Modern Instrumental Methods of Analysis
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Lecture No. # 09
Ultraviolet and Visible Spectrophotometry-5 Instrumentation

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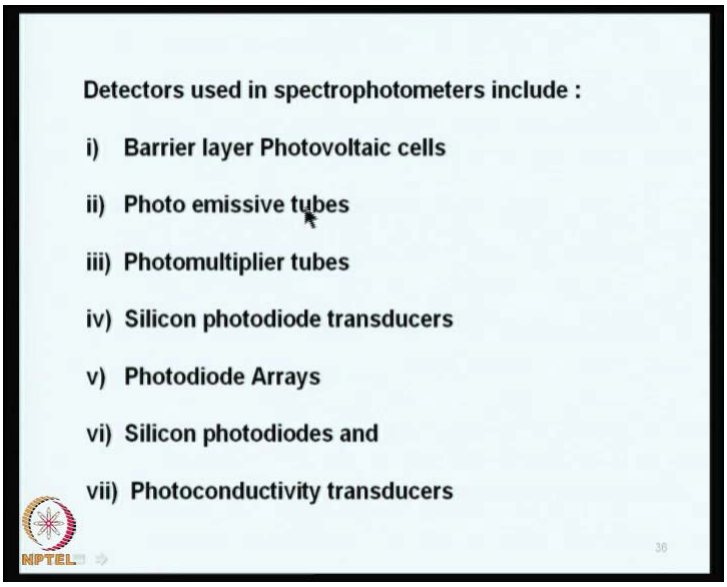

We were discussing detectors used in the spectrophotometers and these includes barrier layer photovoltaic cells, photo emissive tubes, photomultiplier tube, silicon photodiode transducer, photodiode arrays, silicon photodiodes and photoconductivity transducers.

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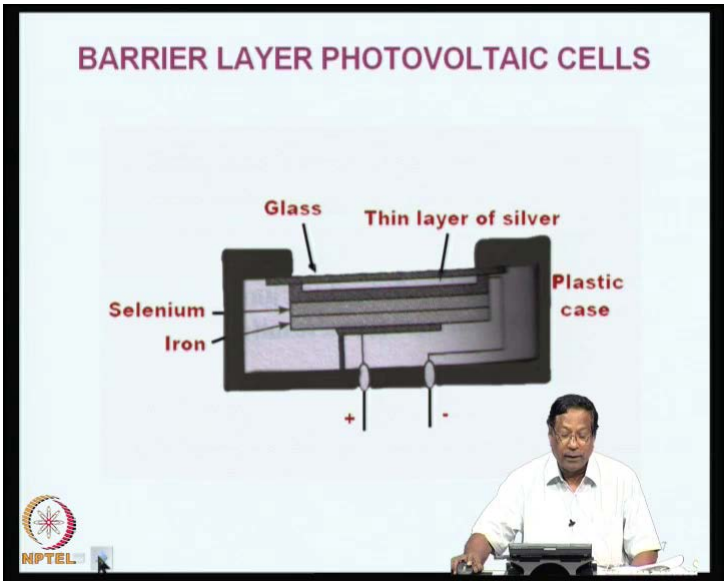

This is a barrier layer photovoltaic cell. Basically it contains two glass plates. One glass plate is here and another is here. It contains a thin layer of silver and a plastic. Everything is coated in plastic and this is a selenium layer which will release electrons when light falls on them and bottom one is iron plate and this iron plate acts as a conductor.

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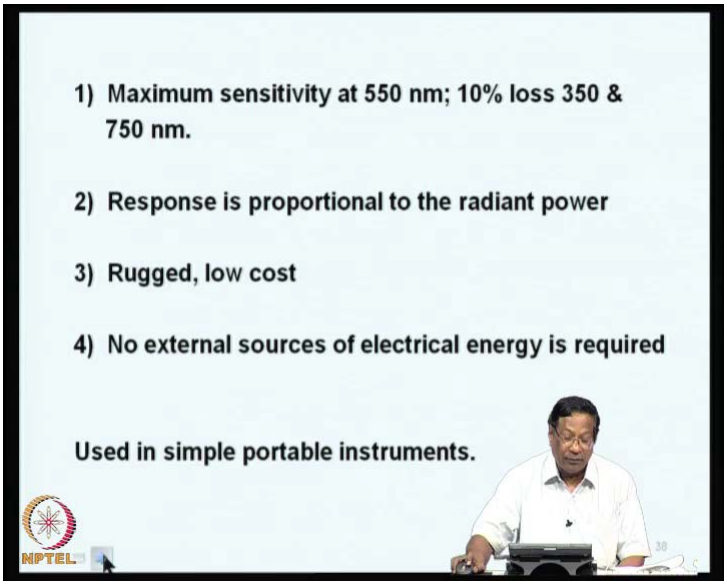

The whole equipment is encased in a glass cell and this photovoltaic cell shows maximum sensitivity at 550 nanometers approximately, 10 percent loss occurs around 350 to 750 nanometers. Therefore, it is basically very rugged instrument and very low

sensitivity and the response is proportional to the radiant power and no external source of electric energy is required. Such detectors are used in simple portable instruments.

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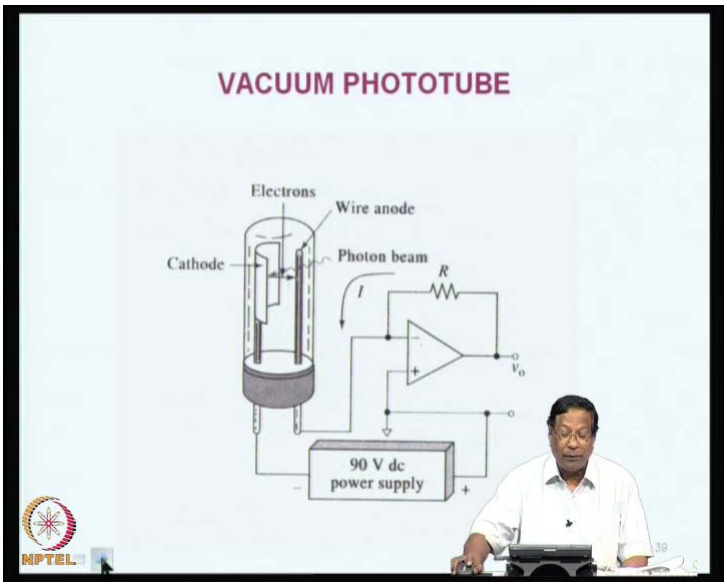

Then, we have vacuum phototubes or photo emissive tubes as they are called. Here you can see that there is a cathode here and wire anode is here. A photon beam falls on that and releases the electrons and the electrons are collected at the wire anode. A whole thing is under vacuum and essentially, its current can be modified and amplified using the 90 volt DC power supply. That means if the sensitivity is low, you should go for vacuum phototubes.

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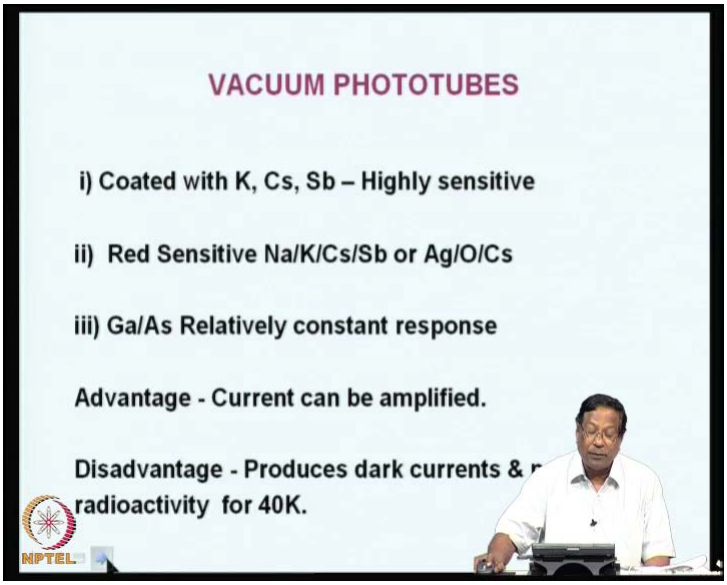

So, the vacuum phototubes cathodes are coated with potassium, cesium, antimony etcetera. These are very highly sensitive for release of the electrons. When the photo, when the light falls on them, red sensitive, sodium, potassium, cesium, antimony are also used or silver and then, oxides cesium etcetera are also used for coating.

Now, the other recently introduced materials include gallium, arsenide, these give relatively constant response. The advantage is current can be amplified, but the disadvantage is it produces dark current and 40 K, 40 potassium, it gives you some amount of natural radioactivity which is not good for usual maintenance.

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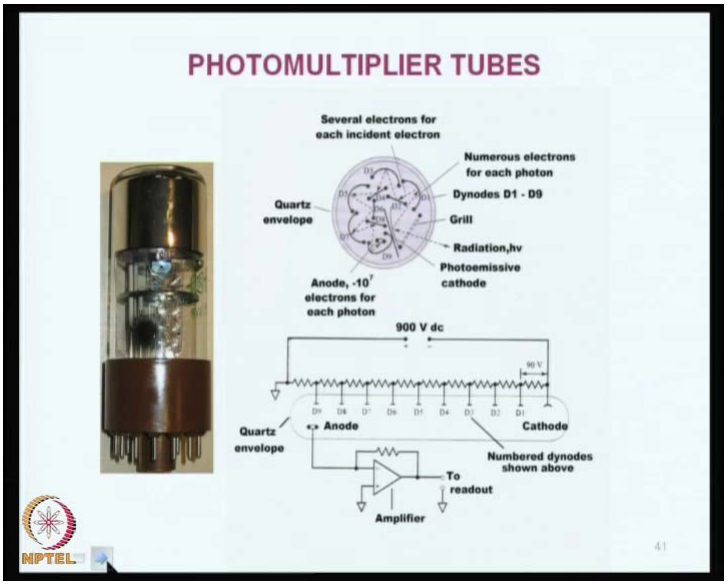

Then, we have a photomultiplier tube. This is used for very low light sensitivity. For example, longer wave length regions. So, here it contains a number of electrodes cathodes called as dynodes.

Here in this figure, you will see that numerous electrons are generated for each photons and the photons are collected on the mirrors, again directed to dynode, another dynode, again collected, again directed to another diode. Like that there could be about 9 or 10 dio dynode which will keep on increasing. Empty tubes, photomultiplier tubes contain a number of dynodes coated with beryllium oxide, gallium, phosphate and cesium antimonite. Each dynode is maintained at these are the dynodes d 1, d 2, d 3, d 3, d 4, etcetera. Each dynode is maintained at a higher potential than the previous one. So, it varies from up to 900 watts DC and cathode is here and anode is here. Numbered dynodes are shown above here and they are all connected in the electro, the electronic circuit which can be amplified. The current can be amplified and it can be taken to read out.

This is the figure of, this is how a dynode looks, P m photomultiplier tube looks and you can see the dynodes are here 1 2 3 4 5 6 like that and several electrons; each dynode releases more electrons than the previous one. So, the electrons will keep on increasing. That means the current also will be increasing. So, these are used mostly for low wave length regions.

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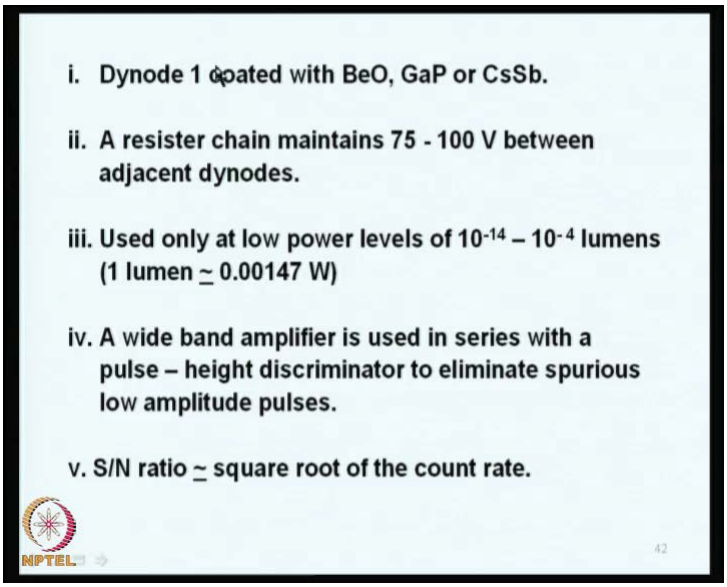

Dynode 1 is coated a resister chain maintains 75 to 100 volts between edges and dynode. That means each dynode is maintained at 75 to 100 volts at higher potential. So, these are used only at lower power levels of 10 raise to minus 14 to 10 rise to minus 4 lumens. You will remember that 1 lumen corresponds to 0.00147 watts. So, a wideband amplifier is also required. It is to be used in series with a pulse height discriminator to eliminate spurious low amplitude pulses. The advantage is signal to noise ratio which corresponds to square root of the count rate.

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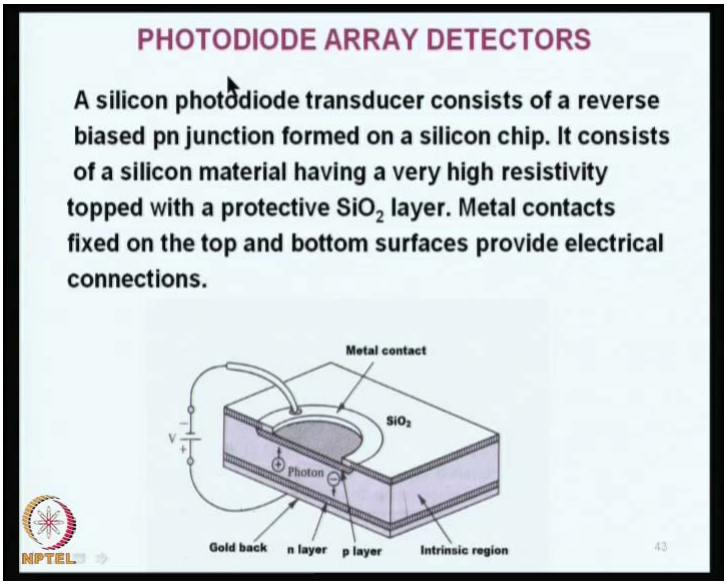

So, nowadays there are photodiode array detectors. These are nothing, but a silicon photodiode transducer consisting of a reverse biased P N junction and that is formed on a silicon chip. It consists of a silicon material having a very high resistivity topped with a protective SiO2 layer. So, metal contacts are fixed here. You can see metal contact is here, another metal contact is here and then, this is intrinsic region. There is N layer and there is P layer. This is the gold bag coated. So, the bottom surface provides electrical connections.

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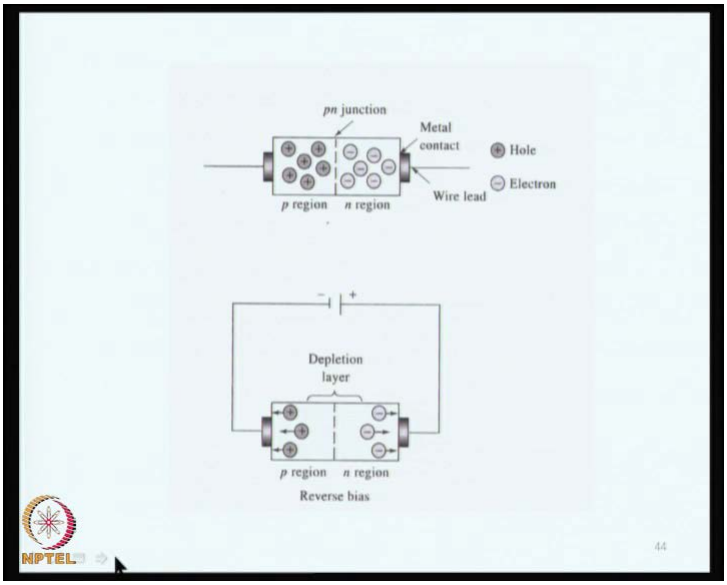

Here, you can see that normal function of a P N junction is like this. There are positive holes and then, negative electrons etcetera and there is a metal contact here and wire lead is taken from this side. These are holes, these are electrons. So, when this is operated under reverse bias, then what happens is this region gets depleted, the P region and N region. So, essentially, there is no current passing through the contacts. Now, when there is contact, there will be electrons and electrons will be released, holes will be generated.

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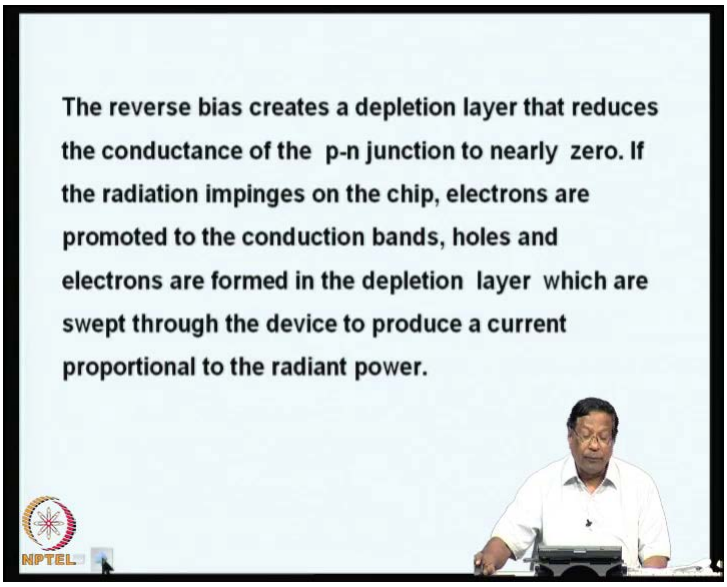

The current will start slowing. Suppose the reverse bias is applied, then the electrons are promoted to the conduction bands and holes and electrons are formed which are swept through the device to produce a current proportional to the radiant power.

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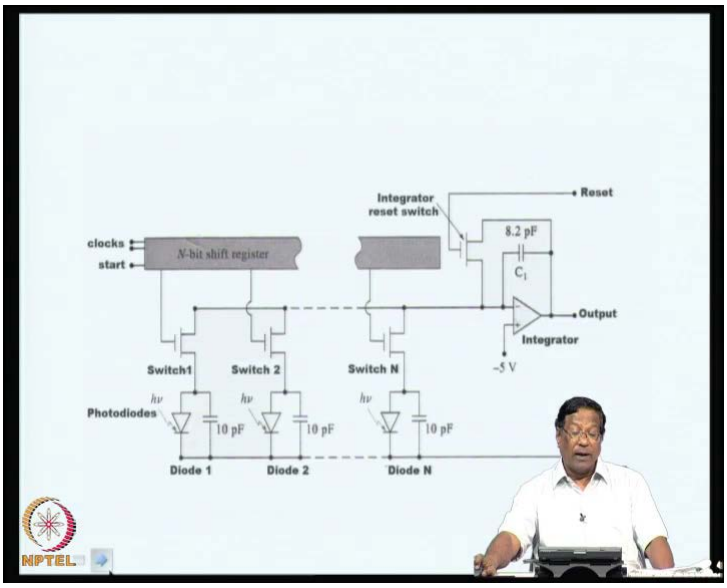

This is the circuit for operating the photodiode arrays. Photodiodes are here and then, they are all operated in series and region in the range of 250 to 500 mille amperes per watt in a per watt inverse across the visible spectrum. So, this is at least one order of magnitude higher than the photo emissive tubes, but several orders of magnitude lower than the P m T tubes. So, silicon chips are approximately of the order of about 0.0 to 5 millimeter square, that is small one and the whole diode is also of the same size. So, a number of photodiodes can be fixed in a two-dimensional pattern on a single rectangular semiconductor chip.

So, the output from each one can be collected sequentially and simultaneously or simultaneously sequentially. Also, you can collect, simultaneously also you can collect using integrated circuit like this and each diode is parallely connected to a 10 P f capacitor and sequentially connected to N bit shift register and the transistor switches.

The shift register you can see here usually close sequentially, closes each of these switches numerically momentarily causing the capacitor to be changed to approximately 5 volts which then creates a reverse bias across the P N junction upon impingement of the radiation in the depletion layer or P region here. In this region, electrons formed will be collected and which partially discharges the capacitor in the circuit. The capacitor charge lost in this way is replaced during the next cycle. So, it can be made working alternately on off on off like that and the resulting charging current is integrated by the

preamplifier circuit. What you have seen here and which produces a voltage proportional to the radiant intensity after amplification, the analog signal is converted into a digital signal and passed on to the computer.

So, basically we are looking at different kinds of detectors. One is photovoltaic barrier layer cell; another is photo emissive tubes and photomultiplier tubes. Recently for extraordinary circumstances when we had to collect multichannel responses, we go for diode array detectors.

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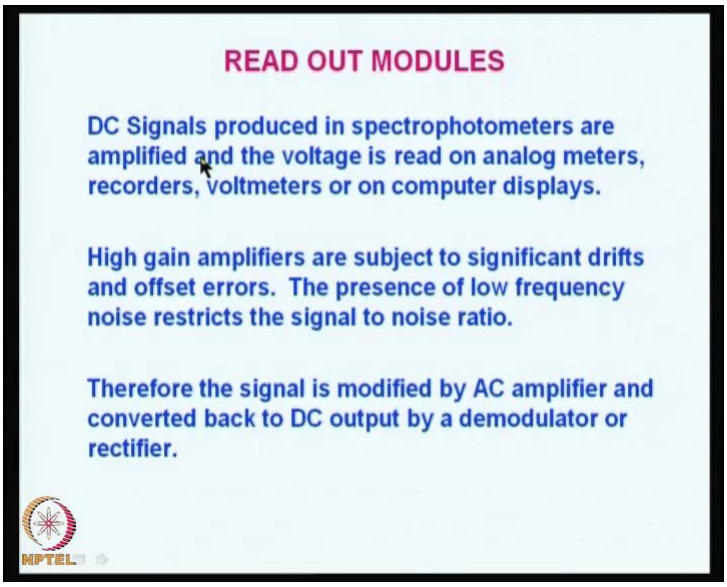

Now, what about the read out modules? How do you read the signal? The DC signals produced in spectrophotometers are usually amplified and the voltage is read on the analog meters or it can be a recorder or a voltmeter or it can be passed on to a computer through RS232 connection and then, computer displays. High gain amplifiers are usually subject to significant drifts and offset errors. Now, the presence of low frequency noise restricts the signal to noise ratio.

This is one of the biggest problems in spectrophotometer because you have to measure the difference between the noise as well as the signal. Therefore, the signal has to be modified by an AC amplifier and converted back to DC output by a demodulator or a rectifier.

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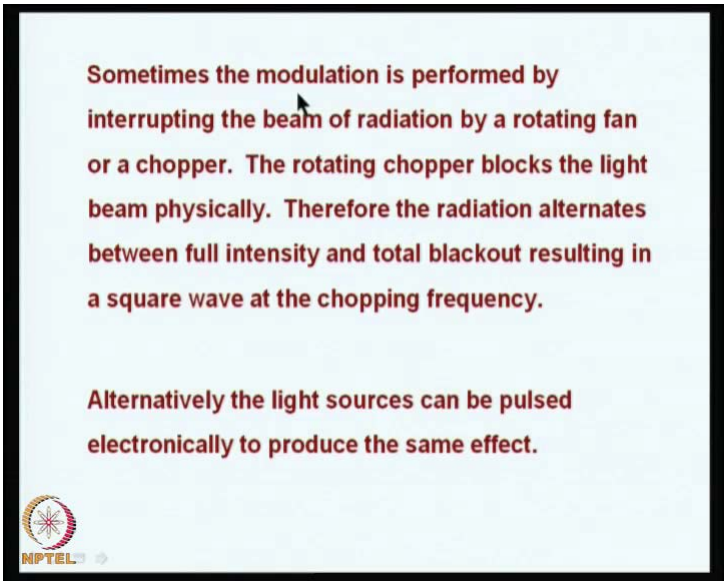

Sometimes the modulation is performed by interrupting the beam of radiation by a rotating fan or a chopper. What you do is the beam or radiation is passing, we just put a fan, so that you can, the beam can be cut physically. You can see the figure here.

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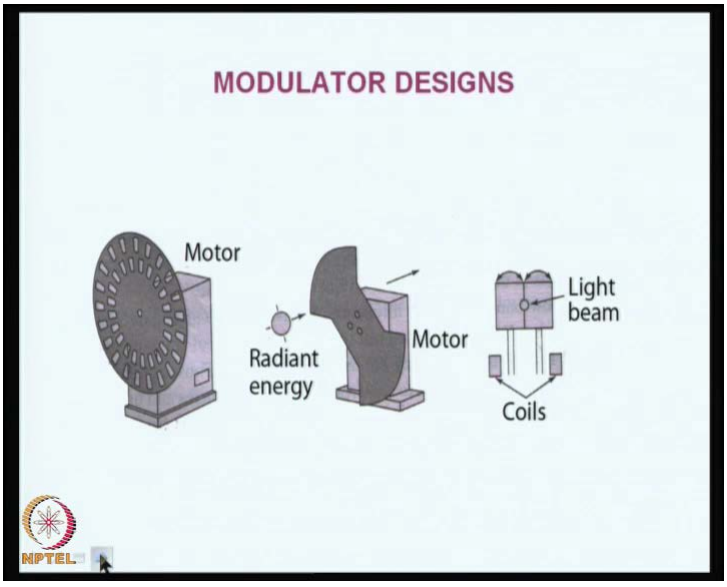

Here, this is a fan. You can see that there are number of holes here and when this fan rotates, light will be passing alternately through this hole and then, it will be blocked again. It will be passed through this hole like that. There are number of possibilities and this is a middle figure is another design or you can do it electronically with a light beam and coils. So, it will just flip-flop here, this side, this side, this side, this side etcetera.

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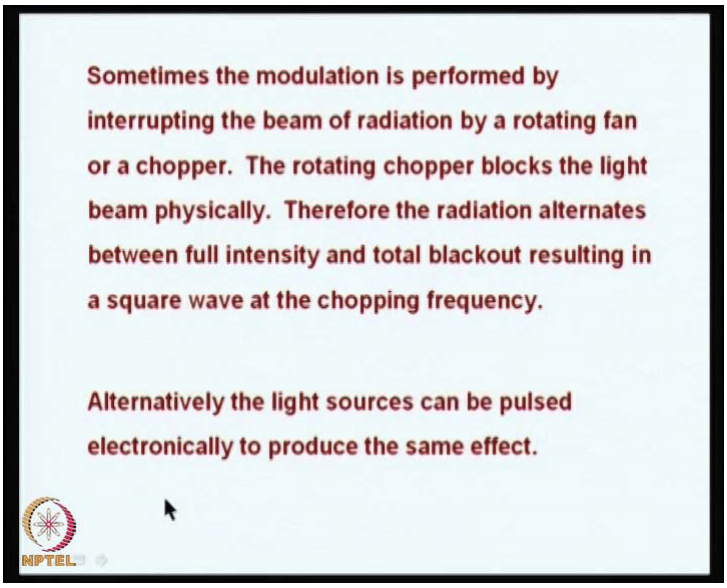

So, this is the type of modulation we normally look for when you use a rotating fan or a chopper. So, alternately they can be done electronically also which we saw that earlier.

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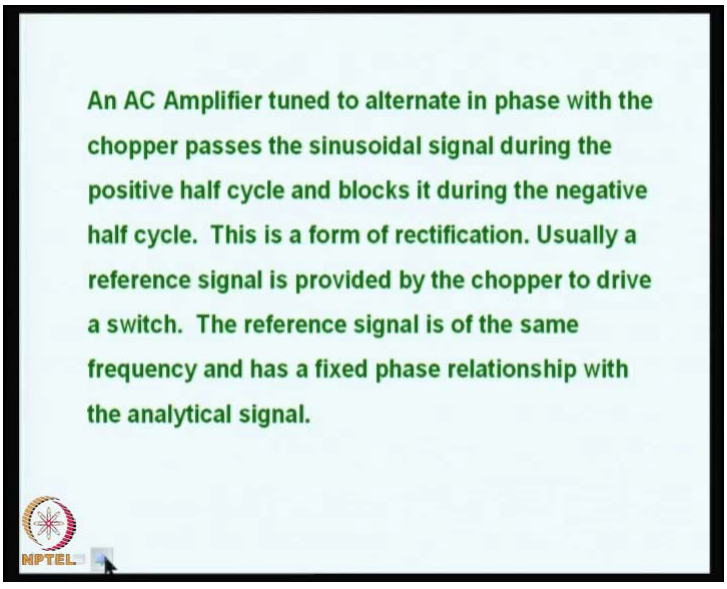

So, an AC amplifier must be tuned to alternate in phase with the chopper that passes the sinusoidal signal during the positive half cycle and blocks it during the negative half cycle. This is a form of rectification basically. Usually a reference signal is provided by the chopper to drive a switch. So, the reference signal is of the, should also be of the same frequency and has a fixed phase relationship with the analytical signal.

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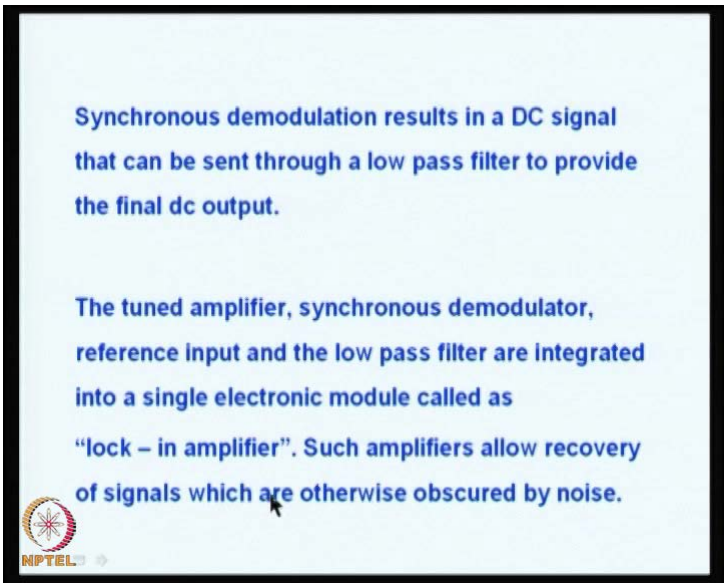

This is very important because unless it is synchronized, you will not get a decent signal at all. So, what you need is a synchronous demodulation and this synchronous demodulation results in a DC signal that can be sent through a low pass filter to provide the final DC output.

The tuned amplifier, synchronous demodulator, reference input and the low pass filter, all these are integrated to form a single electronic module called as lock-in amplifier. Such amplifiers allow recovery of signals which are otherwise obscured by the noise.

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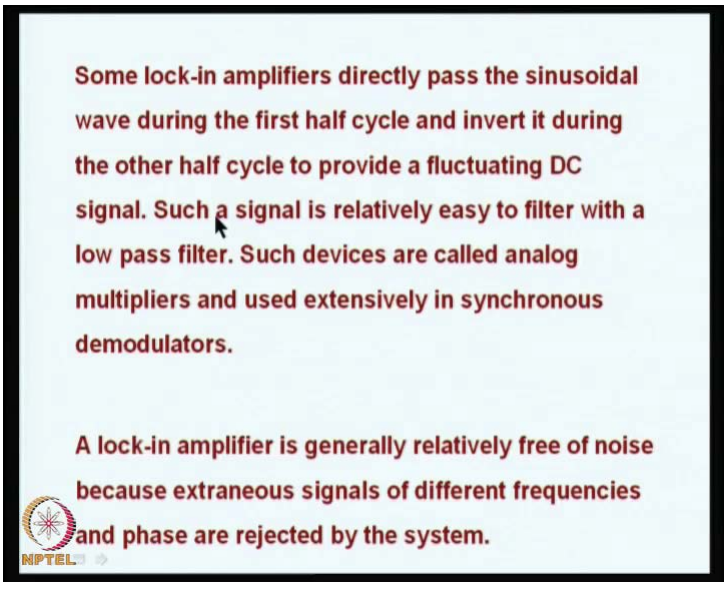

Some lock-in amplifiers directly pass the sinusoidal wave during the first half cycle and invert it during the other half cycle to provide a fluctuating DC signal. Such a signal is relatively easy to filter with a low pass filter. Such devices are called analog multipliers and used extensively in synchronous demodulators.

A lock in amplifier is generally relatively free of noise because extraneous signals of different frequencies are automatically cut off and phases are rejected by the system itself. Now, these are the modulator designs we have already seen.

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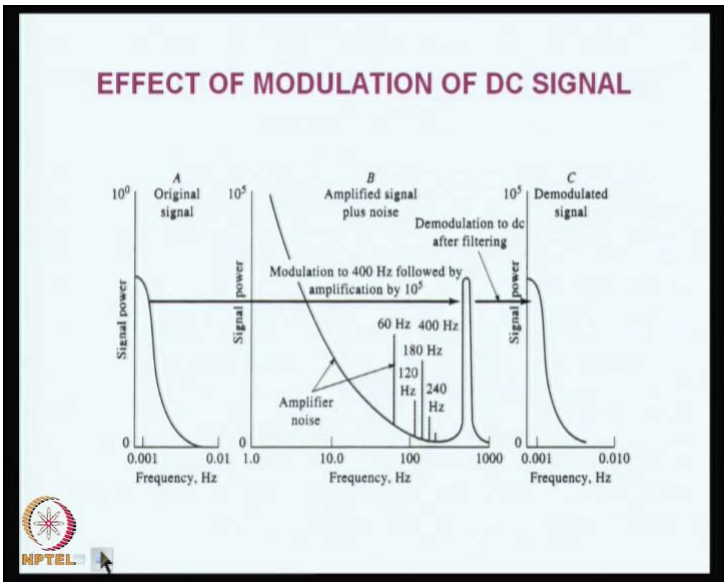

What happens to the signal effect of modulation of a DC signal? I am trying to show you here basically. What I am showing on the left side here is the frequency and signal power and here, it is amplified and then, modulated to 400 hertz followed by amplification by 10 raise to 5. Now, when we will do this, you do see some amplifier noise here a little bit, but all these things will get demodulated. That means removed from the system and then, what you get here is a beautiful demodulated signal and this is the function of the amplifier, lock-in amplifier. Now, there are several possibilities.

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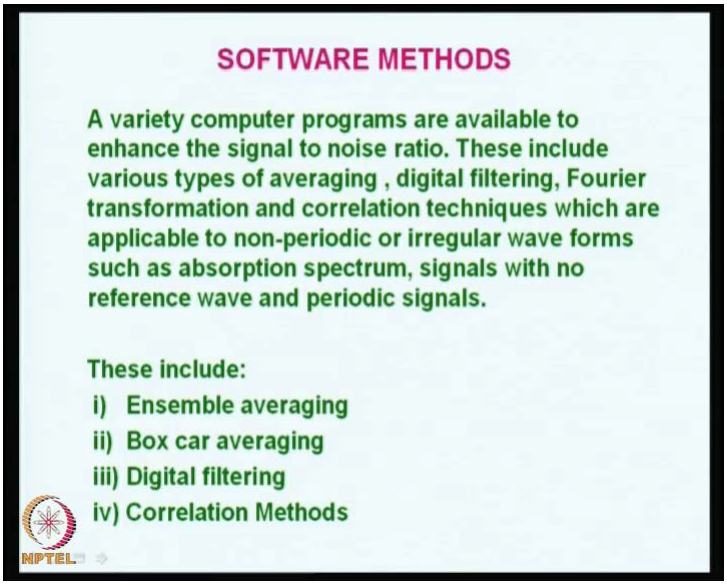

A variety of computer programs are available to enhance the signal to noise ratio. This is important because the signal, the noise determines the basic value of the absorbance what you are going to measure because more than less than that you will not be able to attain at all. So, these include various types of averaging, digital filtering and Fourier transform and correlation techniques etcetera which are applicable to non-periodic or irregular wave forms such as absorption spectrum, signals with no reference wave and sometimes periodic signals also.

So, the techniques include ensemble averaging, box car averaging, digital averaging and correlation methods. We will not go into details of these things except to say that the signals, how they can be used by using different kinds of the filtering.

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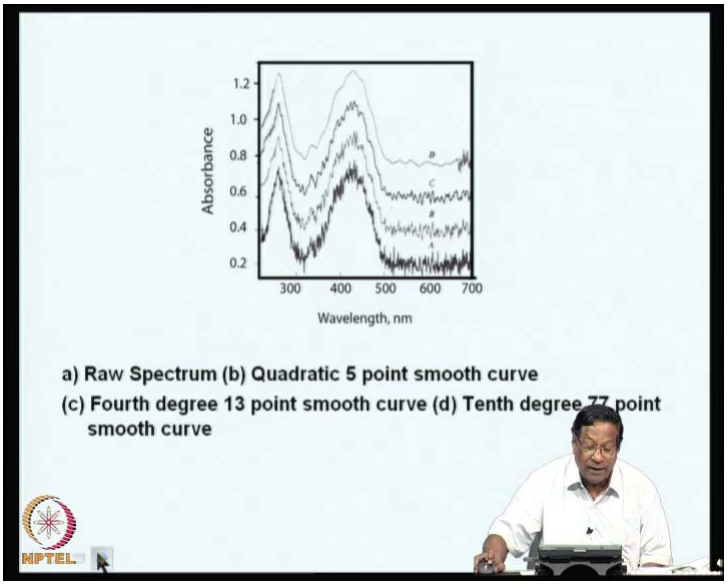

Now, you can see figure A is the raw signal, raw spectrum and figure B, that is this one is a quadratic with 5 point smooth curve. Suppose, we increase it to 4 point fourth degree, then you can see here like this signal is still better and suppose, you increase it to tenth degree 77 point smooth curve, it will give a beautiful signal like this.

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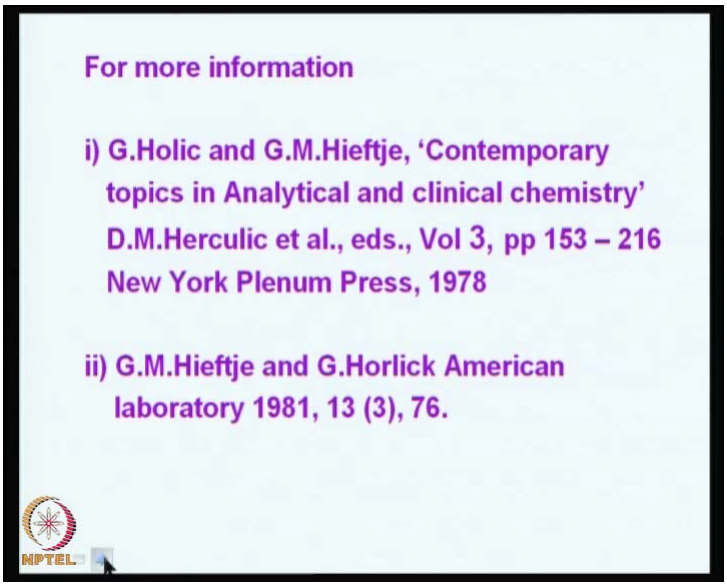

So, the computer information, computer programs will also help in smoothing out the signals. Now, these references what I am giving will give you more details about the techniques and methods of the things what we had discussed earlier, that is G. Holic and G.M. Hieftje contemporary topics in analytical and clinical chemistry that is edited. Another one is G.M. Hieftje and Horlick that is in American laboratory 1981. These two

will give you more details about the electronics and computer methods. How we go about doing handling the signal? That is a very important aspect and that determines how sophisticated your spectrophotometric reagents would be.

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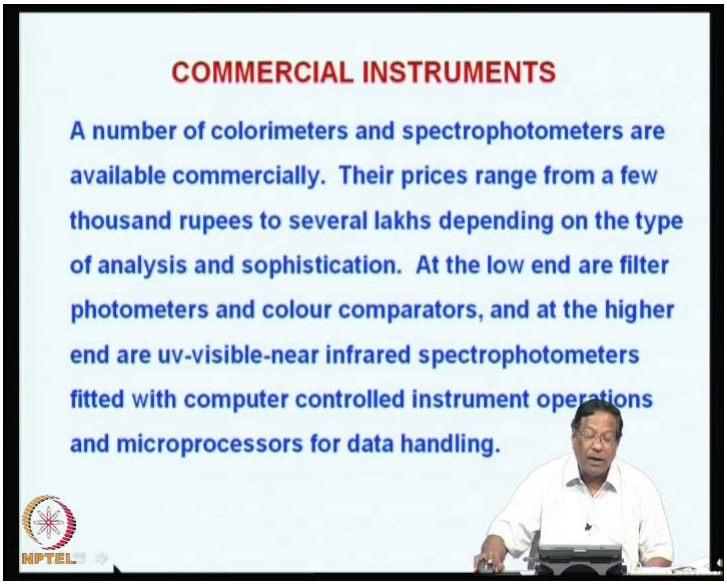

Now, let us look at some of the commercial instructions. There are a number of commercial instructions available in the market. You can see colorimeters and spectrophotometers. They are called, their prices range from a few thousand rupees to several lakhs depending upon the type of analysis and sophistication. You can even make one within a few 100 rupees in India for dedicated analysis that is a calorimeter. So, at the low end are filter photometers and color comparators. These are the cheaper ones and at the higher end are uv-visible-near infrared spectrophotometers fitted with computer controlled instrument operations, process operations etcetera and they are also fitted with microprocessors for data handling.

Now, what are color comparators? These are basically things which will compare the colors of two. If you know one standard, the other standard you can determine only with judgment with your eyes or sometimes charts are available. With the charts you can see the color.

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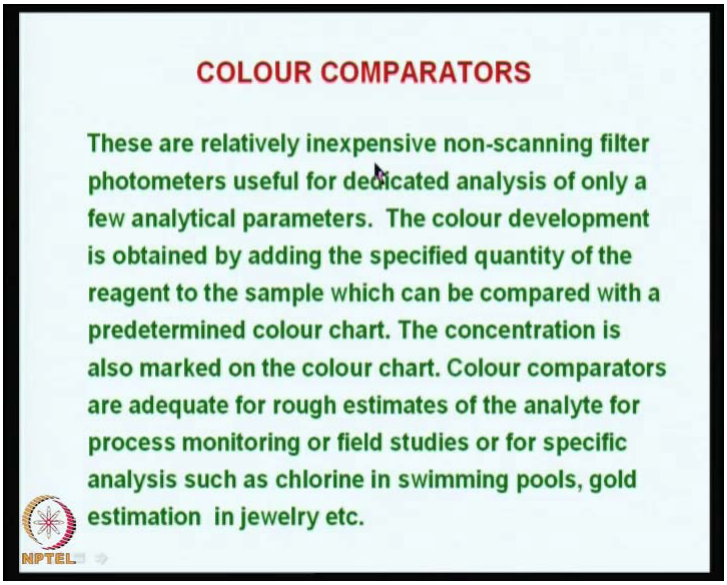

Now, these are basically relatively inexpensive non-scanning filter photometers useful for dedicated analysis of a few analytical parameters. The color development is obtained by adding specific quantity of the reagent to the sample which can be compared with a predetermined color chart. The concentration is also marked on the color chart sometimes to help assessment. Color comparators are adequate for rough estimates basically and then, of the analyte and also for process monitoring or field studies or for specific analysis, such as chlorine in swimming pools or gold estimation in jewelry or vapors acid vapors in electroplating shops. Several things are there available for such small things dedicated application. They are not supposed to be research instruments, but rather application oriented dedicated instruments.

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Now, the next higher end, these are some of the color comparators available. You will see here on this, here you will see two boxes to insert your cells like this. They have shown one green and one red color solutions and these are to be inserted in these places and you can see the color chart provided at the bottom and each color chart refers to some particular concentration and these are the tablets are the reagents which are available in the form of tablets and the color is developed by taking the sample in a beaker and then, add this reagent. It will develop color like this, put it here and then, compare the color and read the concentration. These are color comparators.

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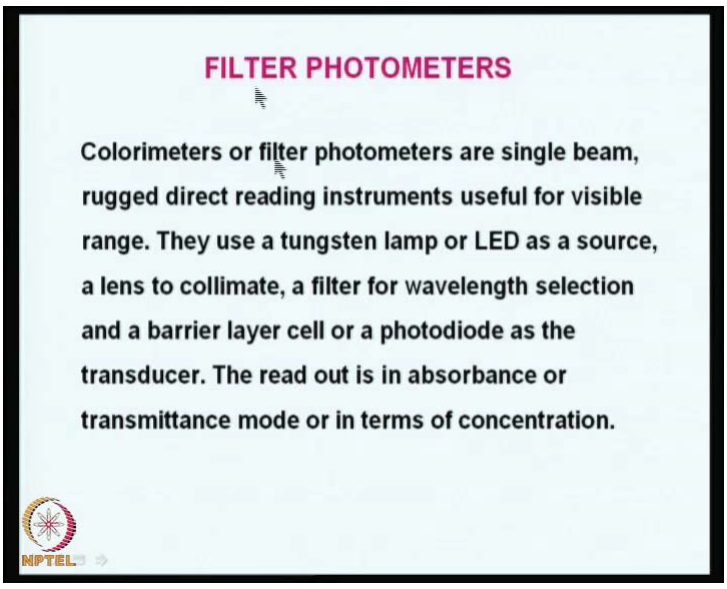

Now, the high, slightly higher end or colorimeters or filter photometers. These are essentially single beam rugged direct reading instruments useful for visible range. They use a tungsten lamp or LED as a source and lens to collimate, a filter for wavelength selection. You do not use prisms or gratings etcetera in for wavelength selection. Basically, it is a filter and the detector is also only a sort of a barrier layer cell or a photodiode as the transducer maximum photodiode, but not PMTS. PMTS are well costly to be incorporated in this because one thing is the cost and another thing is your wavelength selection itself would not be so accurate. So, PMT would serve no specific purpose to incorporate such a high quality detector in this. So, what would be the readout? Read out would be in the form of absorbance or transmittance mode or in terms of concentration in slightly more sophisticated instrument.

So, how do you operate such instruments? The operation is basically very simple. You adjust the 100 percent transmittance or 0 absorbance with the solvent or reference solution by simple changing the voltage, apply to the lamp. So, in modern instruments, reference signal is stored in the memory because they are all single beam instrument. It has to be stored again in the memory and then, you remove the sample, put your sample, measure the absorbance and then, that will be compared with the reference value stored in the memory. Then, the ratio of the sample signal to that of the reference is computed and the absorbance is calculated. Then, it is basically a question of reading the meter or the readout. Now, these are filter photometers.

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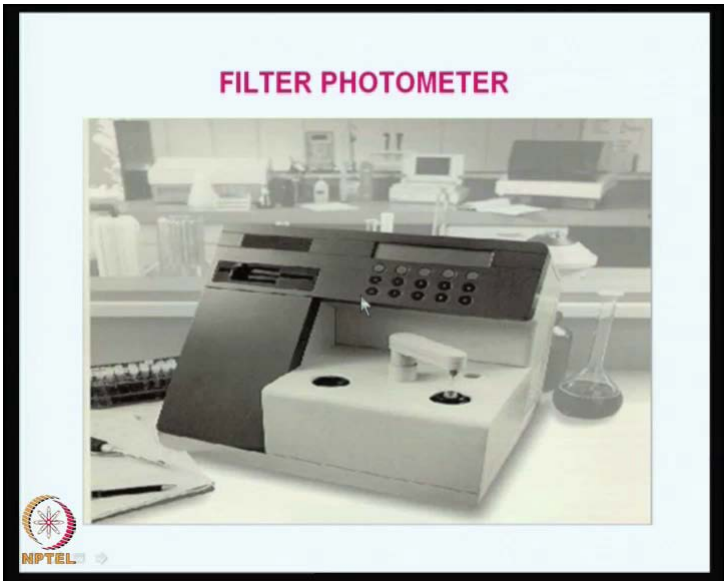

This is the figure of the filter photometer, modern filter photometer. We can see that here are the sample solutions to be inserted and the spectrophotometer is here, body is here, detector would be somewhere inside, the optics would be like this.

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This is a very low led based calorimeter which I was telling that it is something like a filter photometer only, but detected instrument, very low cost and the display here is in terms of concentration. It is a reading 0.035, but it can be any value depending upon the concentration. Now, the slightly higher end equipments are single beam spectrophotometers.

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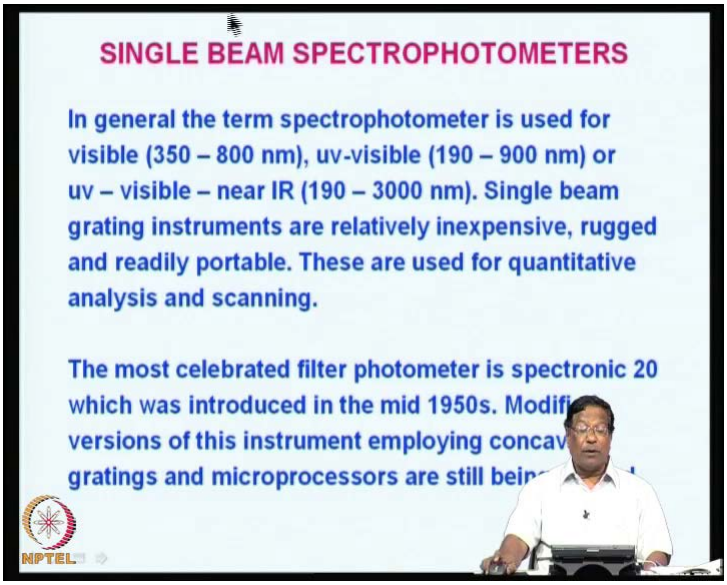

In general, the terms spectrophotometer is used for visible range and that is 350 to 800 nanometers. If you have UV, then the range would be 190 to 900 nanometer. They are also called spectrophotometer, but UV visible spectrophotometers, otherwise 350 to 800 nanometers. What you see here is simply called as spectrophotometers. Then, another modification is near IR. They can have UV visible and near IR range which will cover 190 to 3000 nanometers. Single beam grating instruments are usually relatively inexpensive. They are rugged and readily portable. Nowadays, portable instruments are available. These are used for quantitative analysis as a less scanning. That means with the microprocessors, they can scan the spectrum from 190 to 900 nanometers or even near IR in the case of plastics and other related materials

The most celebrated filter photometer is spectronic 20. That is the model which was introduced in the mid 1950s. During that time, the most of the research in analytical science was being carried out only by this spectronic 20 and it is supposed to be one of the most rugged instrument. Even now you would see in several laboratories such filter photometers still running and modified versions of these instruments are actually available. They are all available in the market. You can purchase any of them and then, you can use them for the measurement of the absorbance.

Usually such instruments employ concave gratings and microprocessors are also they contain. So, it is easier to scan, it is easier to record the spectrum, it is easier to transfer the data and it is easier to read the concentration etcetera.

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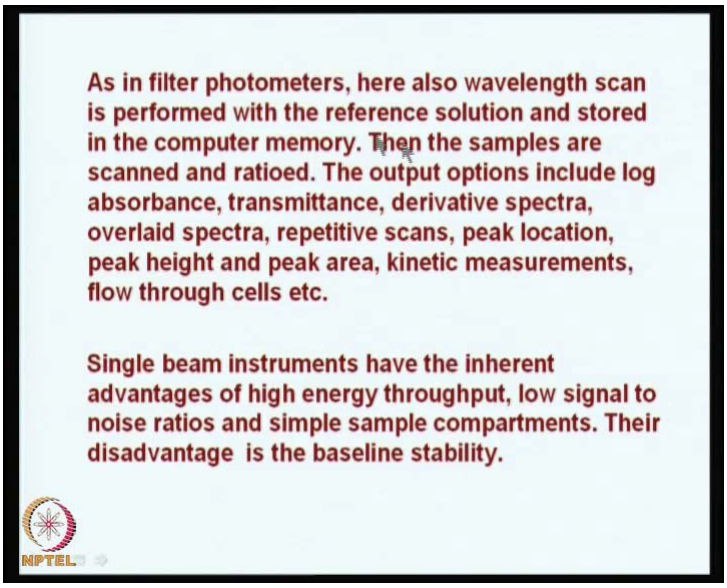

So, as in filter photometers, here also wavelength scan is performed with the reference solution only and it can be stored in the computer memory because if they are mono single beam instruments, there is a need to store in the memory. Then, the samples are scanned and then, signals are ratioed. The output options include log of absorbance, transmittance, you can have a derivative spectra, you can have overlaid spectra, you can have repetitive scans, peak location, you can zoom in and peak height and peak area, you can measure and kinetic measurements are possible through using flow through cells.

So, in general, single beam instruments have the inherent advantage of high energy output because all the signals that comes from a single source is concentrated through the slit beam and then, onto the sample and after a part, it is absorbed onto the signal, but in double beam what happens is the same signal is split into two and then, part intensive is reduced by half. Therefore, the single beam instruments are supposed to be very very sensitive and because of their high energy throughput, but their disadvantage is always the baseline sensitivity. Suppose, you measure now and then store it in the memory, by the time you take out your sample, put another one, the baseline would have shifted or drifted and that cannot be measured simultaneously. So, suppose the line voltage is different, then you are going to end up with wrong results. So, as long as these single beam baseline is steady, then only such instruments will be useful. Otherwise, you will have to go for double beam, but double beam intensity has to be halved. One has to pass through the reference, one has to pass through the sample simultaneously, so that

whatever changes happen in the incident radiation that due to voltage will be automatically compensated in the other two in the sample and reference simultaneously. So, you will get a true picture of the spectrum.

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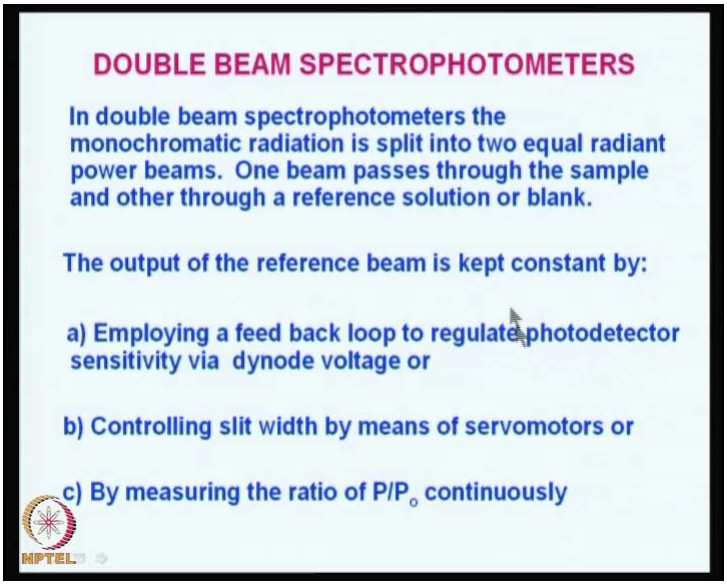

Now, double beam spectrophotometers are always used when the signals are split into two equal radiant power beams. One beam passes through the sample, other through air reference solution or a blank.

So, output of the reference beam is kept constant by employing a feedback loop to regulate photo detector sensitivity via dynode voltage. This is very important concept because the ratio has to be reproducible. So, employing a feedback loop always makes the source current very steady and you get a good signal. Sometimes, you can even control the slit width by means of servomotors and you can measure the ratio of P by P naught continuously. This facility would not be available in single beam instruments.

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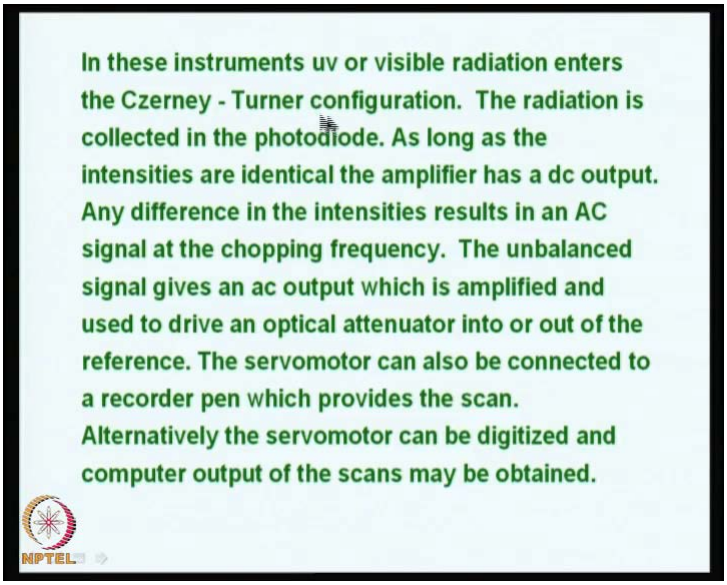

So, in such instrument, the radiation UV or visible radiation enters the Czerney-Turner configuration. Now, the Czerney-Turner configuration who has studied earlier and there we use gratings and the passing on to the mirror and coming back, I will show you a figure of Czerney-Turner once again shortly.

So, the radiation is collected in the photodiode. As long as the intensities are identical, the amplifier has a DC output. Any difference in the intensities results in an AC signal at the chopping frequency. The unbalanced signal gives an AC output which is amplified and used to drive an optical attenuator into or out of the reference. The servomotor can also be connected to a recorder pen which provides the scan. Alternately, the servomotor also can be digitized and computer output of the scans may be obtained.

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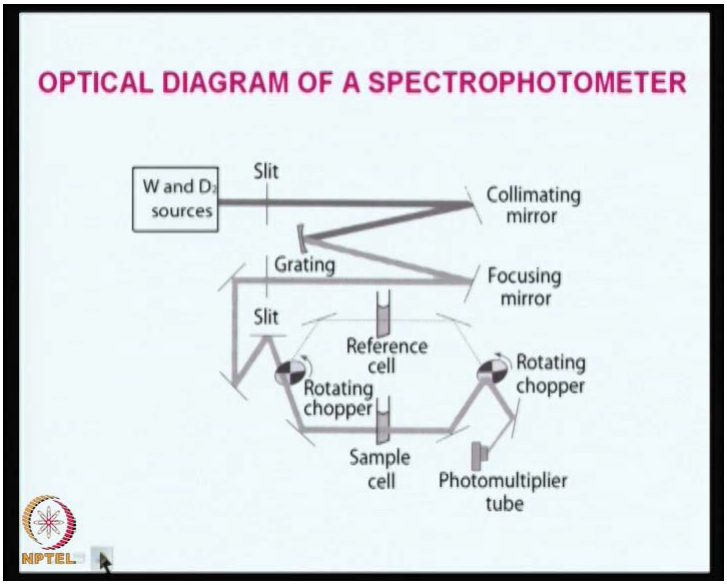

This is also very simple. Here, you can see the optical diagram of a spectrophotometer. Here we have a tungsten and deuterium lamp slit and then, collimating mirror grating and then, focusing mirror. Then, you can see that number of mirror are there 1 2 3 4 5 6 etcetera 7 8. Eight mirrors are there in this figure. You can see that all these things are necessary to the size of the spectrophotometer, a very compact one. Earlier Russian instruments used to come which would be about the size of a big table and nowadays, small tabletop models, carry models, field models, they are all available and this is a single beam instrument because source comes only through one single slit. There is only collimating mirror grating to choose the wavelength. It focuses and then, taken to the sample and this reference comes here. Sometimes you have to remove the reference, put the sample and then, it is put a rotating chopper followed by photomultiplier detector.

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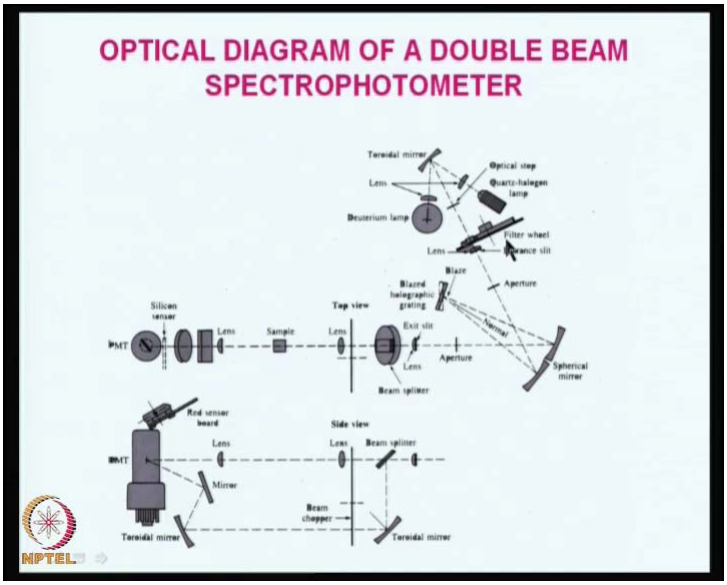

A double beam instrument actually would be much more complicated in terms of optical diagram. So, one can use blazed holographic grating lenses and then, you need a filter wheels quads halogen lamp and optical lamp, optical tops and then, toroidal mirror etcetera. This figure is slightly more completed, but a true representative of the optical diagram and this is the PMT. So, the final, these things it comes to the PMT and then, computer data handling etcetera, there those things are separate. This is only the optical diagram.

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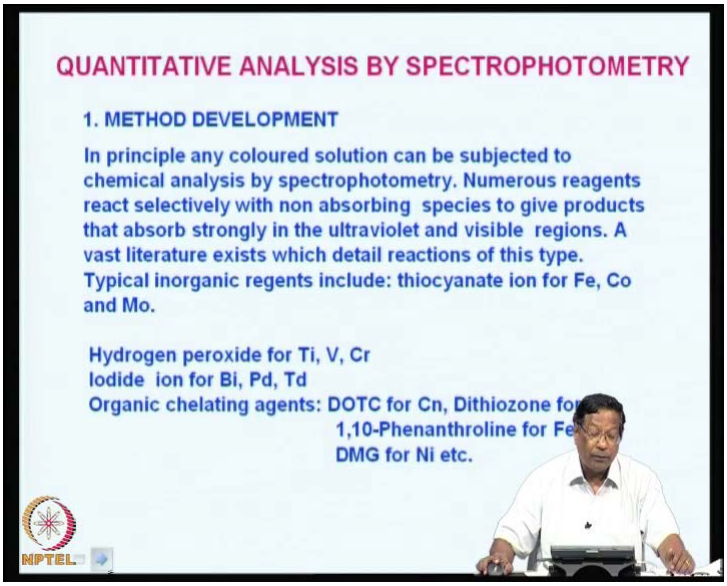

Now, what to do? So far I have taught you how to choose a spectrophotometer for your purpose. Suppose you want to use them for only specifically for one hour two

parameters. That means you want to buy a detected one like a swimming pool or somewhere etcetera. Only one or two parameters you want to monitor amount of chlorine or something and then, it is better to go for a simple calorimeter based on led because the person who operates the instrument would not know much about the chemistry and concentrations etcetera. He just has to run to take a sample, put the tablet, develop the color and measure the concentration. If the color is not there if the correct concentration is not obtained, then he just has to change the water etcetera to get these things.

So, a person who is not well versed in chemistry or spectrophotometer can handle field instruments provided you give him a readymade chart, readymade reagent, readymade instrument etcetera. Now, at higher end, suppose for teaching aids you may need a filter photometer because they are all single beam instruments. Lot of people can be taught using that how to use the instruments etcetera theory and then, for limited amount of research and more accuracy, for a filter photometer, for very high quality research, you should go for spectrophotometers, UV visible, IR near, IR followed by computer microprocessor, assess data handling etcetera depending upon your requirement.

Now, how do you use a spectrophotometer? In principle, there are method development procedures. Any color solution can be subjected to chemical analysis by spectrophotometers. As long as you have a colored spectrophotometer, colored substance, it can be measured using a spectrophotometer. Numerous reagents react sensitively with non-absorbing spaces to give color complexes that absorbs strongly in the ultraviolet as well as visible region.

Now, a vast literature exists which detail reactions of this type. Typically reaction inorganic reagents include thiocyanate ion for iron, cobalt, molybdenum etcetera. We can use hydrogen peroxide for titanium, vanadium, chromium. Iodide ion for bismuth, palladium, tellurium etcetera and number of organic reagent cheating agents dithyocarbamite for copper, dithizone for lead 1, 10 phenanthroline for ion etcetera, dimethylglyoxime for nickel etcetera. Number of reagents actually if you want to take a look at spectrophotometric methods available for the metals, you would be surprised that for each metal, there would be about 200 to 250 reagents available and methods are there recorded which you have to scan and choose the applicable method for your requirement.

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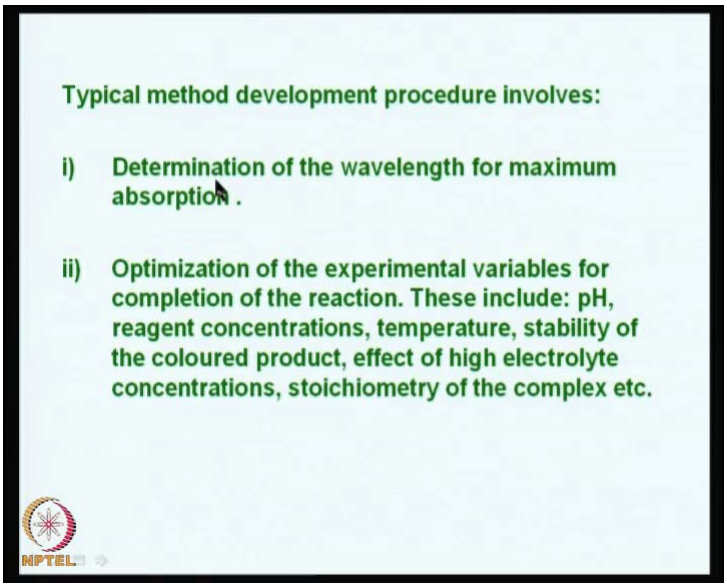

So, typical method development. How do you do that is that you determine the wavelength for maximum absorption. So, in the maximum absorption at that wavelength, you should optimize the experimental variables for completion of the reaction. These include pH reagent concentrations, temperature, stability of the colored product, effect of high electrolyte concentrations and then, stoichiometry of the complex etcetera.

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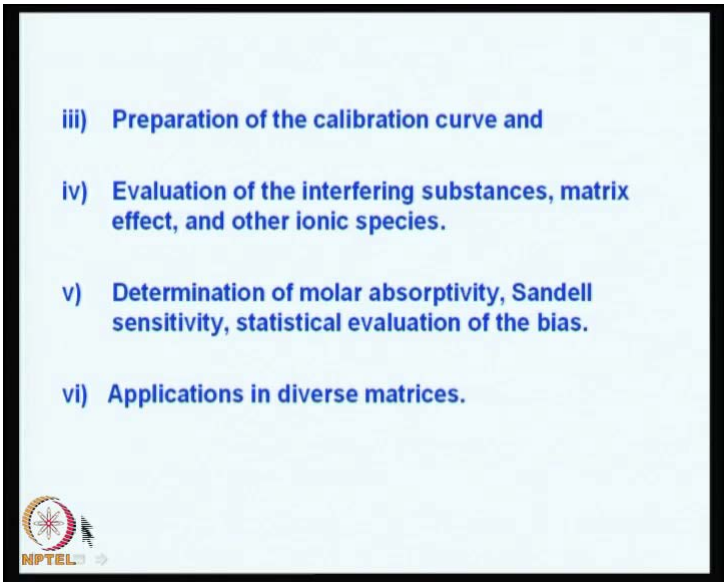

Now, what is the next step? Suppose, you are able to optimize the reaction conditions, then you should go for the preparation of a calibration curve. How long the calibration

curve is followed? So, the linearity of the spectrophotometer you have to determine. That means for up to 5 P p m, 10 P p m, 20 P p m depending upon the system. Now, you can evaluate the interfering substances and matrix effects and other ionic species. For example, you may like to determine gold in seawater. That is one type of complex. You would know that there are about 3 to 3.5 percent of salt in seawater. So, that will have a different effect on the spectra. Suppose, you want to determine gold in jewelry, then gold may be the major component or most probably the only component along with silver. May be some copper, but suppose you want to determine gold in a mobile cell, then in that mobile cell, it could be just a coating provided on the sample of a FW microns.

So, the base metal would be iron or tantalum or something like that along with that some coating material followed by gold determination. So, matrix effects and interfering substances always create problems and these things need to be evaluated continuously. Now, you can determine the type of the complex. You have to determine the molar absorptivity. This will tell you how sensitive the material is. So, the method if it is very highly sensitive, molar absorptivity would be very high. The order of about between 10 raise to 4 and 10 raise to 5. Suppose, the substance is having very low molar absorptivity, the substance would not be colored.

So, you would also like to determine sandal sensitivity. That is the sensitivity at the detection limit of 0.001 absorbance. What would be the concentration that gives you so much of absorbance and then, you may also like to evaluate statistically the bias and if you would develop a method using all these aspects including all these aspects, then you would like to determine the element in diverse matrices. That is another part of the research that you would like to be interested in.

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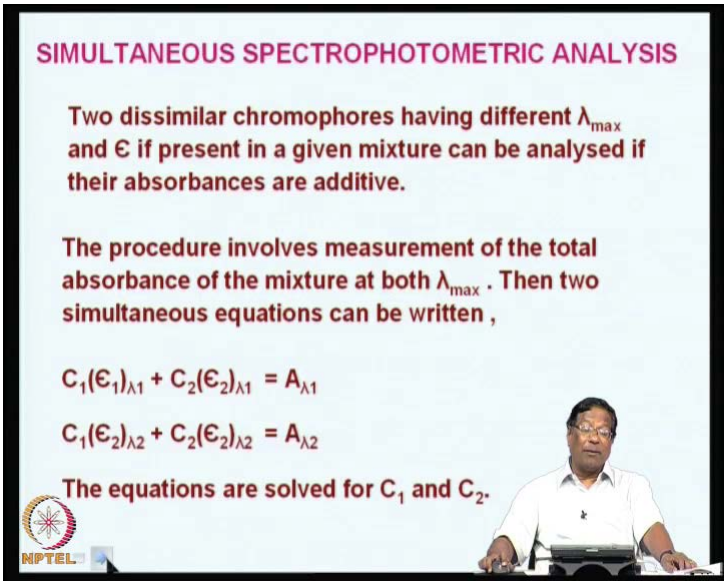

So, sometimes what happens is there may be two substances which are having two different colors and you would like to determine the concentration of two different substances in a mixture. So, two dissimilar, this slide shows you two dissimilar chromophores having different lambda max and molar absorptivity epsilon if they are present in a given mixture. They can be analyzed if the absorbances are additive. This is a very important concept

Now, the procedure involves measurement of the absorbance at the lambda max of both substances. Then, two simultaneous equations can be written. You have C1 first component absorbing at lambda 1. Its molar extinction coefficient is epsilon 1. Similarly, at the same lambda 1, the second substance also absorbs. So, that is C2 E2 epsilon 2. That corresponds to the total absorbance of a lambda 1. Similarly, C1 epsilon 2 at lambda 2 plus C2 epsilon 2 at lambda 2 would give an absorbance at lambda 2. So, we know the absorbance.

So, there are two equations and two unknowns, C1 and C2. So, they can be solved algebraically. For the correct determination of the mixture, for this you will have to remember that both absorption peaks should not occur at the same wavelength. There should be minimum 30 nanometers difference between the lambda max of the first substance as well as the second substance. So, if they are very near, you will not be able to determine. Suppose, you have three samples which are having 3 lambda max and three mixtures would you be able to determine the three concentrations in a given mixture? The answer is yes. They can be. All you have to do is you have to write one more

equation involving three parameters, that is C1, C2 and C3. These equations you will have to write C1 epsilon 1 at lambda 1 plus C2 epsilon 2 at lambda 1 plus C3 epsilon 3 at lambda 1 followed by C1 epsilon 2 at lambda 2, C2 at lambda 2, C3 epsilon 2 at lambda 2. Then, third one also you can write. All the three simultaneous equation you should be able to solve and then, determine the different concentrations.

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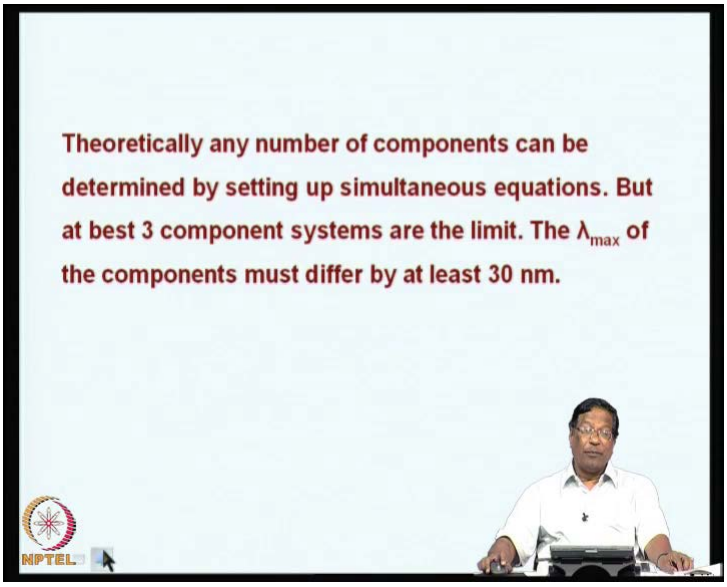

So, theoretically any number of components can be determined by setting up simultaneous equations, but for all practical purposes, one can handle three component systems. More than that it becomes slightly difficult and because the lambda max of the components also have to differ by at least 30 nanometers.

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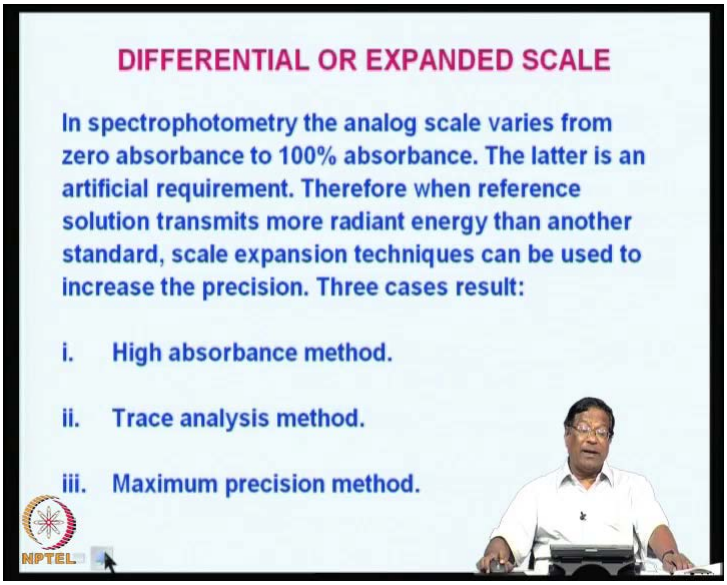

So, simultaneous equations can be done and then, differential or expanded skill here. What do you do is we basically change the absorbance range and we call it high absorbance method, trace analysis method and maximum precision method.

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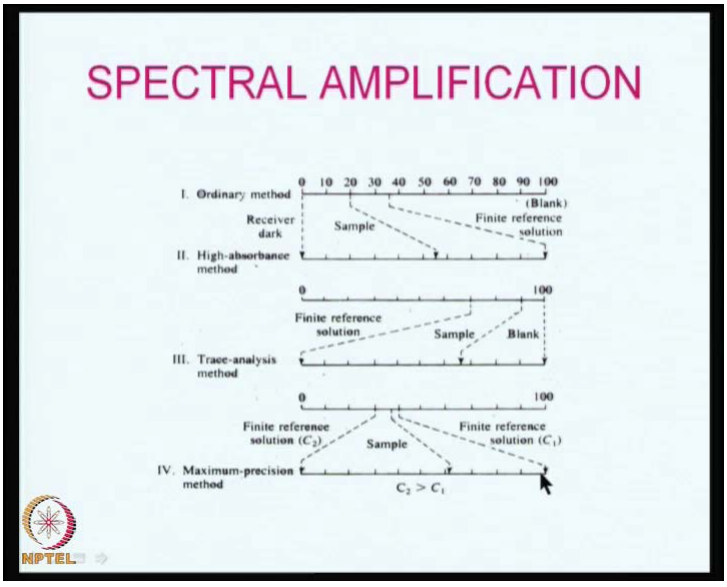

So, when we are able to change the absorbance is like this. See the first one here. What I am doing is I am taking a sample between the two different concentrations here. On the top, you will see that there are three figures about 35 percent transmittance. I am adjusting to maximum absorbance and around 20 percent I am adjusting to these things. So, 0 is always 0. So, I am expanding the scale from somewhere around 35 to read the full scale, that is absorbance by opening the slit more.

So, if my sample is somewhere around 20, it will cover the whole range, but appear somewhere here. Similarly, if the substance is too low concentrated, very low concentrations are handleable. Then, I adjust the 100 percent transmittance and 0 percent absorbance. So, my sample is my standard. I allow it to go up to this point and 100 percent is sale and in between my sample is there. So, in such cases, accuracy would be better than the standard method because here the sample is low concentration.

Sometimes what we would like to do in precious metals etcetera, we need still more accurate. We are neither on the higher absorbance scale nor on the low absorbance scale. In that case, what do we do is we take two standards. One standard is here, another standard is here. So, this one, these two (( )) for 0 percent and 100 percent transmittance and then, the sample would be covering a small portion of the standard would cover the whole absorbance range and these things are known as maximum precision method, trace analysis method and high absorbance method, but you would be surprised that this can be done only in filter photometers, not in spectrometers.

So, filter photometers still provide you some amount of freedom to play with the absorbance and get better results, but not in spectrophotometers because it is not possible for you to play with the uptakes and upper chair etcetera because most of them are usually controlled by the computer.

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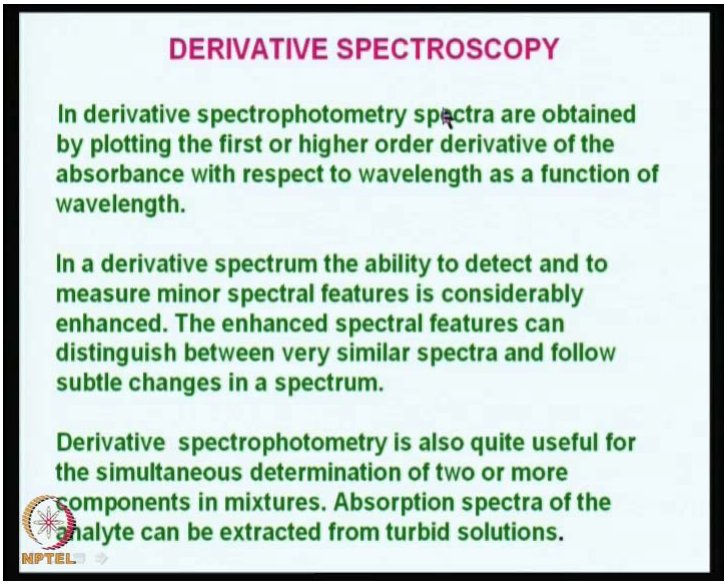

So, another aspect is derivative spectroscopy. In derivative spectrophotometry, what do you need to do is spectra are obtained by plotting the first or higher derivative of the absorbance with respect to wavelength as a function of wavelength.

Now, what do we mean by that? We take the spectrum and this helps. We take the first derivative and it helps in the derivative spectrum the ability to detect and to measure minor spectral features that is they are considerably enhanced. The enhanced spectral features can disguised between very similar spectra and follow subtle changes in a spectrum.

So, derivative spectrometry is also, spectrophotometry for that matter is also quite useful for the simultaneous determination of two or more components in the mixtures. So, absorbance spectra of the analyte can be extracted even from turbid solutions. So, this is the application.

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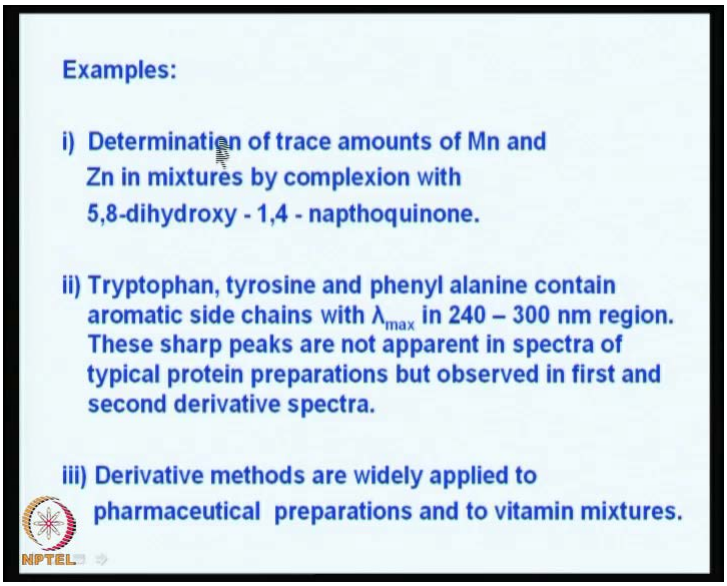

For example, we can determine the trace amounts of manganese and zinc in mixtures by complexion with 5, 8 dihydroxy, 1, 4-naphthoquinone.

So, tryptophan, tyrosine and phenylalanine, these are aromatic amino acids. They contain aromatic side chains and with a lambda max in these range 240 to 300 nanometers range. These sharp peaks are not apparent in spectra of in ordinary spectra, but if you take first

and second derivative spectra, all these three amino acids tryptophan, tyrosine and phenylalanine, they can be distinguished very easily.

Derivative methods are usually applied to pharmaceutical preparations and vitamin mixtures. So, applications include quite a lot.

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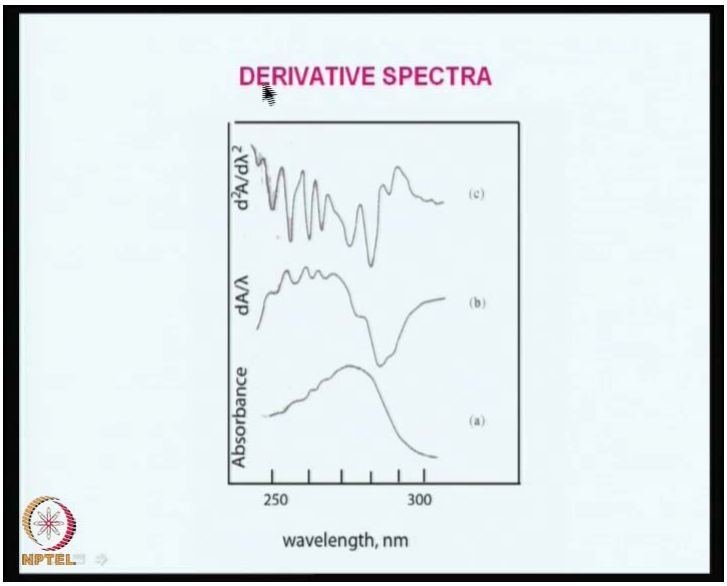

Here, you can see the derivative spectra. First one is just absorbance that is at the bottom and at the top is first derivative. You can see how the spectrum changes.

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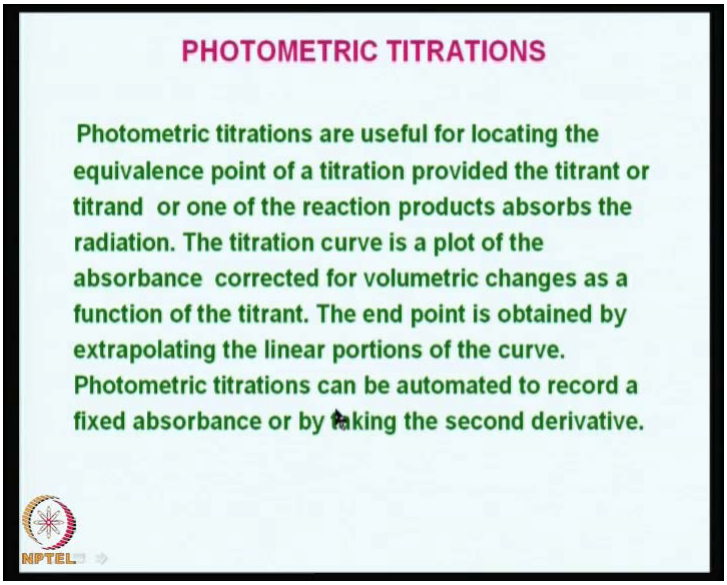

If you do this second derivative spectrum, you can see so many fine aspects of the spectra and the mixtures also can give you directly concentration determination provided you do the second derivative spectra.

Now, another aspect is photometry titration. It can just like ordinary titrations, you can do photometric titrations. All you got to do is take a burette, take a pipette and then, keep on adding the reagents and then, it will give you color and you take the substance in a 1 centimeter cell or 5 centimeter cell. Measure the absorbance and the plot absorbance versus volume of the sample titrant. So, photometric titrations are useful for locating the end point of a titration provided the titrant or the titrand or one of the reaction products absorbs the radiation. This is the foremost requirement

So, the titration curve is basically a plot of the absorbance corrected for volumetric changes. That is very important. You have to correct for the volumetric changes as a function of the titrant. The end point you can obtain by extrapolating the linear portions of the curve and the advantage is you can overshoot the titration. Still you should be able to determine the absorbance by plotting and taking the tangents at different portions of the spectrum. So, photometric titrations are basically advantageous in that. You can overshoot the end point and still get the correct end point. Another thing is they can be automated to record a fixed absorbance or by taking the second derivative, now you can conduct different kinds of spectrophotometric titrations and the instrument you will have to modify slightly.

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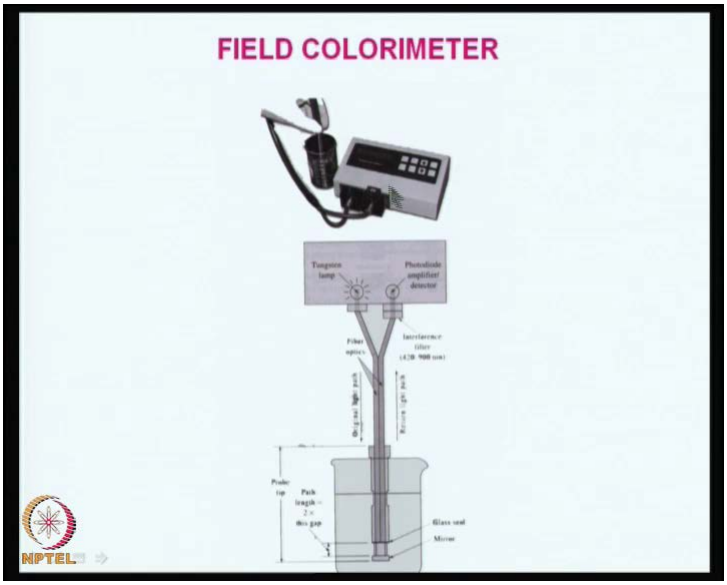

Here, I have shown you a photometric titration in this and here you are adding the reagent and you can take the sample and reference through this and then, put it there and then, measure the absorbance. So, these can be directly connected to the computer to record the absorbance versus volume of the titrant added.

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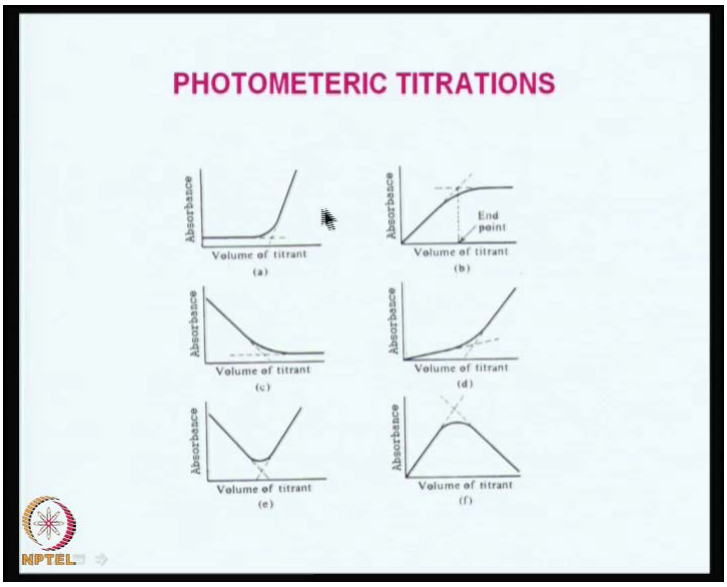

So, I am showing you here some of the titration curves. Here, you can see that the volume of the titrant and absorbance curves is plotted. The first one, this one shows that initially the color is not changing. At the end of the titration, the complex is formed which absorbs and the absorbance keeps on increasing. Now, the end point would be obtained by taking a tangent like this and it can be determined. This could be a case

something like copper titrate being titrated with EDTA. For example, ammonia because copper solution in very low concentrations are very dilute and they do not give a absorbance, but when it combines with ammonia or EDTA, it starts forming a blue colored complex which can be monitored by measuring the absorbance.

Now, the second one is an example of sample also being colored, but the complex is also colored, but sample, the titrant is slightly less colored. Once it forms the complex, the absorbance does not increase. This is something like arsenic with bromide because bromide is also color complex. So, the color will keep on increasing. At the end of the reaction, the absorbance will attain a constant value.

Now, the third one is the example for (( )) in butanol with perchloric acid. Now, you can see that absorbance is very low here. That means the product is not colored. So, the (( )) is colored and as the titrant, you keep on adding color comes down and then, becomes 0 again. As usual you can extrapolate these two parts of the curve and then, find out the end point of the reaction. So, the titrant can react with the titrand to produce colored product absorbing at the same wavelength. This is number d. Here also it is forming a color, here also it is forming a color, but the absorbance keeps on increasing. So, here the sample would be completely titrated at this point itself.

Now, the last one, last two. So, in the last one last, but one we have color titrate being titrated with the titrant to produce colored, produce absorbing at the same wavelength. Here also you can see the color component is being utilized. At the same time, the product is being colored. So, the endpoint can be easily determined.

Now, the last one is, suppose it forms a complex and then, it forms another complex. That means the first complex is colored, second complex is not colored, but the absorbance ratio where the second complex starts forming can be determined using the tangents being drawn from different parts of the absorbance spectrum. So, this is one of the another important applications of spectrophotometric titrations, where there is no danger of overshooting the endpoint. We can automate it, we can use it for various colored reaction etcetera.

We will continue our discussions on the applications of spectrophotometry in the next class. Thank you.