Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 15
UV-Visible spectrophotometry, instrumentation-III

Greetings to you: once again we start where we left off in the last class. I think I was discussing about the optics of the instrumentation in the last class that was about the spectrophotometry.

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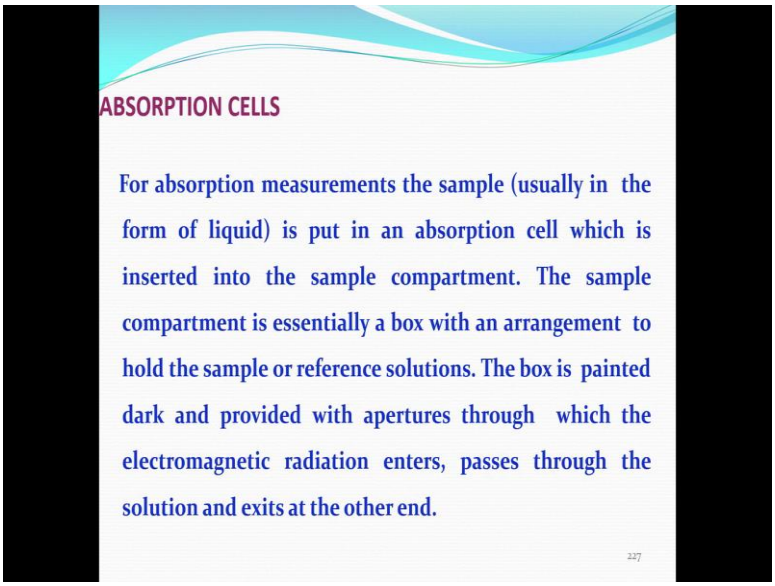

I think I had shown you this slide where I had explained to you that the absorption measurement for is always done in an absorption cell which is inserted into the sample compartment. The sample compartment is essentially a box with an arrangement to hold the sample and the cell reference solutions. The box is painted black and dark provided and provided with apertures through which the electromagnetic radiation enters from one and passes through the solution and exit is at the other end.

So, in the process absorption occurs. So, basically the sample compartment is an essential component of the instrumentation, but the design is nothing very great anybody can do that. Only thing is you should make sure that these absorptions box the sample box sample compartment should not have any stray radiation, wholes or something like

that and it should be completely dark when we close their sample for close the instrument for measurement that is a requirement.

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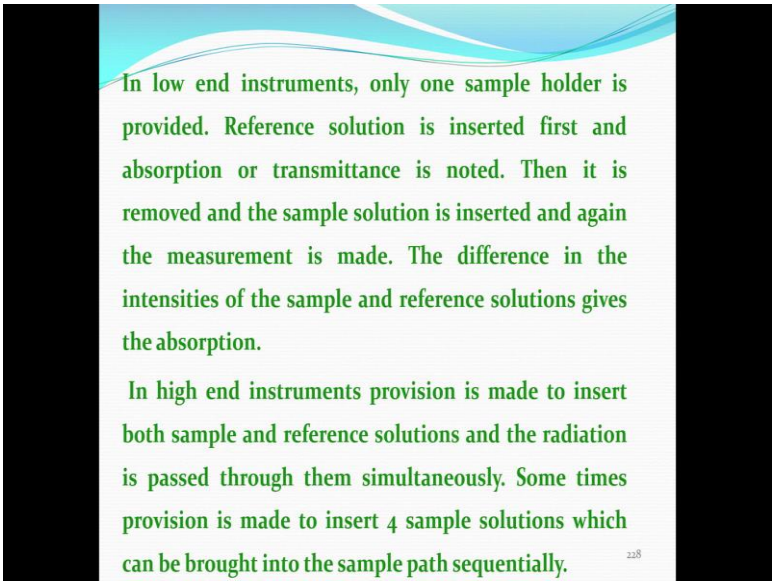

So, in low end instruments, what happens is there will be only one sample holder and you have to insert the reference first and then remove it record the absorption or observation and then put your sample and then again record the absorption or transmittance. Then you have to remove it and the sample solution the difference in the in intensities of things sample and reference solution use the absorbance or transmittance whichever way we prefer to make the measurement.

In high end instruments what happens is the normally put the sample and reference solution in the same compartment. And split the incoming radiation, one to pass through the reference solution all the time, and the sample solution during the measure another part is may to pass through the sample solution. Sometimes provision is made to insert 4 samples 5 samples. Nowadays with automation taking place we have even about00 cells, sample compartments. Through we will be passing through one by one and the measurements will be made. So, subsequently both the sample and reference beams are brought into the sample path sequentially and separated.

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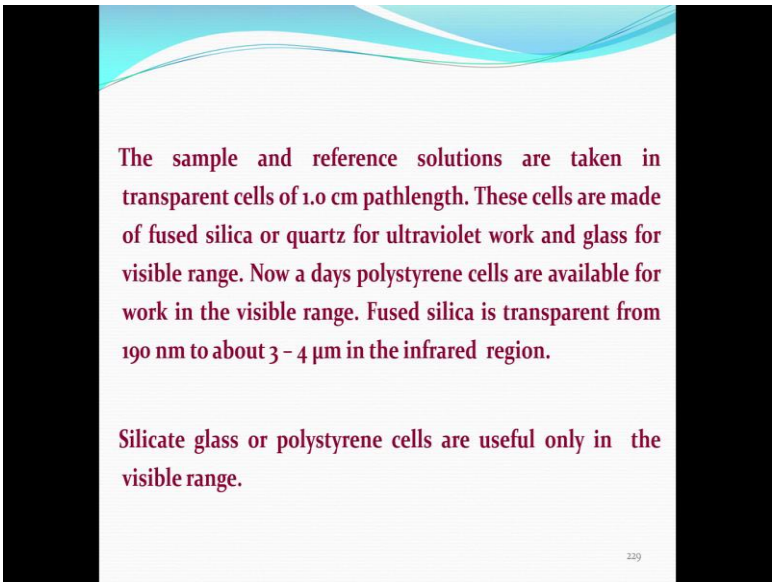

So, the sample and reference solutions are normally taken in cells of about one centimeter path length that is 10 mm. These cells are made of fused silica or quartz. The quartz sample cells are useful for ultra violet range. Of course, they will be useful for visible range also, but the glass once is not useful for quartz, and glass once are not useful for ultra violet range, that is 180 to 350 nanometers, but the for that we need only quartz glass will be useful from 350 to 900 or 1100 or whatever we wish to measure the in the visible region of the electromagnetic radiation. Nowadays what we do is there are some polystyrene cells which are also crystal clear and useful for work in the visible range. These things will cost hardly about 2 rupees 3 rupees and they can you can use them a throw away cells if you are measuring the bacteria bacterial cells and other things.

But they are also useful for visible range. Now fused silica is basically transparent from 190 to nanometers to about 2 to 4 micrometer in the infrared region also. So, now, a days several spectrophotometers are available which will permit you to make measurement in the near infrared region. That region is very useful that is from 900 to 1100 or 1200 nanometer range. 99 percent of the instruments operate from 350 to 700 that is visible 180 to 700 or 900 that is UV visible and if you want to include this thing the range is extended up to 1200 nanometers for infrared region. That region also you can make measurements using a spectrophotometric instrument.

Only thing is that will be useful for solids and then polymers and then leave samples reflectance and all other related measurements. So, silicate glass or polystyrene cells are useful only in the visible range.

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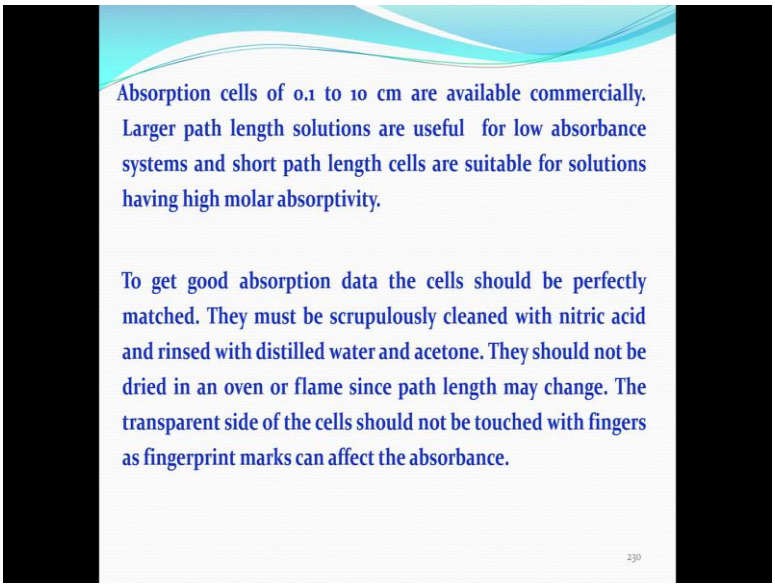

Now, absorption cells this you should know, the abs normal absorption cell I had already mentioned to you that it would be about 0.1 centimeter, but there are cells of both UV visible range which will measure from 0.1 to 10 centimeter cells. So, 0.1 millimeter that is 1 millimeter to 10 milli 100 millimeter range are available commercially. Depending upon the color you can choose the cells. And sample holder is designed to take all these cells in the sample compartment by slight adjustment of the path length.

So, larger path lengths are useful for very low absorbance. And short path lengths are useful for solutions having very high molar absorptivity. So, higher absorption goes for smaller range and go for smaller cells of about 0.5 m 0.5 centimeter point 2 centimeter like that. And larger once 5 centimeters 2 centimeter 5 centimeter 10 centimeter cells are useful for visible rain, for substance visible range as well as a UV, but the absorption absorbance must be very less. Then only the beer lamberts law will be come for you can handled it comfortably. So, to get good absorption data the cell should be perfectly matched. This is one important concept I want you to understand.

What is matching? In general, you must assume that any glass material is inhomogeneous. So, unless they are prepared from this both absorption cells are prepared

from the same batch there will be certain anomalies in the composition. Even though they look the same transparency is the same etcetera, but still there will be some amount of absorption for this what you have to do is you have to match the cells. The matching of the cells means essentially that if you put make the spectrum measurement from 180 to 900 or whatever it is for 2 cells the absorbance should be exactly same at all wavelengths. This is known as matched pair of cell cells. So, whenever you buy an instrument spectrophotometer you must always ask for a matched cell couple. And then if you are buying more than all of them should have been prepared from the same batch.

This is important and I do not have to over emphasize that the cells must be scrupulously cleaned with nitric acid and rinsed with distilled water and acetone to remove any color or dies etcetera. Before every measurement these cells must be cleaned nicely. So, they should not be dried in an oven or a flame since path length may change, because glasses are known to have now inelastic expansion. The transparent side of the cell should not be touched with fingers because even we our finger marks can affect the accuracy of the absorbance. Quite often if you are our hands fingers are having a res a bit of a residual oil, in our skin and that can leave fingerprint and this will affect the absorbance.

So, we normally are very careful that these matched cells. 2 sides are made into frosted side in which you can hold the sample hold the cell. And other 2 sides are used only for transparent making measurements. The side which is very clear we should not be touching with our fingers.

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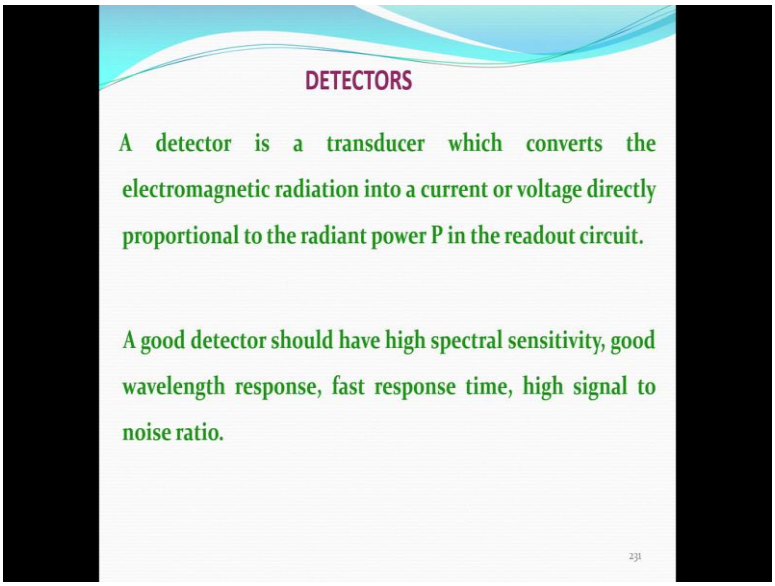

So, now we come to the detectors because that is all I can tell you about this sample compartment etcetera. Now once the radiation passes through a sample compartment, I told you that the both reference and example beams are brought into focus into a single this thing, and then that needs to be the sent to the detector to find out what is the absorbance.

Now, what is a detector? A detector is a transducer which converts electromagnetic radiation into a current or voltage, which is directly proportional to the radiant power in the read out circuit. Basically it converts electromagnetic radiation that is falling on a detector on the detector means a transducer which will convert into electromagnetic into current or voltage. So, a good detector what are the qualities of a good detector. It should have high spectral sensitivities, very small change in the wavelength the detector should be able to sense the change in the wavelength, and absorbance and then immediately record it. So, it should have good wavelength response and it must have fast response time it should it should there should not be any lag between measurement and the change that is known as response time.

So, the fast response time is also very important. And then what we need for a good detector is a high signal to noise ratio. So, if the noise is very less signal will be high; so the aim of all detectors is basically to generate signals which are having very stable current with the reference. Of course, with the sample also, but given the optics and

detectors this thing the noise will be essentially same for a blank reference sample as well as the res as the actual sample.

So, one of the important thing is high signal to noise ratio. Several 100 people have worked on this aspect in various detectors. Lot of data has been available and fantastic improvements have been made to reduce the signal to noise ratio. That is to increase their signal to noise ratio to reduce the noise and increase the signal.

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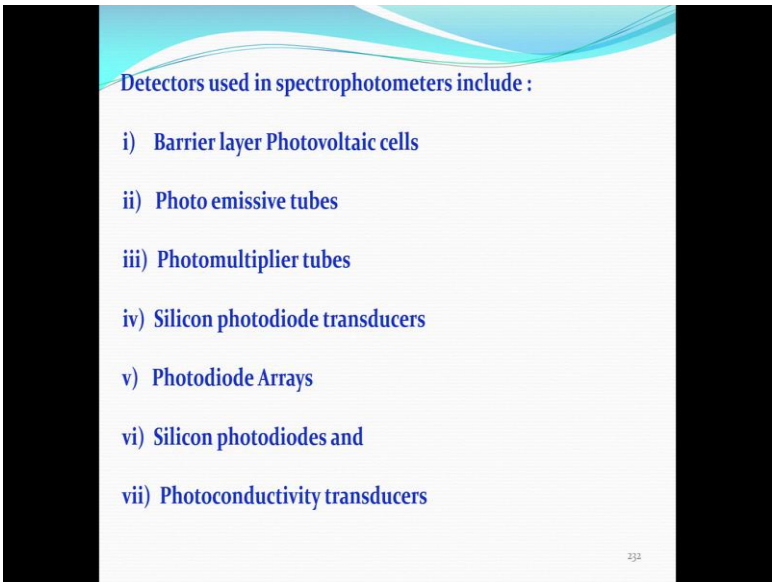

Now, what are the different types of detectors used in a spectrophotometer? There are about I had listed here about 7 detectors. And these 7 detectors are used singly or in multiples in any given spectrophotometer. All these are the various types and one is barrier layer cell, and second is photo emissive tube third is photomultiplier tube and then silicon photodiode transducers photodiode arrays silicon photodiodes and then photoconductivity transducers. All these detectors have got advantages as well as disadvantages in a given spectrophotometer. And many of them are high and also. So, whenever you need a specific function, you may have to go for different kinds of transducers in spectrophotometric cells.

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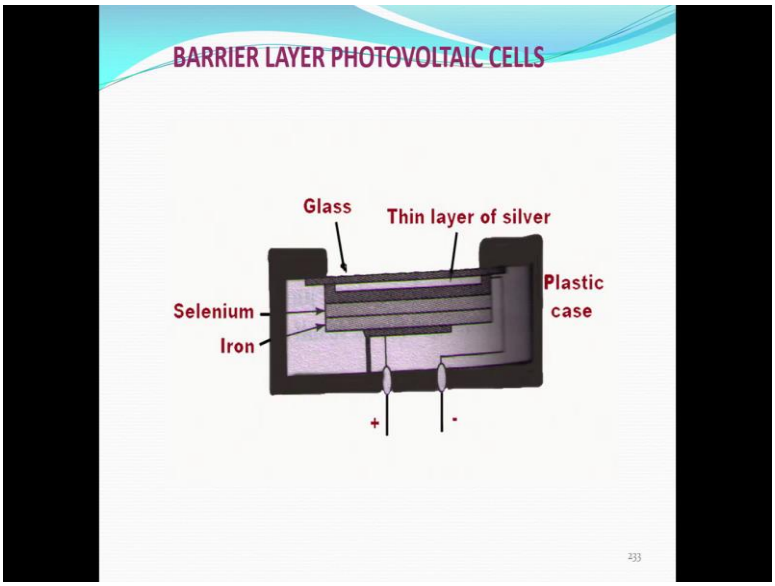

Now, let us look at some of these detectors and try to understand how they function. So, this is the picture of a barrier layer photovoltaic cell. This is a very low end equipment, a small transducer here you can see that there is an iron it is all and contained in an iron box on the outside and then inside I have an another box containing selenium and this is the base plate is iron and then the one more iron based plate and this there is an electrical connection from here and from the top. Here I have a glass plate and below that on the plastic on the selenium, selenium is coated on a glass plate and then, that is again covered with another glass thin layer of silver, and then closed with a glass and then whole thing is encased in a plastic cover.

So, one is a cathode another is an anode cathode is the bottom plate that is I have shown here and anode is from the top that is the selenium silver layer. So, how does it function if I know what it is?

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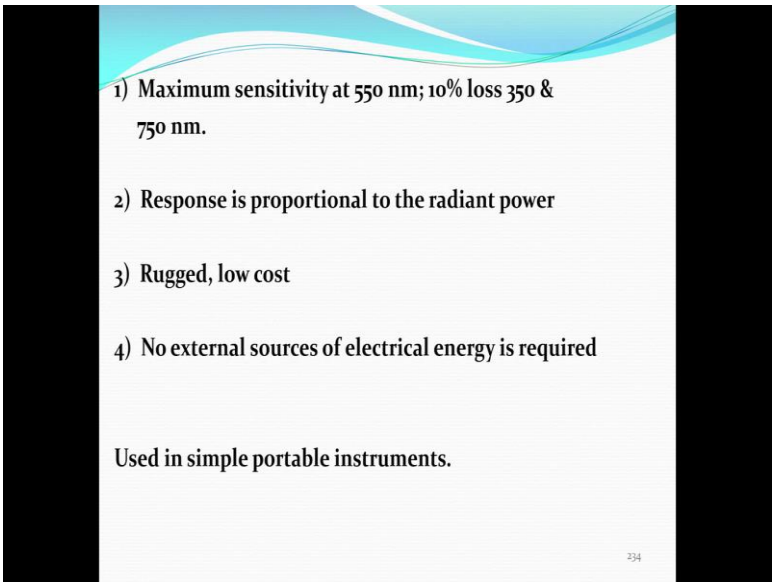

Then I can find out what happens is when the incoming radiation, when the incoming radiation is allowed to fall on the semiconductor plate, the radiation falling on the semiconductor plate will generate electrons. These electrons are carried out through an external circuit to the cathode. So, thermo current is generated and this current or voltage whichever is re required it will be measured in the detector, that for that you need another connection of measuring current or voltage, that I have not shown you in this because full circuit is not necessary because it is all part of electronics, which is advanced quite a lot.

Since last 50 to 60 years and the essential qualities of a barrier layer life. That I want to tell you now, if you look at this next slide the first quality is maximum sensitivity is only around 550 nanometers in a barrier layer cell. Usually at 350 10 percent loss is there and even about 30 to 35 percent loss is there up to 750 nanometers. So, basically it shows a maximum sensitivity, but you can still use it is a low end detector. So, response is proportional to the radiant power that is understood and it is rugged and very low cost. You can probably buy it in your town itself a barrier layer cell if you go to an electronic shop and then use it in your equipment.

So, no extra this does not require any source of external energy; that means, you do not have to supply any power for the material to function. So, this is therefore, this is used in simple portable instruments, so because it does not require any additional electrical

energy etcetera. So, high end it is not very accurate because loss of radiation is approximately 10 percent around 350 and 750 nanometers.

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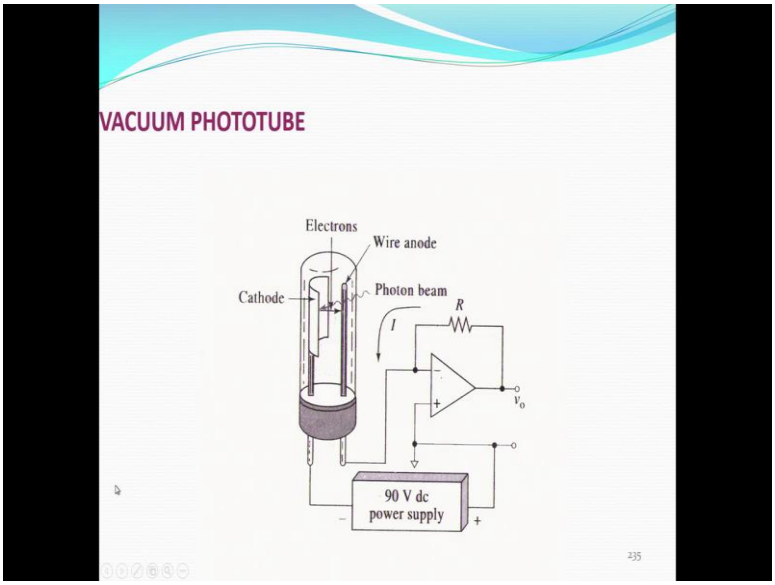

So, the next improvement came in the form of vacuum phototube. So, this is a slightly advanced version, where the electromagnetic radiation is made to fall on the anode and then this is the wire anode this is the cathode. The electronic radiation the electromagnetic radiation will be coming and falling on the cathode and depending upon the coating of the cathode work function of the material with which it is coated electrons will be released which will be connected collected on the wire anode. Anode is in the form of a wire, and cathode is in the form of a small cone concave plate. And the electrical connections from outside are made and then what you need is a 90 volts' dc power supply for this.

So, the essentially the mechanism is same when you are supplying this much of energy work function of the material is exceeded. So, whenever any radiation falls I have shown it through a wave here proton photon beam, it falls on the detector electrons are released they are collected and then they are they enter the circuit. And the whole thing is gives out a read out in the form of voltage or a current change in the voltage or current is noted.

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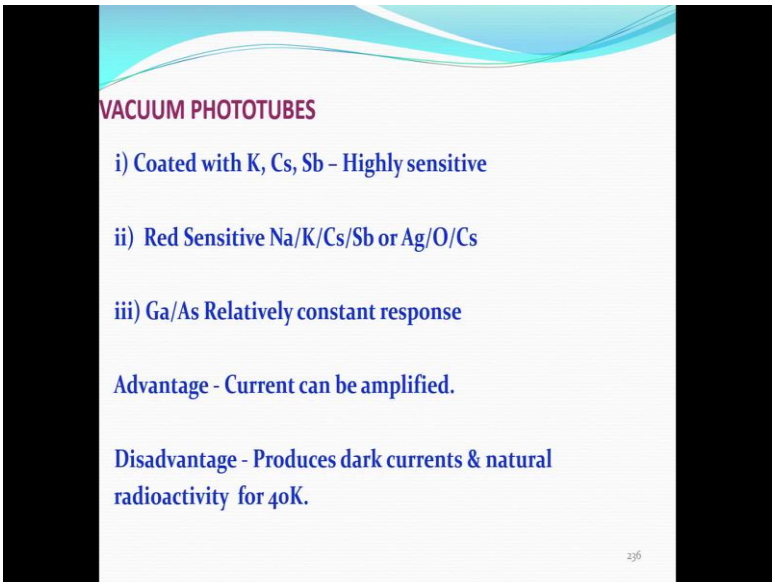

So, what are basically materials used in a vacuum photo tube. The I simple materials like potassium cesium antimony. Such materials are very useful highly sensitive also. And if you use a mixer of sodium potassium is same antimony or silver oxide and cesium you will get very good sensitivity in the red sensitive range. The red sensitive range covers the near infrared. It is not useful for UV or something.

And gallium arsenide, arsenic arsenide is a good detector used in several other instruments also not only spectra photometers, but several as infrared and things etcetera. And relatively it shows a constant response all over the electromagnetic radiation. The advantage is I can because I have a electronic circuit like this, I can amplify the current. So, the current can be amplified where as in a barrier layer cell, we cannot amplify the current. Therefore, the measurement for low concentrated solutions are possible in this, but it is also it also suffers from a disadvantage that it produces dark currents you do not know where the current is coming from and sometimes natural radioactivity also is possible.

So, but still the many of the in a calorimeters bench top calorimeters are fitted with vacuum phototubes. So, it is not a choice for low end instruments. Because barrier layer cells are not very useful, and vacuum phototubes are useful. So, it most of the instruments low and instruments come with phototube vacuum phototubes.

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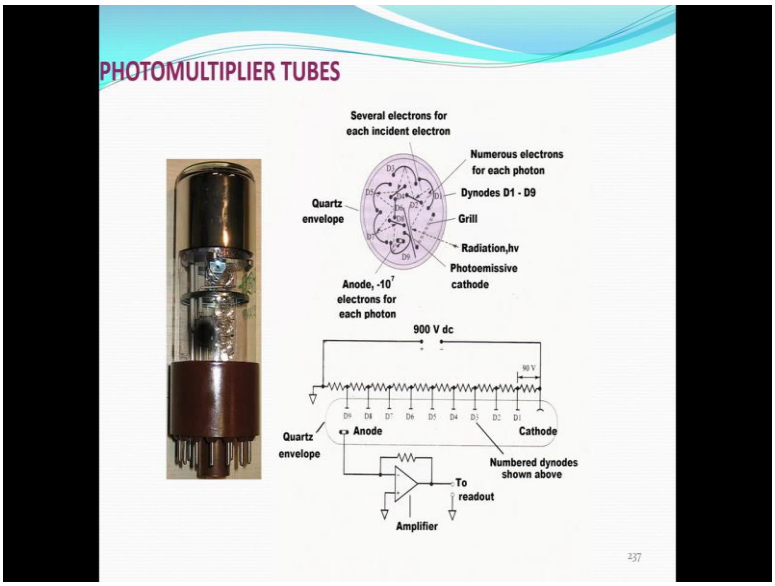

Now, the next bigger advantage is advance, is from photo multiplier tubes. Here what I have is essentially the same thing as last time photo metal here instead of one cathode I have number of cathodes in the same photo size same vacuum tube and connected to the power supply, but maintained at different voltages. So, what happen the radiation falling on the photomultiplier tube is array the phototubes cathodes are arranged like this 1 2 then 3 4 5 6 several dynodes. These are known as dynodes. And for each electromagnetic radiation coming from here it falls on the cathode and then it goes falls on a dynode.

And this dynode is maintained that a slightly positive value of voltage, here I have shown you 10 dynodes arranged in and bottom figure 1 is D 1 D 2 D 3 D 4 etcetera. And then what I have is and dyne anode here quartz cube hear these are all maintained at diff 90 volts difference. And then the difference keeps on increasing. The anode will reconnect the electrons and these collected electrons are again sent back to another electrode there is another anode which is at higher potential. So, this generates more electrons. And then it goes to D 3. Then again it comes back then it goes to D 4, D 5 like that several anodes maintained at different voltages there will there will be more and more number of electrons will be generated and very small absorbance differences can be calculated. Because here approximately 10 raise to 7 electrons are generated for each photon.

So, this is one of the most used detector not only in spectrophotometer, but in several other instruments also.

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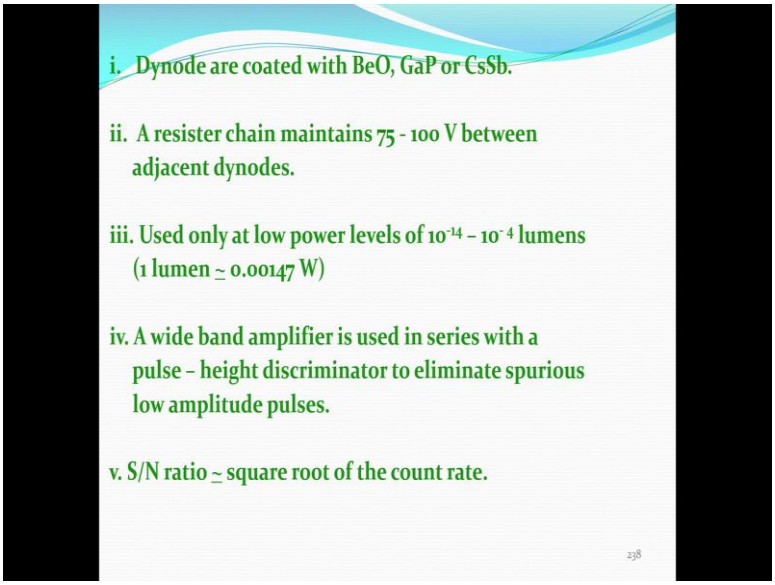

So, dynodes are normally coated with beryllium oxide, gallium phosphides, cesiums are antimony etcetera. Cesium antimonite and then we also have a register chain maintaining 75 to 100 volts between adjacent dynodes. So, they are used only at low power levels of 10 raise to minus 14 to 10 raise minus 4 lumens. And basically lumen is the measurement unit for the electromagnetic radiation 1 lumen corresponds to approximately 0.001475 watts. So, you can calculate from this figure what would be the lumen compound, if I tell you 3 watt bulb you can always calculate what would be the lumens.

So, it is a lumen is also a tool to evaluate the different kinds of bulbs which are used extensively in bulb industry. So, another thing is a wide band amplifier is used in series with pulse height discriminator to eliminate spurious low amplitude pulses. So, this is an important advancement. And in this case signal to noise ratio is approximately equal to the square root of the count rate. Now you remember that we have about 10 raise to 7 electrons per photon. So, s by n ratio would be essentially very good in such photomultiplier tubes. Even now 90 percent of the instruments use this photomultiplier tubes they are popularly known as p m t tubes photomultiplier tubes

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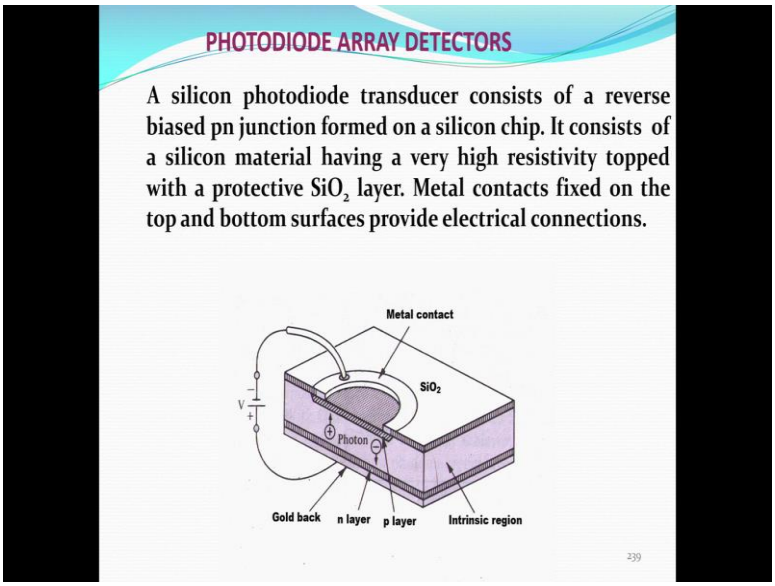

Now then the next is advance is photodiode array detector. Many of the instruments nowadays come with photodiode array detector because these things work at several wavelengths simultaneously. So, how does it work? Here I shown a picture it is a basically p n p semiconductor and there is a p layer there is 1 layer there is an in between layer and intrinsic region, and then there is a collector here metal contact and then a SiO 2 is used and there is a power supply for this. And then there is a gold back here at the bottom.

And this is how it works. A silicone photodiode transducer consists of a reverse based p n junction on a silicon chip. That is why we have a silicon dioxide chip here. It consists of a silicone material having a very high resistivity topped with a protective layer of SiO 2 silicon dioxide. Metal contacts are fixed on the top and bottom that is why we need a gold here gold back to which one collector is there and they put provide the electrical connections.

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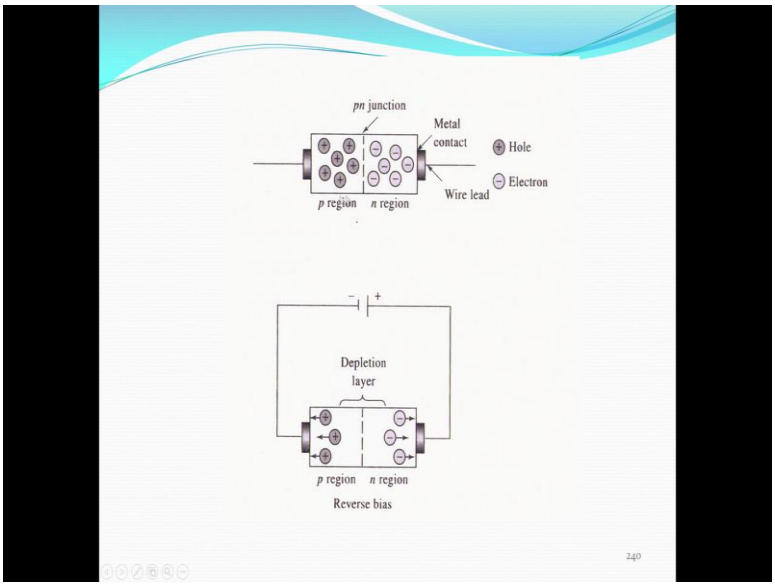

So, the mechanism is like this. Whenever the machine is switched on we have a p n junction, the electrons will be going one side and protons will be going on the other side. So, whenever these 2 are the contacts at the end on the top figure, I am talking about. And then there is a wire lead and then here there are holes in the p reason and electrons in the n region. So, whenever an electron leaves it leaves a positive charged hole. So, it is known as hole and the holes you will travel towards the anode and the electrons will travel to the cathode and the there is a depletion layer in between.

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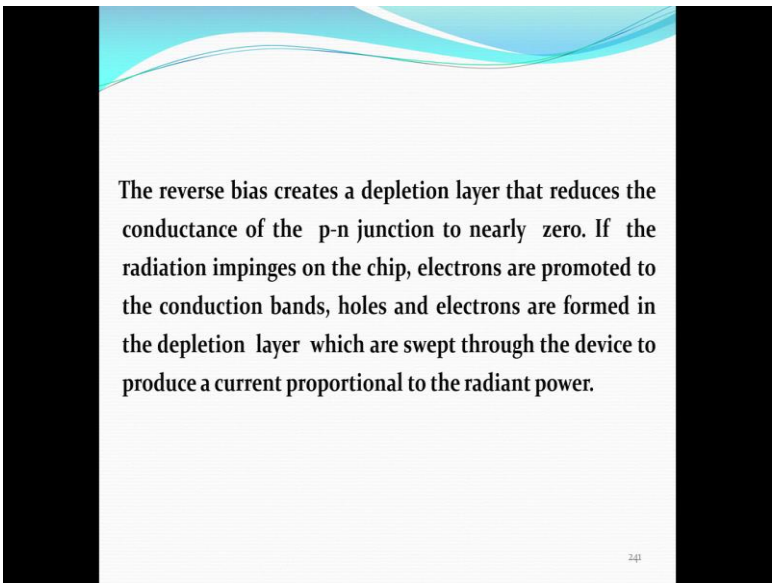

So, the current the reverse bias, this reverse bias where there is depletion is known as reverse bias. And this reverse bias rates a depletion layer that radius is the conductance of the p n junction to nearly 0. Because you can see that in the previous this thing the region where are all the holes and electrons are there, it is no more there here in this case at the bottom in the bottom figure.

So, the p n junction when the radiation impinges on the chip electrons are promoted to conduction bands, and holes and electrons are formed in the depletion layer, which are swept through the device to produce a current proportional to the radiant power. Very simple mechanism and it is basically a physics concepts n p junction. So, you can read more about it in physics textbooks. What are the p conductor and semi n semiconductor p n p junction transistors etcetera and very well-known technology, but it has been used in the instrument only since last 20 years?

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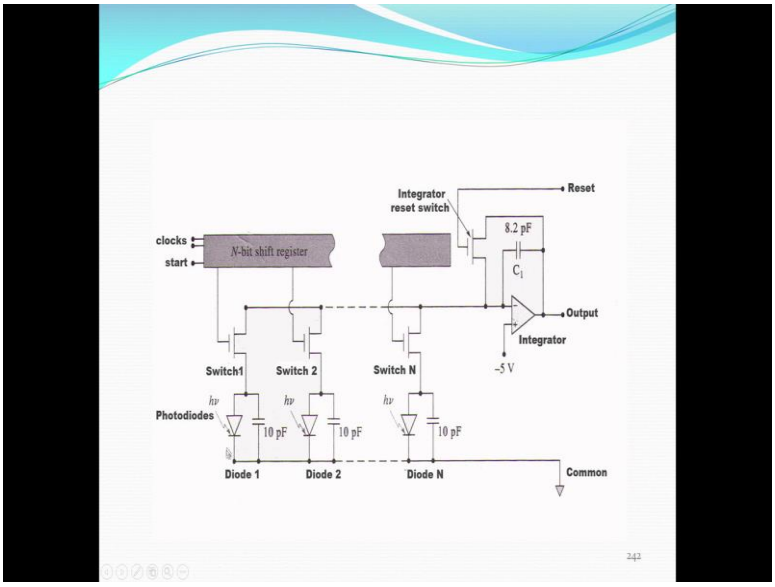

So, this is a sort of electronic configuration that is required for number of diodes, I can you have to remember that each diode is about 0.5 centimeter. It is a small button like thing and all the electrical connections are made like this, in this like what I have shown here switch one switch 2 switch n and number of switches, I can have number of diodes I can have and each diode can take up the current and the measurements can be made simultaneously at different wavelengths.

So, subsequently whenever you want to determine the spectrum, it have these it the radiations must be separated at each wavelength, and that is done by a Fourier transform method which I will not go into details, but the details are available in any standard mathematics textbook you can look it up.

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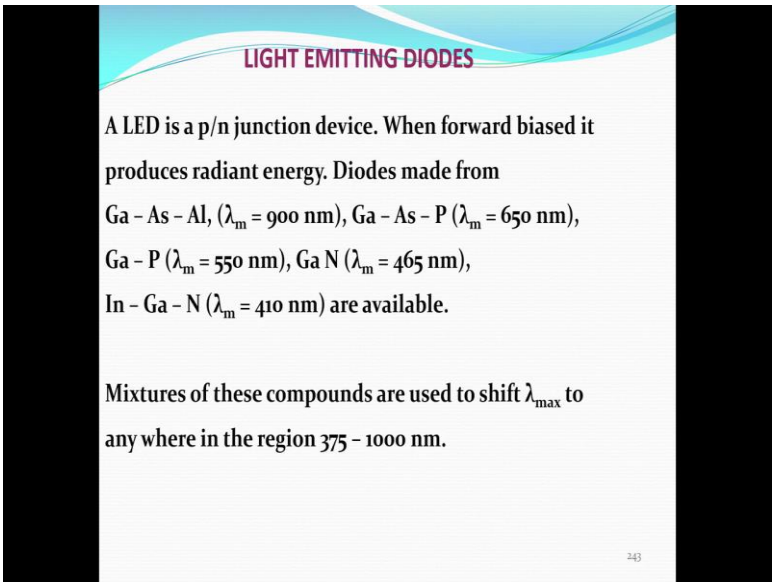

And then there are light emitting diodes which are essentially a p n junction again. The when forward base forward based it produces radiant energy, and these are the gallium arsenic aluminum gallium arsenic phosphide. These are all different coatings on the cathode. So, mixtures of these compounds are used to shift the lambda max to anywhere in the region 375 to 1000 nanometers.

So, light emitting LED, I think many of you know LED is from some other applications in your day today life. And now a days LED is have become available all over. And these light emitting diodes will emit radiation of specific wavelength. They will not be able to measure the whole electric cover the whole electromagnetic radiation that is a difference.

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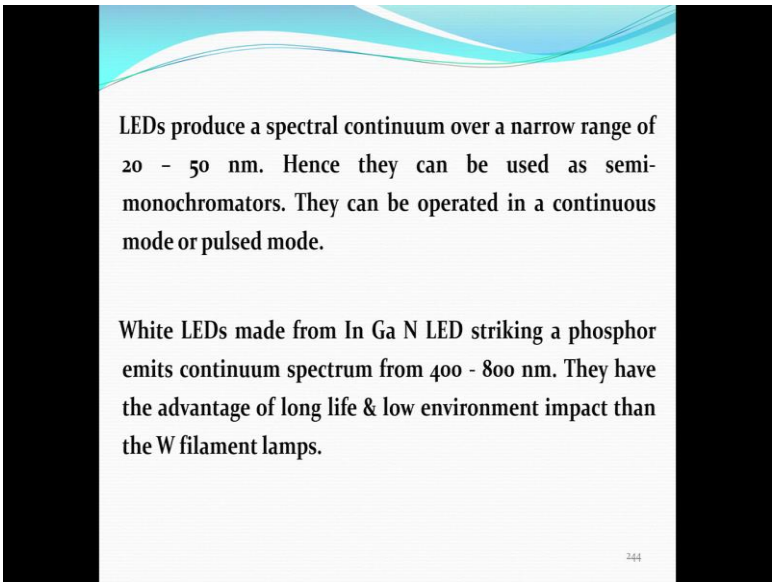

So, if you have a white LED then probably you may be able to measure the absorbance over a wide range. So, LED s normally produces a spectral continuum over a narrow range of 20 to 50 nanometers only. Hence they can be used a semi monochromators. They can be operated in continuous mode or pulse mode and I was talking to you about the white LEDs they are made from gallium and nitrogen LED striking a phosphor emit is a continuum spectrum for from 400 to 800 nanometers.

That is why now a day in a spectrophotometer the radiation sources are also made from LED which give continuum spectrum for 400 to 800 nanometer. I am not aware of LEDs giving you ultra violet light. That is a still a bit further away, but LED s emitting 400 to 800 nanometer have an advantage of long life and low environment impact, then the tungsten filament lamps. Of course, nowadays we understand that tungsten filament lamps are very energy guzzling instruments lamps and many of the radiation sources have been changed to white LEDs.

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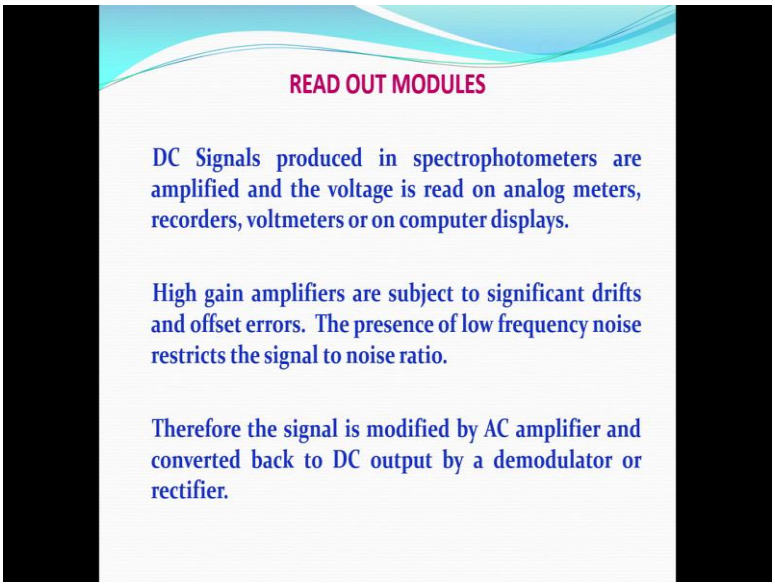

So, once we know all these kinds of detectors, the next job of a spectrophotometer that it should do is to give out absorbance or transmittance data right. So, what is important is the DC signals produced by the spectrophotometer are amplified. And the voltage is read on analog meters recorders voltmeters or on computer displays. These are the read out ways; that means, it is basically a display of the absorbance or transmittance whichever is occurring. So, normally there is a certain amount of electronics involved in read out modules also. And high gain amplifiers are sometimes employed. The presence of low frequency noise of course, restricts the signal to noise ratio.

So, the signal is modified by an AC amplifier and converted back it to DC output, because what we need whenever we need a spectrophotometer measurement, we need a DC output not AC output. So, that is done by a demodulator or a rectifier. So, you may read more about the demodulator and rectifier in the electronics and physics books, but they are very advantageously employed in spectrophotometers for measurement of read out.

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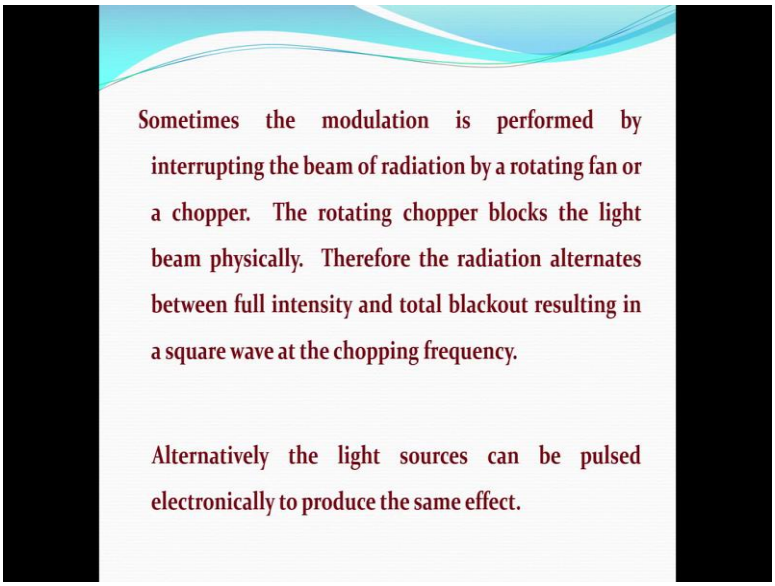

Now, sometimes the modulation is performed by interrupting the radiation by a rotating fan or a chopper. The rotating chopper blocks the physical beam blocks the beam physically. And the radiation alternates between 0 intensity and full intensity. And total blackout sometime you know on and off on and off on and off like that. And that results in a square wave at the chopping frequency.

So, this square wave can be have converted into DC. The light sources can be pulsed electronically also to produce the same effect. So, instead of having a mechanic rotating chopper which is a mechanical device they can be converted into auto electronic this thing for better the signal handling.

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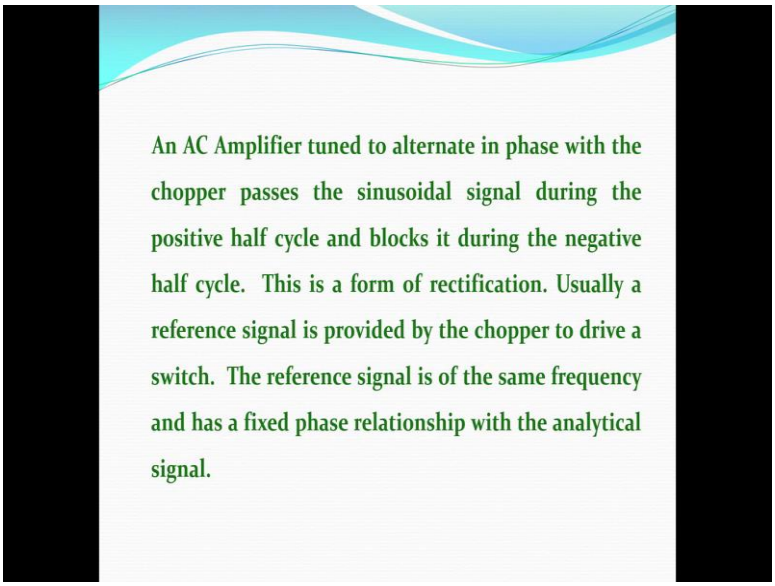

So, an AC amplifier how do you do it we take an AC amplifier tune it to the alternate to the alternate phase with the chopper that passes the sinusoidal signal during the positive half cycle and blocks it during the negative half cycle. This is some form of rectification only usually a reference signal is provided by the chopper to drive a switch. So, this switch is made automatic the reference signal of the same frequency has a fixed phase relationship with the analytical signal which we will keep on giving you the same amount of absorbance over a several seconds measurement.

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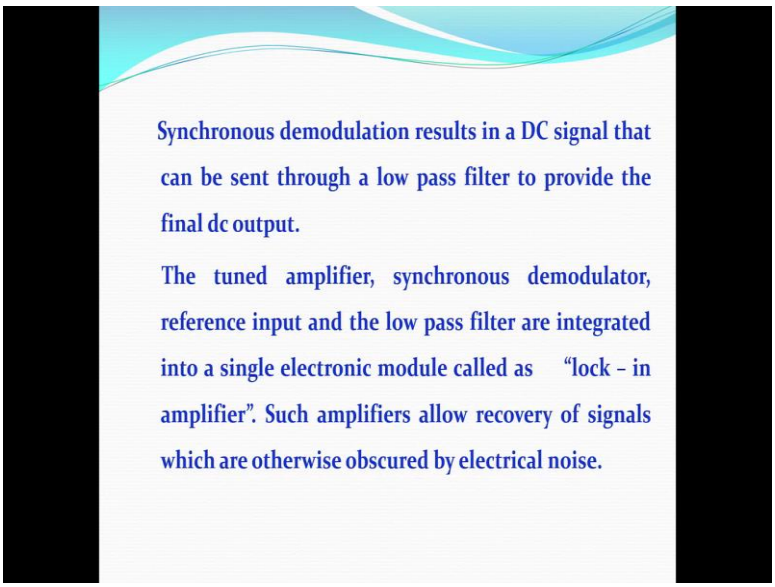

So, synchronous demodulation and also results in a DC signal. That can be sent through a low pass filter to provide the final DC output. So, sometimes what we do is that we make a compact device using a tuned amplifier synchronous demodulator reference input and low pass filter they are all integrated into one small single electronic module called as lock in amplifier. So, whenever you want to buy an instrument sometimes the instrument seller will say serve we have got lock in amplifiers. So, you do not have to worry much about the signal display and signal quality such amplifiers normally allow recovery of signals also which are otherwise obscured by the electrical noise. This is an advantage lot of people claim.

But essentially, So, long as you have the modulation done by chopper or the or this lock in amplifier unit essentially the result is same.

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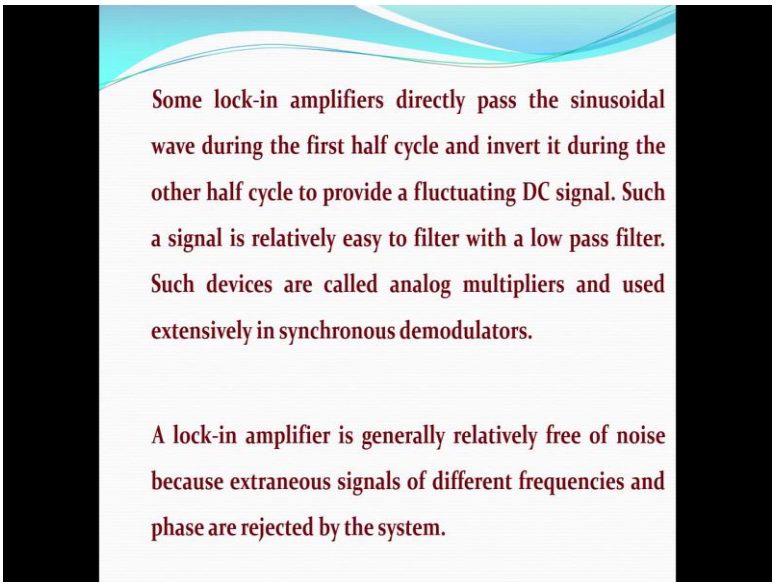

So, some locks in amplifiers directly pass and others are doing not. It is generally relatively free of noise because extraneous signal of different frequencies and phases are rejected by the system itself during demodulation. So, this is the advantage.

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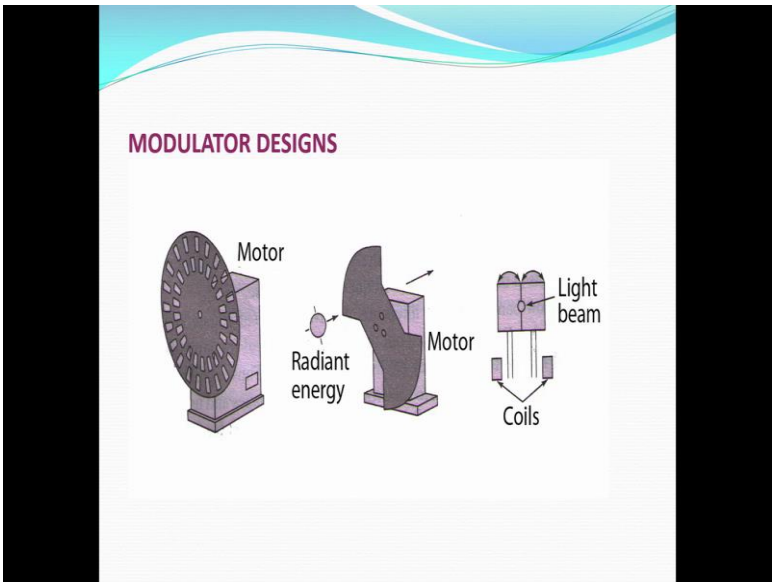

So, these are the some of the modulator designs. I have shown you here and this is first one is a motor second one is a this is radiant energy, this keeps on rotating this is a wheel which will give you alternate slots and blank spaces. And here it is the fan like arrangement and the light beam is shown here, with coils this is electro for electronic measurement.

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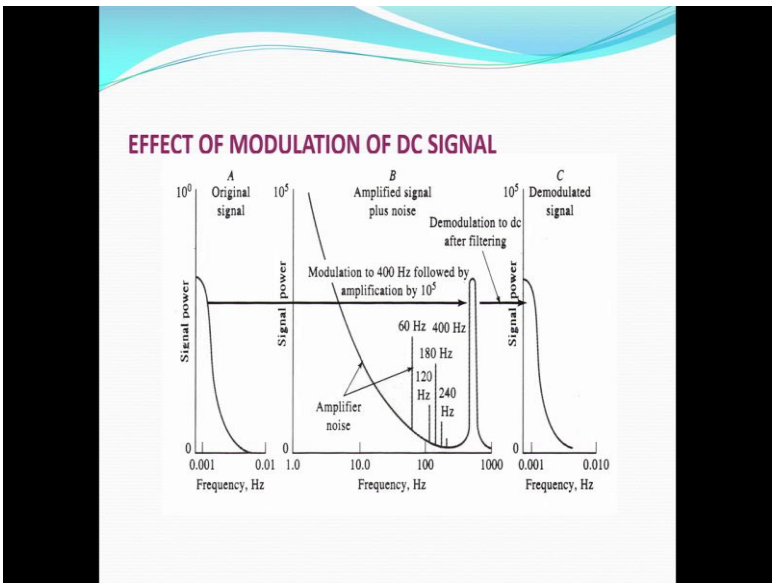

So, effect of modulation or if DC signals I have shown here. And you can study this for different wavelengths that are formed that are required over a signal. Here I have shown

the original signal on the left side amplified signal and noise and demodulated signal also I have shown here. We can see that the lot of problems associated with amplified signal and noise are removed in the final demodulated phase in the third part that is in the signal.

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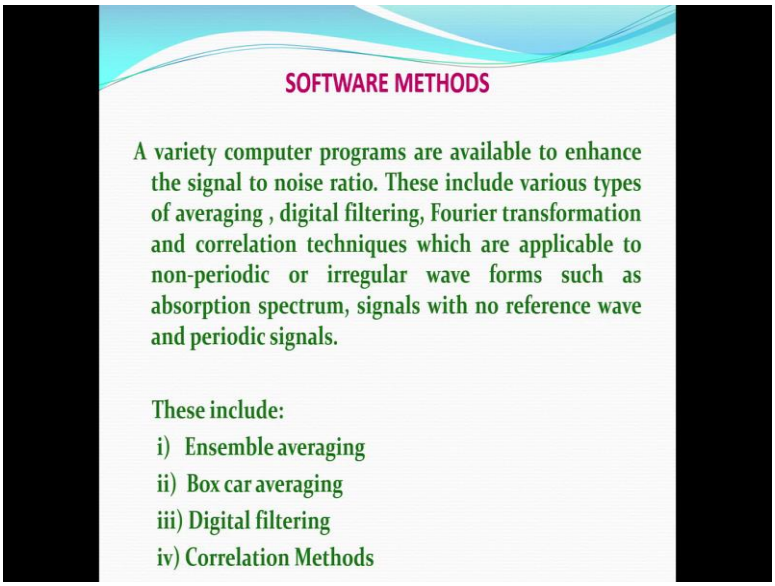

So, once you have the data, then the lumen or voltage or whatever you measure the data is processed averaged digital filtering is done Fourier transforming for transformation is possible, and correlation techniques and all these things are applicable to non to non-periodic or irregular wave forms such as absorption spectrum. So, signals with no reference wave and periodic signals are also can be handled because the signals with no reference mean they would have stored the reference signal and then it will be used for averaging. So, there are several types of averaging a spectra spectrum that is known as ensemble averaging box car averaging digital filtering and other correlation methods. I will not go into the details of this because basically this course refers not to the instrumentation, but the technique has a whole.

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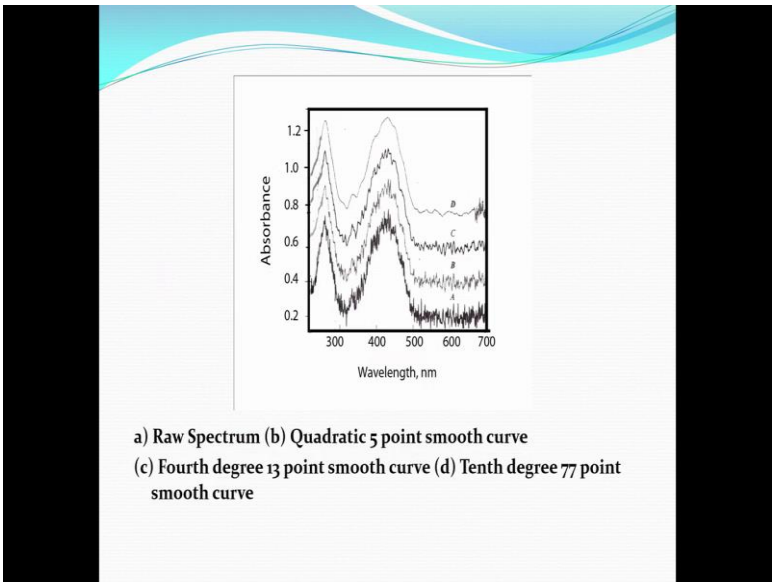

So, we will continue our discussion, but before I can close this session, I want you to see the raw spectrum here in this figure. And then b is a quadratic 5 points more than more than curve and c is a 13 point, and d is a 27th degree, 77 degrees 0.1. See how smooth the curve looks in this case.

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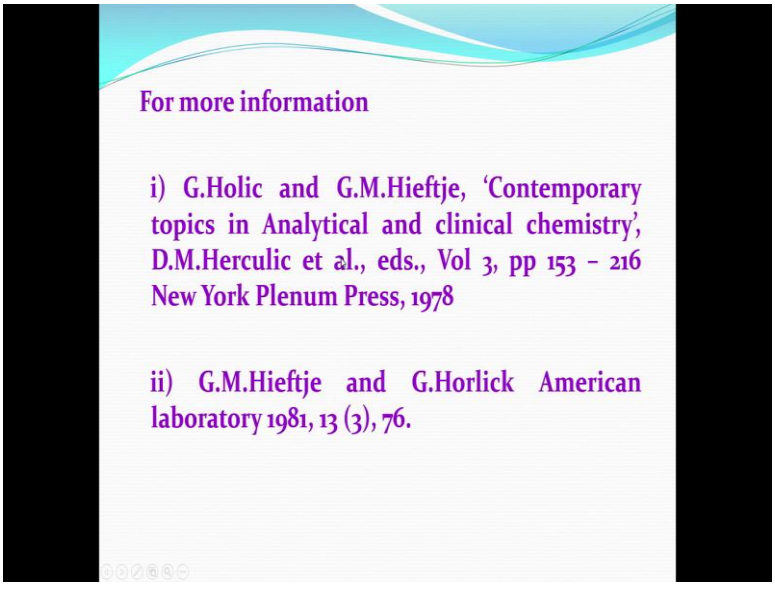

So, the for more information, I refer to a beautiful paper by Hieftje G and G Holic, that is contemporary topics in analytical and clinical chemistry and American laboratory also in 1981.

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So, we will continue our discussion with the commercial instruments and spectrophotometer as a tool in the analysis after sometime.

Thank you.