Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 12
Beer-Lamberts law

We continue our discussion on the theory of spectrophotometry. Now the idea is to derive a relationship between concentration and absorbance.

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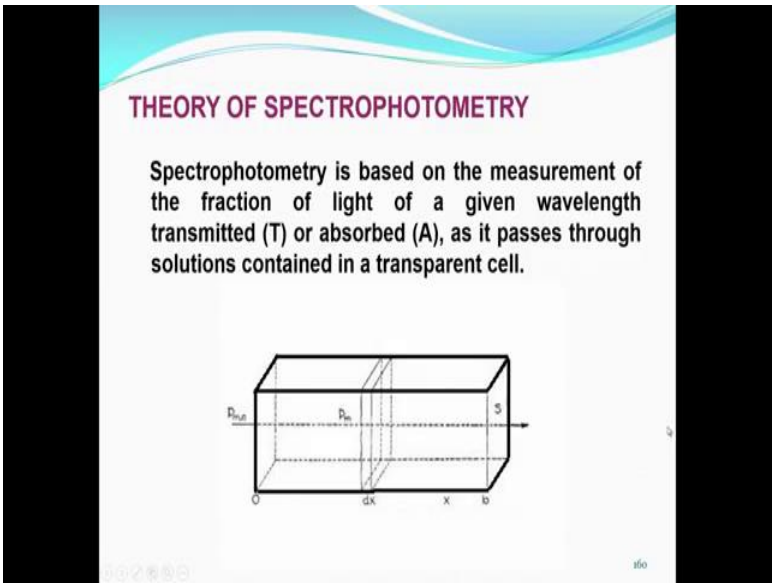

So, this is a session with a number of equations and I hope you will be up to it is not very complicated, but in generally it requires a little bit of knowledge of a mathematics and integration and derivation etcetera; I hope you are all up to it. So, basically spectrophotometry is based on the measurement of the fraction of light that is coming that is of a known wavelength. Part of it should be absorbed because whenever there is absorption of light the electrons can get excited to next higher energy level.

So, any UV visible peak whether it is n to pi star, sigma to sigma star or pi to pi star or n to pi star all of it must result in the decrease of the incident light; that is if I take a known amount of energy make it incident on the sample, the energy coming out after passing through the sample should be less; that means, part of the energy is absorbed. The observed energy is used in the electronic transitions like what I had already told you. So,

the spectrophotometry basically involves the measurement of transmitted light and incident light and the difference between the two.

So, how do we go about doing it is to image in a solid block like this, assume that this whole block is representing a chemical with a definite width and length. So, here I have P m o and P m and this is yes. So, I have the initial radiation coming through this block part of it will be observed, what comes out would be having energy less than what is less than P m o. And what for our purpose what I do is I take a small cross section of this having a length of about d x. So, I then I extend the derivation to the whole box that is the idea. So, you have to consider a beam of parallel monochromatic radiation with power P 0 passing through a transparent cell having a path length of b centimeter containing and absorbing solution.

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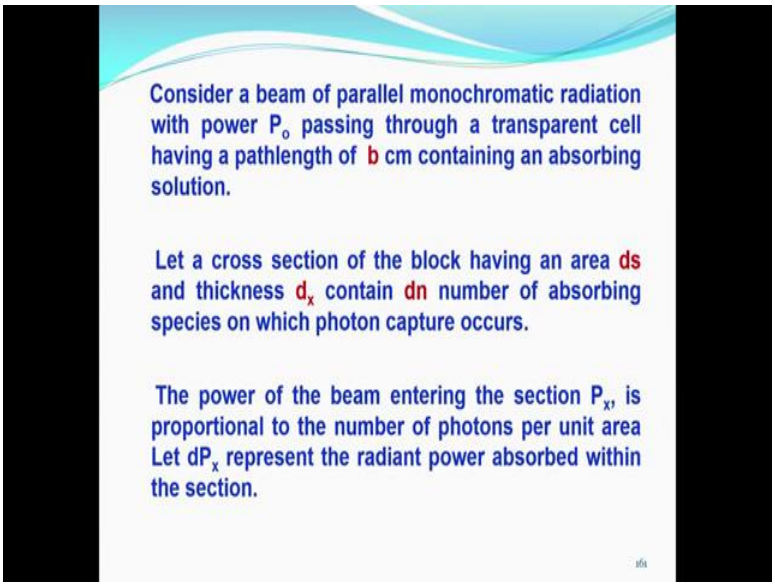

So, this box contains an absorbing solution the path length is 0 to be that is b, and this is the initial radiation this is the initial radiation. So, the same thing I am I have written in the word format. So, a small cross section of the block having an area d s and thickness d x, area is thickness d s and thickness is d x let it contain n number of molecules that is instead of n, I am taking d n a small fraction. So, on which photon capture occurs photon capture means incident radiation is absorbing. So, the power of the beam entering the section P x is proportional to the number of photons per unit area, more photons more absorption.

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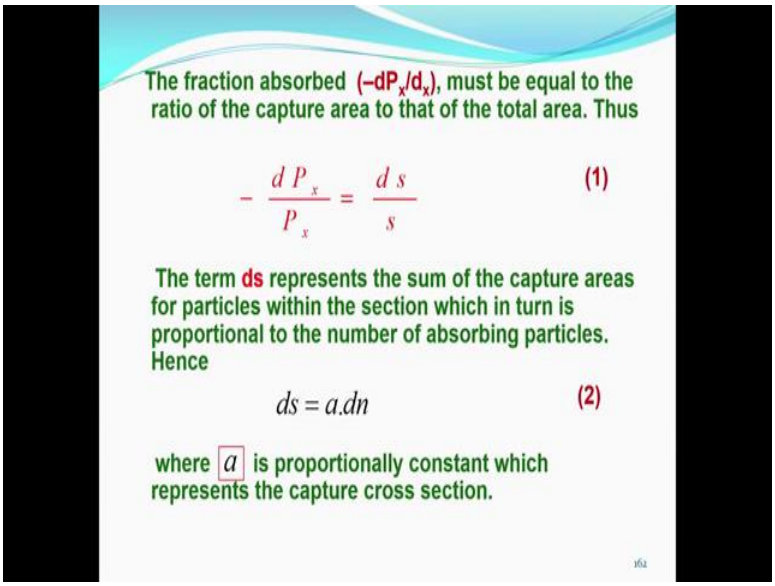

So, let d P x represent the radiant power absorbed within the section what we are considering. So, the fraction observed would be minus d P x by d x. So, it must be equal to the ratio of the capture area to that of the total area. So, I can write an expression like this minus d P x by P x should be equal to d s by s, and here the term d s represents the sum of the capture areas for particles within the section which in turn is proportional to the number of absorbing spaces. So, I can write d s is equal to is a turn containing two parameters, one is a and another is d n; d n are we already know that it is it represents the number of molecules, and a is a proportionality constant which represents the capture cross section.

Remember we have talking about a single molecule as of now. So, capture cross section corresponds to they are almost the diameter of the analyte.

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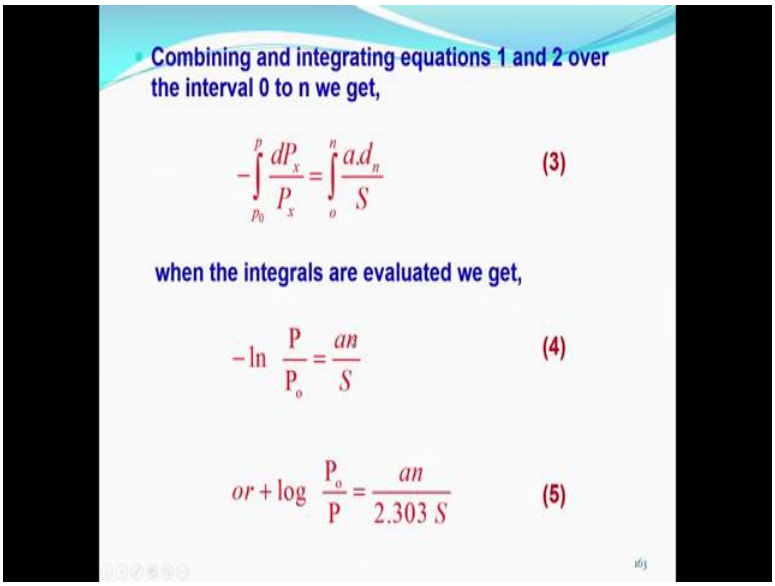

So, now what I should do, is I should combine all the sick all the small small boxes and then calculate the total amount. So, what how I do it, that is the by integration. So, I combine the equation one and two and integrate where do I integrate? I integrate from 0 molecules to total number n of molecules. So, I get I write an expression like this integral sign and when the we evaluate the integrals, I get minus l n P by P 0 is equal to a n into a n upon S or I convert this into log P naught by P is equal to I simply interchange the numerator and denominator on the left side.

So, that changes the sign. So, log of P naught by P is equal to a n upon s and since I am changing from l n, I had to include a factor of 2.303. So, actually l n is minus 2.303 log. So, that comes as a denominator here that is our equation number 5.

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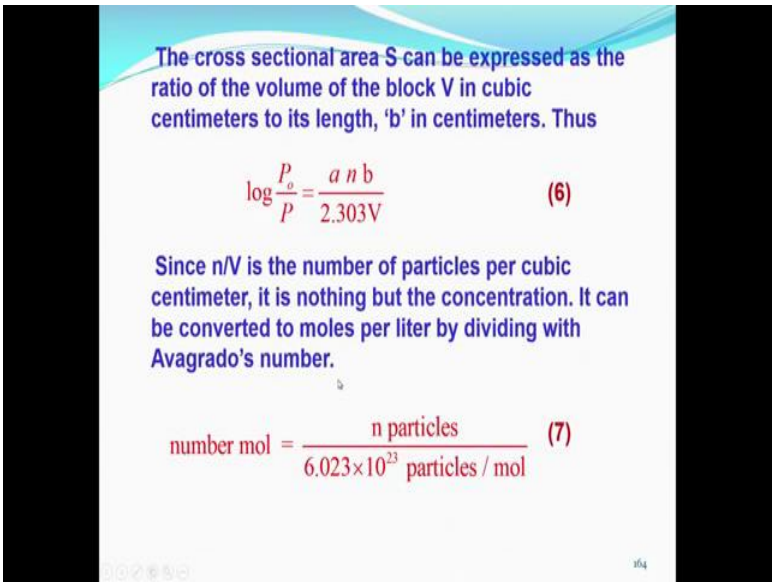

And then the cross sectional area I can also express as the ratio of the volume of the block, in cubic centimeters or path length in centimeter. So, I add one more factor that is b that is the path length, I can take 1 centimeter path length, I can take 5 centimeter path length, I can take a 10 centimeter box or I can take 0.1 centimeter box, so whatever it is I introduce a factor known as b. Since n by v here you see n this is the total volume. So, n total number of atoms divided by total volume is the number of particles per cubic centimeter that is nothing, but concentration.

So, I can replace n and v by a simple term that is concentration. So, concentration it can be expressed as moles per liter by dividing with the Avogadro's number. So, number moles are equal to n particles divided by 6.023 into 10 raise to 23 that is Avogadro's number particles per mole.

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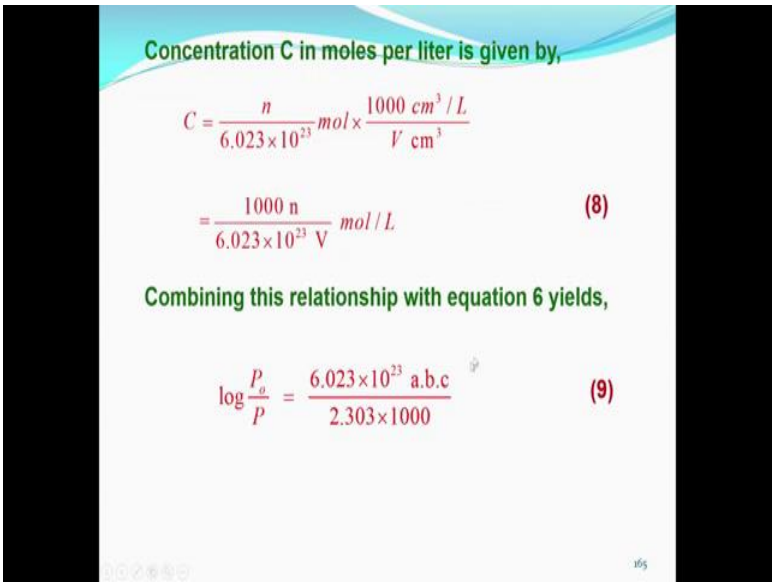

And if I do that the concentration I can express it as n by 6.203 multiplied by 1000 liters divided by total volume, and that way I can convert it into moles per liter. So, they combining this equation again I go back to equation 6 that is log of P naught by P. Here I simply introduce what we have derived just now.

So, log of P naught by P is equal to 6.3 not 0 to 3 multiplied by 10 raise to 3 into a into b into C divided by 2.303 into 1000. So, these are all essentially by derivations following from one equation to another.

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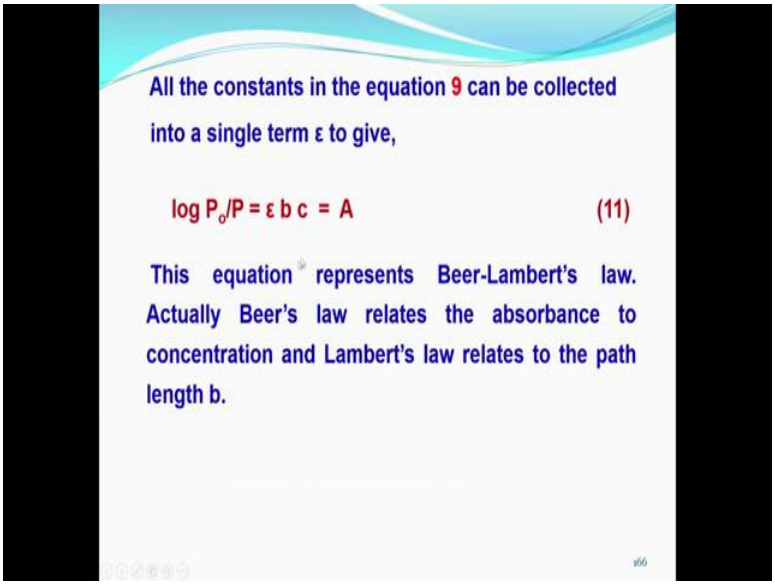

So, all the constants in this equation that is in the previous equation this is the constant this is constant this is only variables are a b and c. So, all this represents some sort of a number which is proportionality constant. So, what we do is, we put the proportionality constant as epsilon and then log we write the expression log of P naught by P is equal to e to the power epsilon b c, and that is known as absorbance this absorbance is a defined quantity.

So, the difference between the incident and the radius radiated light transmitted light logarithm of that is defined as absorbance. So, basically what we are talking about is absorbance is proportional to concentration and path length and epsilon is a constant. So, depending upon the molecular size, molecular structure molecular concentration not more concentration only molecular size it depends upon it is a species specific quantity, depending upon a molecular cross section and the number. So, this equation is known as Beer-Lamberts law, actually there are two laws involved in this one represents the proportionality with respect to concentration, another represent proportionality with respect to the path length.

So, Beer's law represents relates the absorbance to the concentration, and Lamberts law relates to the path length. So, since both of them relate in the same way by the same expression epsilon, we simply say it is related to both b and c path length and concentration.

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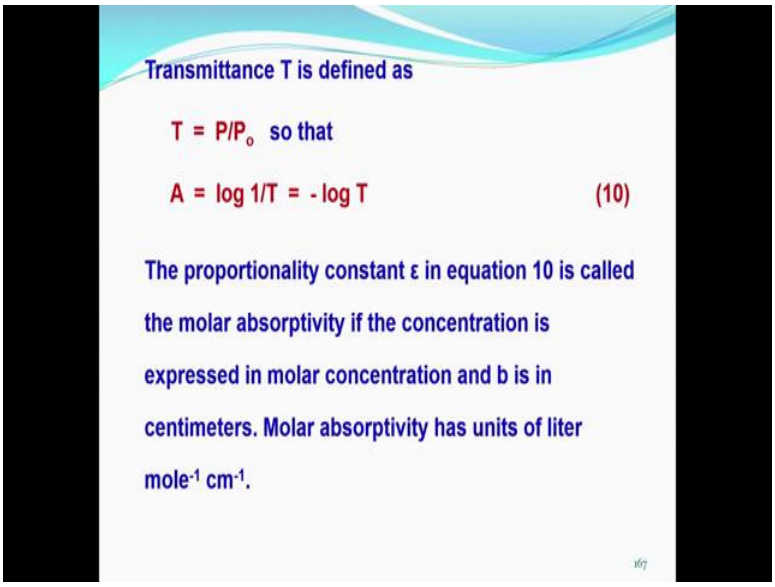

So, what we do transmittance now we define a term what is known as transmittance. Transmittance is what is what comes out of the substance after the initial radiation process through. So, that is again a ratio turn in terms of light a power of the light that is P divided by P naught, and absorbance is P naught by P that is absorbance log. So, absorbance is basically if you look at these two equations that is 10 and 11 abs you will see that absorbance is logarithm of one over transmittance or negative log transmittance, this expression it is very important for us to follow because in all our discussions henceforth.

So, it is very clear it should be very clear to you that whenever we are talking of absorbance, we are talking about negative log of the transmittance; that is transmittance says P by P 0 incident light intensity of the incident light to that of the transmitted light to that of incident light. So, the proportionality constant in this equation is called the molar absorptivity, if you express the concentration in terms of molar concentration and b has to be in centimeters. So, molar absorptivity has units of liters per mole per centimeter that is how we write it. So, this is also an important parameter with respect to every absorbing spaces understood.

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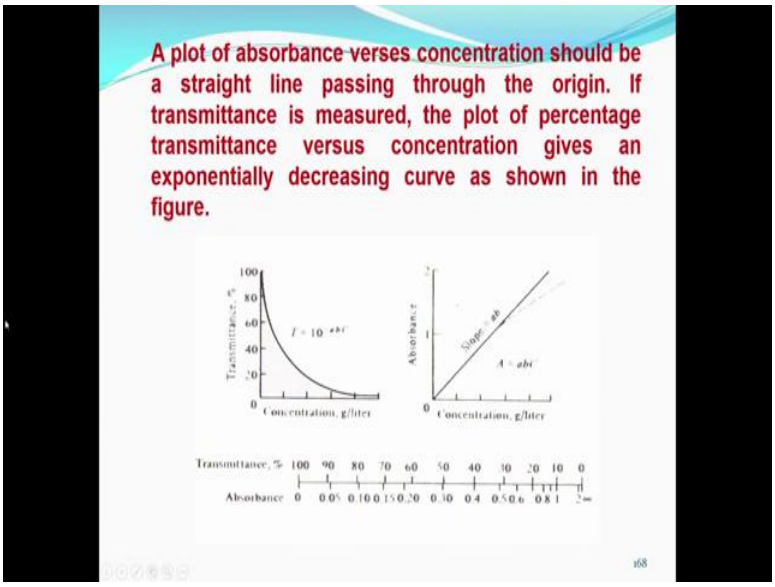

So, what happens if I plot this go back I have a I measure a quantity what is known as absorbance, and I measure a quantity what is known as path length, I major a quantity what is known as path length that is size of the cell and I measure a quantity what is

known as concentration. So, because it is proportionality constant I must always get a straight line if I plot A versus log of P naught by p. So, this is, but if I plot A versus P naught by P alone instead of logarithm turn I get a different curve.

So, that is what I am trying to show you here a plot of absorbance versus concentration log of P naught by P, log term gives you a straight line with a slope epsilon with a slope of epsilon b and we are drawing concentration. If b is one centimeter I will get the slope should correspond to only a that is molar absorptivity, and if I plot percent transmittance because transmittance is basically P naught by P or P by P naught whichever way you look at it, transmittance if it is not absorbing anything transmittance should be 100 percent right if it is absorbing all of it transmittance should be 0.

So, if the transmittance is 0 absorbance should be maximum, and if the transmittance is maximum absorbance should be 0. So, these two figures you should always try to remember and a plot of transmittance versus concentration we will give a curve like this on the left side what I have drawn, and if you plot absorbance versus concentration it has to be like this. I can you device a scale something like this transmittance 100, 90, 80, 60 etcetera 0 to 100, but absorbance transmittance 100 percent should correspond to absorbance of 0; but absorbance is in the log scale so same 0 to 100 percent should represent 0 to 2; why 2 why it should be 2? Because the absorbance can be only 100 percent and logarithm of 100 is 2.00 or something. So, the scale defines itself if I talk about 0 percent transmittance and 100 percent transmittance, then the absorbance scale is automatically defines itself with a with a range of 0 to 2.

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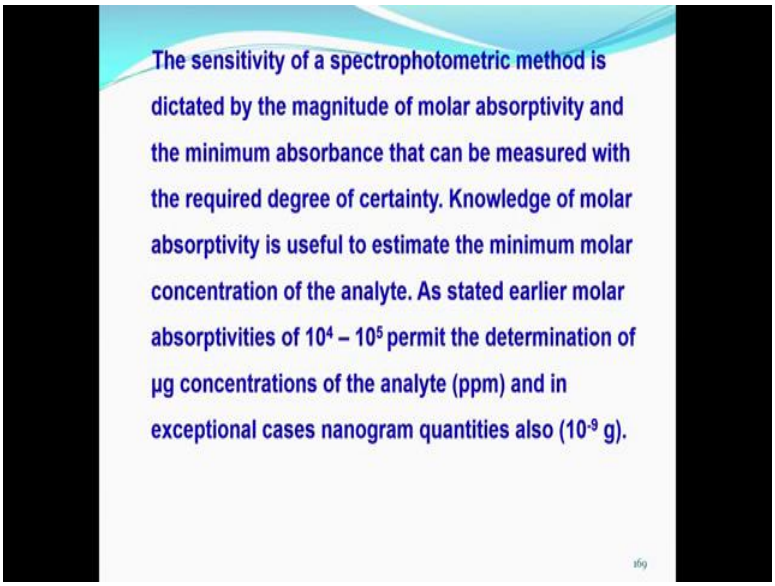

So, these are the different types of curves what we get and the sensitivity of a method spectrophotometric method is dictated by the magnitude of the molar absorptivity, this I have already explain to you earlier if the molar absorptivity is high the color is very high and very small quantities can be determined with a required degree of certainty. So, knowledge of molar absorptivity is very very useful to estimate the minimum molar concentration of the analyte. So, analyte what do you mean by analyte? Analyte is what we want to determine it may be an organic substance having sigma to sigma star, n to pi star, pi to pi star, n to pi star transitions or choice transfer complexes or any other coloured compound and that is the analyte, we what we want to analyze is known as analyte and if the analyte has a molar and every organic compound it has got a molar absorptivity as a characteristic.

So, as stated earlier the molar absorptivity is of number of compounds have been calculated and determined and put in a compendium it is available in the textbooks or internet or in Google or in the laborate or in the journals, research journals etcetera and books something like that, and the molar absorptivity should remember that if it is in the range of 10 raise to 4 to 10 raise to 5 the determination of microgram quantities is concentration of the analyte is possible that is parts per billion. One part in one million that is 10 lakhs, we can determine by spectrophotometric and if the absorbance molar absorptivity is very low then they obviously, the detection limit can be higher.

So, in exceptional cases if the molar absorptivity is really very high, then I can go to sub parts per million that is nanogram quantities also. So but this requires very high instrumentation because at nanogram quantities the instrumental error becomes almost equal to measurement. So, the error percentage increases at that level, for that we need a better electronics and how do we go about doing it? We will study in the discussion on the instrumentation; right now for the time being what I want you to understand is high molar absorptivity means very low detection of the substance, low quantities and that is what we want we do not want to determine like a bucket chemistry in high quantities for that titrations gravimetry and several other methods are available, but the pollutants have got a characteristic that very small quantities can affect us in a very large manner.

So, that is where the crux of the problem is there, and that is why we are talking about you visible spectrophotometry as a technique for pollution control monitoring. So, if I can determine these quantities in parts per million or parts per billion, the government also can impose conditions for us not to release concentrations higher than p p m levels that are how the whole thing is sort of supportive of each other.

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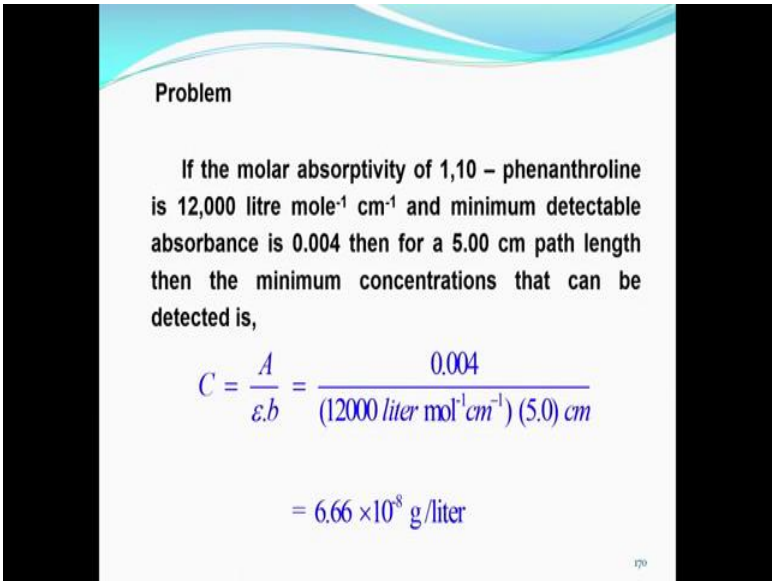

Now, I want you to look at a small problem as an example. So, the example is the reads like this molar absorptivity of 1,10 phenanthroline is 12,000 liters moles inverse centimeters inverse and minimum detectable absorbance of the instrument is 0.004, and

suppose I use a 5 centimeter path length I take a box of 5 centimeter and fill it with a solution of 5 m l.

So, the minimum quantity that can be detected is how much; it is a very simple relation a is equal to epsilon b c. So, if I write the equation C should be equal to A by epsilon b 0; I want the slide look at the slide once again C is equal to A by epsilon b c if this is A is equal to epsilon b c. So, if I put these numbers what I can determine is 6.66 into 10 raise to minus 8 grams per liter this is in some p p m level. So, it you can imagine the power of spectrophotometry for the minor test quantities that can be determined with certain accuracy that is the beauty. So, now, that is over that was only an example what I wanted to talk to you.

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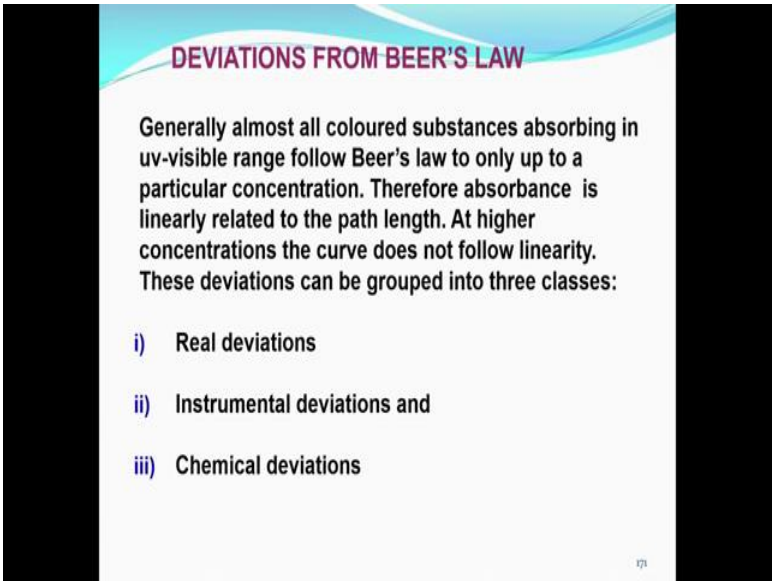

Now, look at it this way I want you to think about A is equal to epsilon b c. So, if you plot a absorbance versus concentration you should get a straight line, passing through the origin. Now if all substances systems follow Beer-Lamberts law I must get a straight line, but do I get a straight line that depends upon several factors; suppose the concentration keeps on increasing they on their near they on the log scale there will be large quantities, but all of them will be can show all it can show is 100 percent absorbance, so the at higher concentrations they will all be bunch it together on the logarithmic scale. On the lower side if the concentration is very low it is not using to

determine because the instrumental noise which determines log of P naught by P could be almost equal to that.

So, the error will be more. So, there is only a certain concentration range in which Beer-Lamberts law operates. So, the Beer what does Beer-Lamberts law say the absorbance should be proportional to the concentration, it does not say up to what level concentration it does not say up to what level the linear it is obtained. So, because the molar absorptivity is different and compounds are different, matrix maybe different. So, there is always a certain range for any given system in which the molar absorptivity will be linear. So, what happens to the range when you cross that range, will Beer-Lamberts law still be followed; that means, still will you get always a straight line at any concentration, very low very high somewhere there should be a limit.

So, that we are going to discuss now and the derivations deviations from Beer-Lamberts law are quite a few. So, almost all coloured substances absorbing in UV visible range follow Beer's lamberts law, but only up to a particular concentration therefore, absorbance is related to path length. So, at higher concentration the curve does not follow linearity, these deviations can be grouped into 3 classes and these include they are real deviations and some could be induced by instrumental factors, and some can be induced by chemical factors that is number three.

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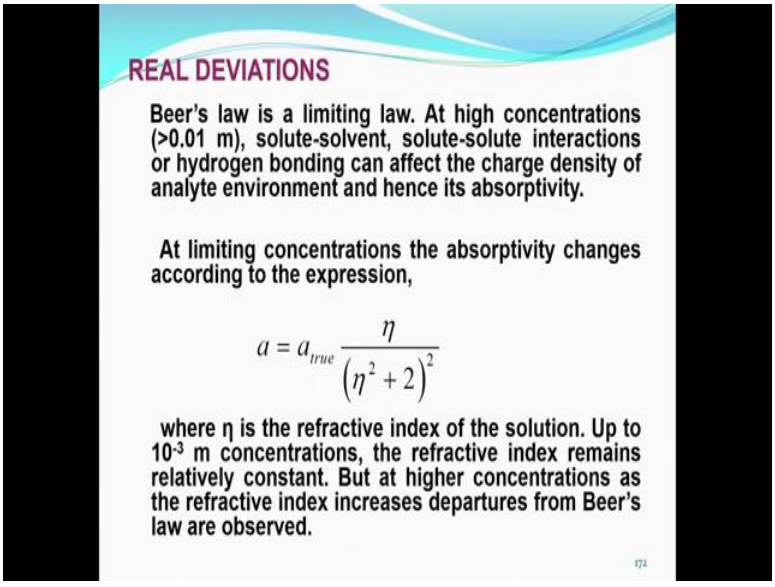

And all these things combine to give you a very small range in which Beer-Lamberts law is followed. So, let us look at the deviations.

So, how do how does the deviation look like; a Beer-Lamberts law you may have to understand now that Beer-Lamberts law is only a limiting law, at high concentration the solute, solvent, solute, solute all kinds of interactions will take place in a given solution. So, they can that can affect the charge density of the analyte environment, what is charge density mean it means absorption absorptivity. So, the actual concentration in which the absorptivity changes is not directly related to concentration, but to the refractive index of the solution; the actual concentra actual relationship is given by a is equal to a true value, multiplied by a factor which represents n refractive index that relation is given by n by n square plus 2 whole square, and up to 10 raise to minus 3 concentration this works.

But higher concentration the absorb refractive index increases and increase of the refractive index causes departure from the Beer-Lamberts law; that means, the straight line graph will not be obtained anymore.

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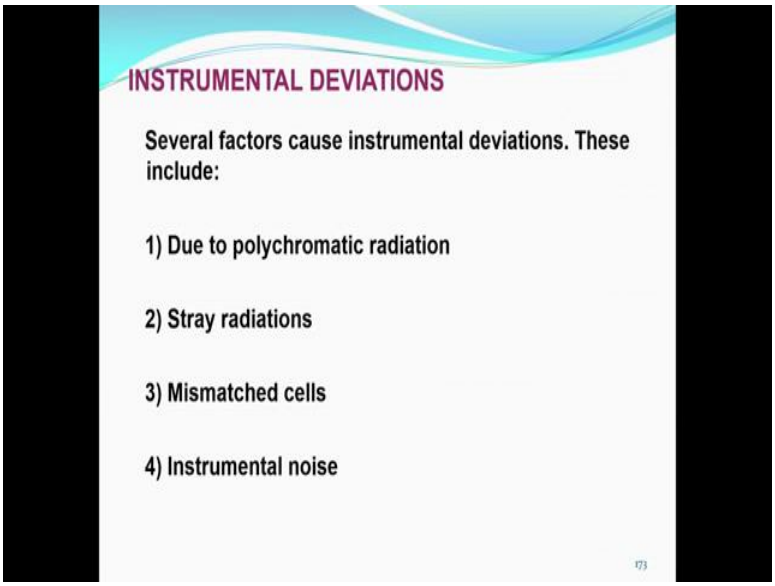

So, the other factors cause deviation for example, look at your screen now that is number one; one is polychromatic radiation whatever we may talk about; however, much you may talk about it is almost impossible to get the single wavelength of single radiation wavelength radiation of single wavelength it is impossible in the whole world.

So, what I actually get when I take a radiation from a lamp, I get a bunch of radiations which is called poly chromatic, but not the monochromatic. For Beer-Lamberts law I need a monochromatic radiation, but that is not possible. So, anytime the Beer-Lamberts law is obtained only has a limiting law not as a definitive law. So, the more monochromatic radiation it is or the less polychromatic light it is, the better will be the Beer-Lamberts law that will be followed; that means, I must try to get single wavelength plus associated wavelength percentage of associated wavelength should be very very very less, otherwise it is not possible to get; that is why I cannot use a tube light or I cannot use a tungsten light, all the light that comes because it is a polychromatic wavelength, the radiation coming out to number of wavelengths associated and coming out as a group.

So, I need in all spectrophotometers I need a device which can separate out all other wavelengths except the one which I need to use for my system. So, if I need 243 nanometer I must isolate 243 nanometer only and, but it is not possible when I isolate 243 plus or minus 3 nanometer I always get, I have explain to you earlier just go back and look at the P half width peak.

Then now second is stray radiation. So, you cannot on the whole you cannot on the whole get a radiation in a box which is completely dark, there will always be some amount of radiation coming from different sources that means, you cannot find a perfect black body in which you can send the monochromatic light and no other light.

So, there is always a see whatever is the amount of technique you try to seal a box in which through to send the monochromatic light, you will always failed; there is certain amount of extraneous radiation not from the lamp, but from the surroundings you will always get. So, that is known as stray radiation, whenever you buy an instrument you must always look at what is the stray radiation component in a given system. So, it is a one of them least stray radiation means better instrument. So, stray radiation again contains many other wavelengths. So, it is not possible for us to say it is monochromatic light at all. So, sometimes what happen us I talked about us taking the sample in a box right?

So, the box I need to measure once twice thrice etcetera, once I need to measure what is incoming and once I need to measure what is out coming how do I do that? The

incoming light I must pass it through an air. So, measure it and out coming light I must pass it through the sample, and I must measure what is the out coming. So, the cells the box in which the sample is kept this should be matching because they should not introduce errors on their own, I want to measure the concentration of the substance taken in a cell, but not the cell material; cell material may be a glass it may absorb part of it, it may be a cords, it may be at may absorb part of the radiation, it may absorb many other radiation so mismatched cell. The cells should be exactly matching each other that is why it is very important how we take the sample, and then instrumental noise as I have already explain to you higher concentration, the bunching takes place lower concentration error is more.

So, there is always certain amount of radiation that is moving here and there, which does not allow Beer-Lamberts law to be followed perfectly that is how we should understand.

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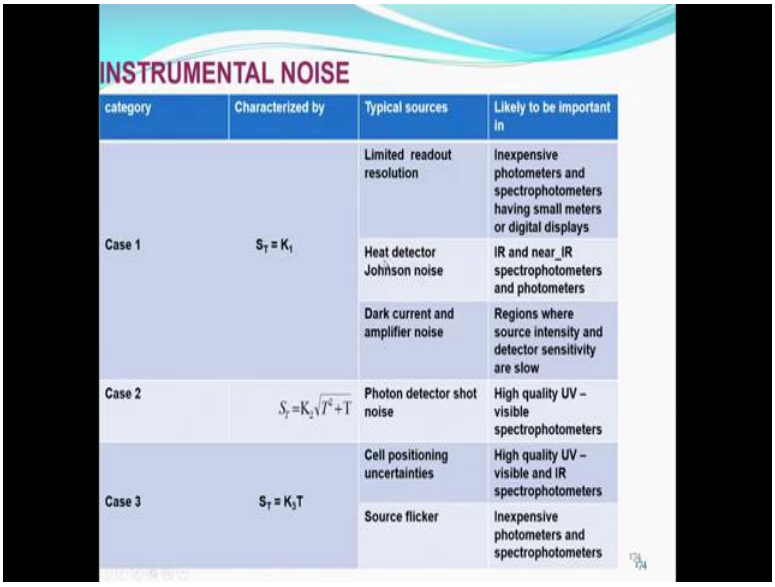

So, the instrumental noise quite often is quantized can be calculated quantified. So, one is heat detector these are the different kinds of noises instrument, one is limited read out resolution and then Johnson noise, amplifier noise, photon detector shot noise and then cell positioning uncertainties, source flicker so many possibilities of errors in the instrument when you design an instrument you must avoid all these. I am not going into details of these kind of instrumental noise because that is not for us at this stage, but

what is important is we understand what are the different sources of errors that can creep in the determination of a substance by Beer-Lamberts law.

So, these are the different things, and then I have some more slides which will tell you about more about the instrumental part that causes an error.

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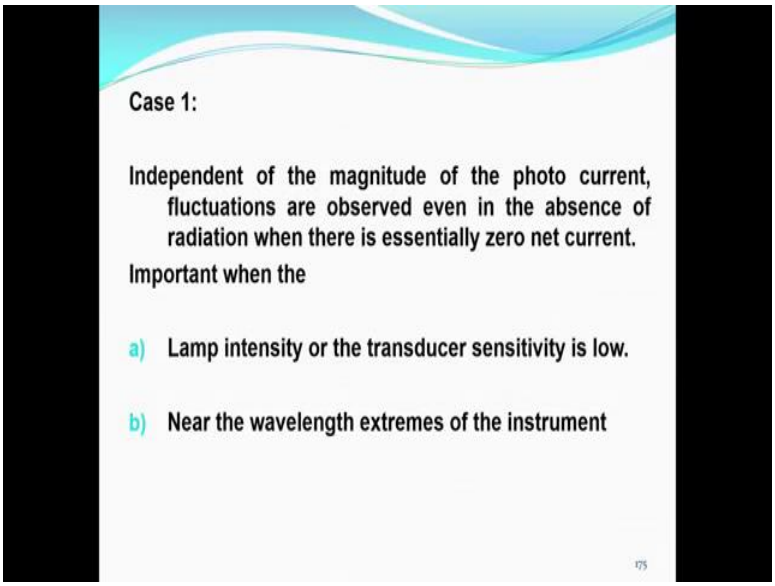

But I am not going into details for example, and there are about 3 cases one is source flicker noise short noise etcetera.

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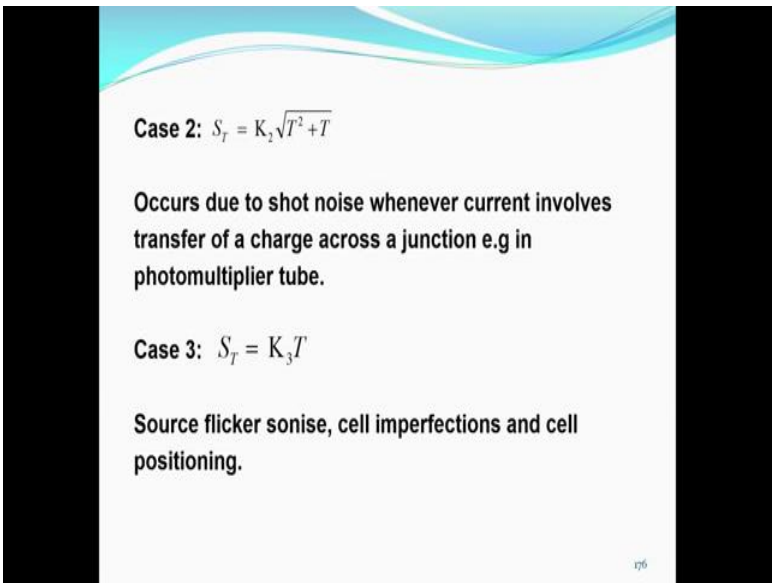

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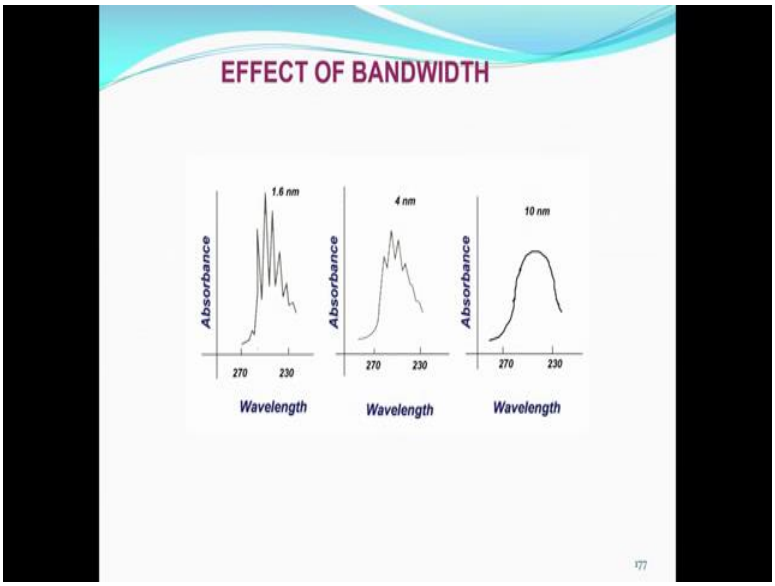

They can all be quantified, but the effect of bandwidth also is important, because I have as I have already told you the selection of a monochromatic light is important.

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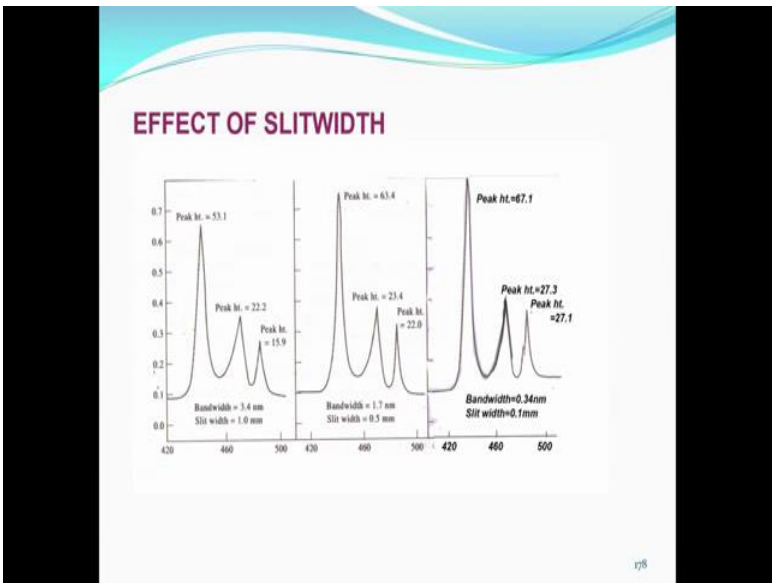

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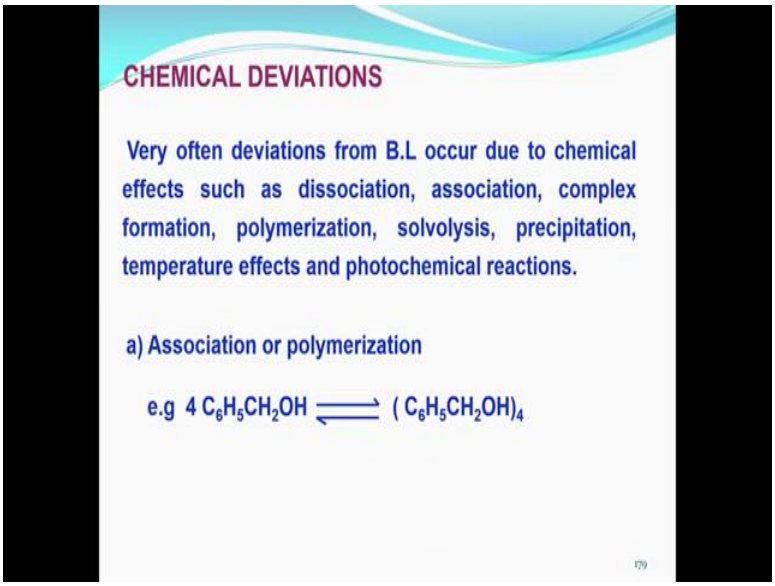

And the effect of slit width how big a slit is again important I will explain to you this in the next class and chemical deviations are also possible. So, we will talk about the chemical deviations and we will talk about the instrumental noise etcetera how they really affect the Beer-Lamberts law, and we will also see how to minimize the error in the measurement of Beer-Lamberts law and also how to avoid the chemical errors coming from chemical deviations that we will see in the next class.

Thank you very much. Have a nice day.