptical reflector at the back so that, whatever radiation is following on the back side is again reflected and again taken to the taken front. now meters.

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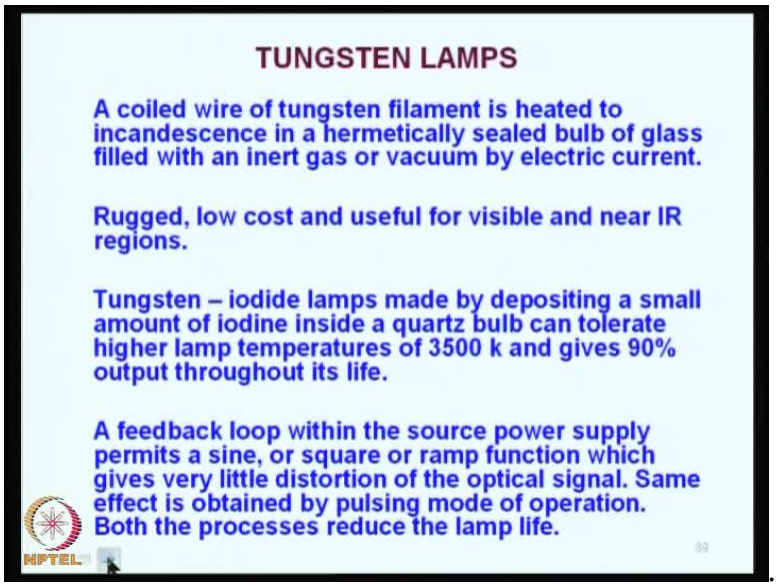

Now, tungsten lamps is nothing but what I have told you a coiled wire of tungsten filament is heated to incandescence in a hermetically sealed bulb of glass filled with an inert gas or vacuum by electric current. So, rugged low it is very rugged, low cost and useful for visible and near IR regions.

Sometimes we use tungsten iodide lamps that is tungsten cope mixed with a… in the bulb, some amount of iodine is put by depositing a small amount of iodine inside a quartz bulb that can tolerate higher lamp temperatures of about 3500 degree kelvin and gives you about 90 percent output throughout its life.

So, you can have a feedback loop within the source power supply per that permits a sine wave or square wave or the ramp function which gives very little distortion of the optical

signal. Same effect is obtained by pulsing mode of operation; both the processes unfortunately reduce the lamp life.

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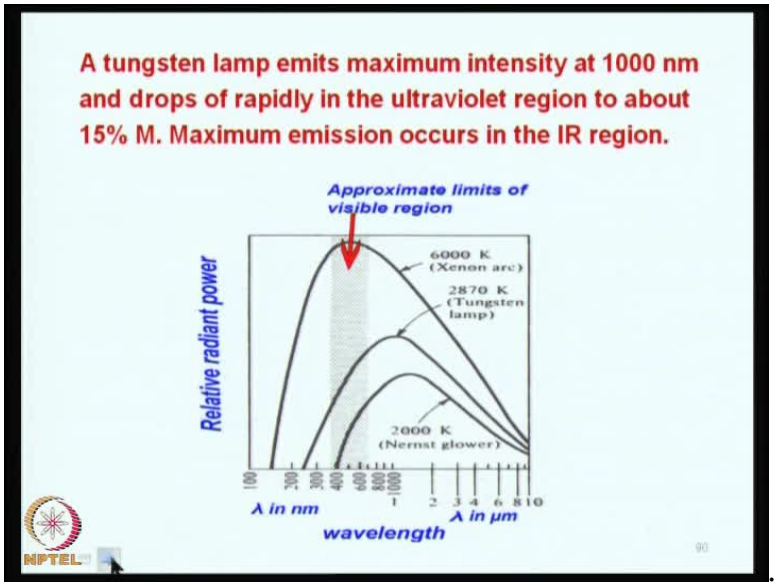

Now, you can see that the tungsten lamp emits maximum radiation in this infrared region; it is like a black body. So, around 6000 degrees this is xenon arc; this is tungsten lamp; the maximum intensity of the tungsten lamp is here, whereas in the visible this is where you are need maximum intensity. This is the small portion; that is this portion is the visible range, but lambda max is always passed in tungsten; so the only above 15 percent of the total radiation false in this range.

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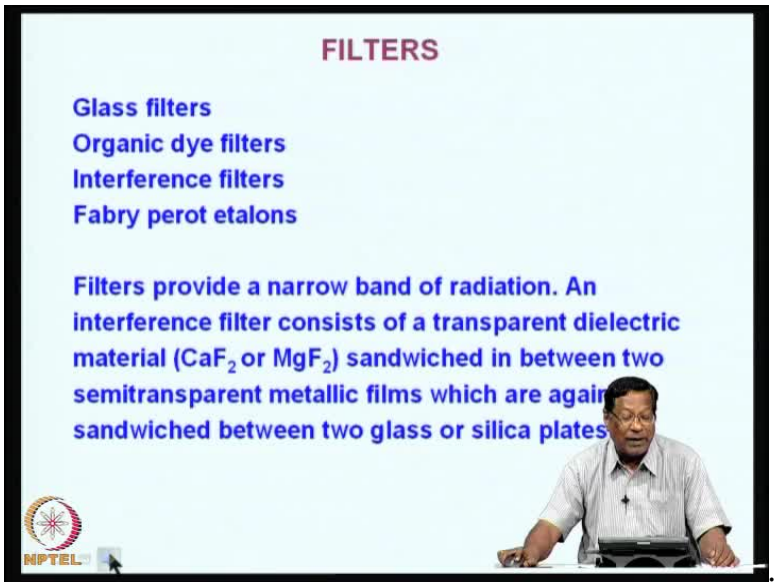

So, that is not very good, but still it serves lot of purpose using xenon lamp, more than xenon lamp tungsten and hydrogen lamps. So, these two you can use. We will continue our discussions on the instrument subsequently in the next class.

Thank you.