Chemical Process Instrumentation
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Lecture – 53
Miscellaneous Measurements: Composition (Contd.)

Welcome to lecture 53. In our previous 2 lectures, we have talked about gas chromatography and liquid chromatography as tools for concentration measurement. In today's lecture we will talk about spectroscopic method for measurement of concentration. Specifically, we will talk about ultraviolet visible spectroscopy for measurement of concentration. Ultraviolet visible spectroscopy or in short as it is called UV-Vis spectroscopy is widely used and simple inexpensive method of measurement of concentration. UV-Vis spectroscopy finds it is use in research laboratories, in industries, in quality control centres, in hospitals so on and so forth.

So, today's lecture we will see how UV-Vis spectroscopy can be used for measurement of concentration. We will first go through the basic principles of UV-Vis spectroscopy and then we will see how to measure concentration.

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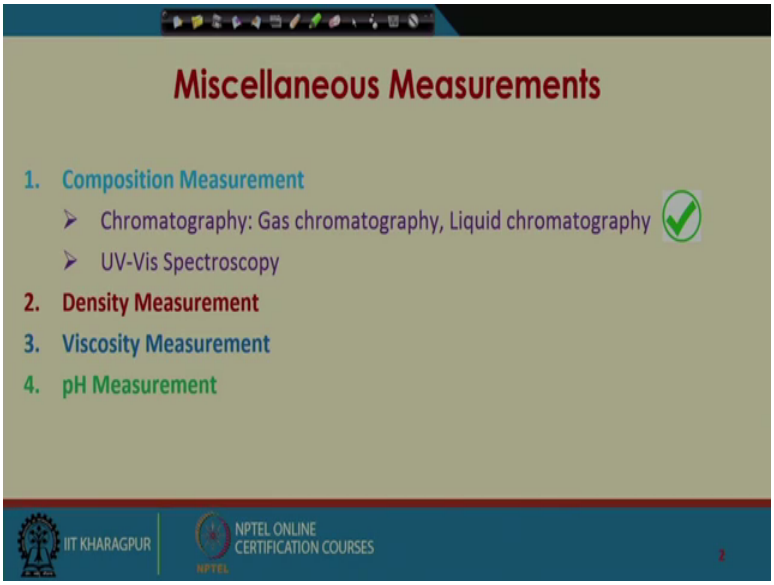

So, we have talked about the gas chromatography and liquid chromatography. And today we will talk about UV-Vis spectroscopy, or ultraviolet visible spectroscopy.

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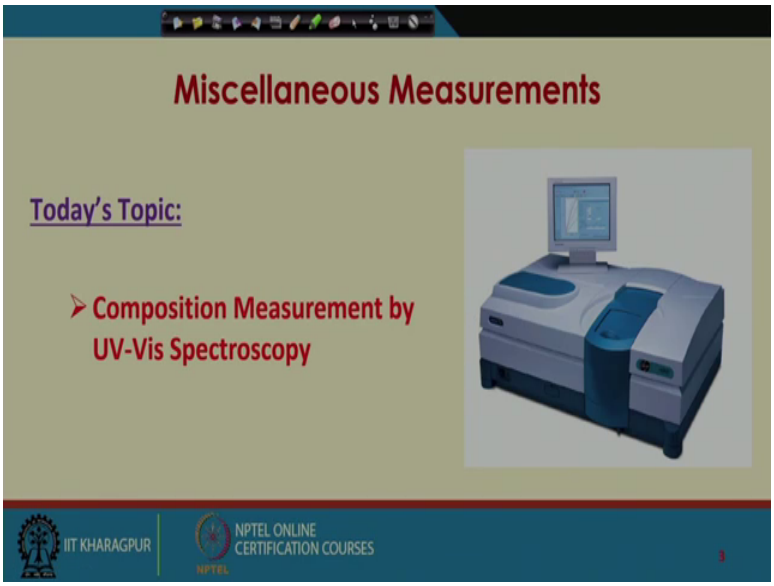

So, todays topic is composition measurement by UV-Vis spectroscopy, and in the screen, you see how a UV-Vis spectrophotometer looks like.

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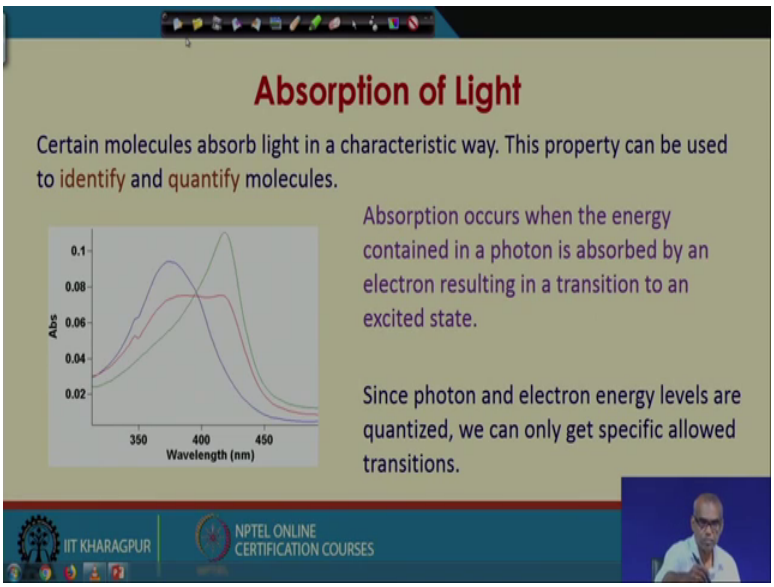

Certain molecules absorb light in a characteristic way. This property can be used to identify and quantify molecules. Absorption occurs when the energy contained in a photon is absorbed by an electron resulting in a transition to an excited state.

Since photon and electron energy levels are quantized, we can only get specific allowed transitions. So, in the molecules absorb light we can have a spectro or spectra or

spectroscopy which is nothing but a plot of absorption versus wavelength. Absorption corresponds to the amount of light that is absorbed and it is plotted with various wavelengths. So, we will see more about it as we proceed in the lecture.

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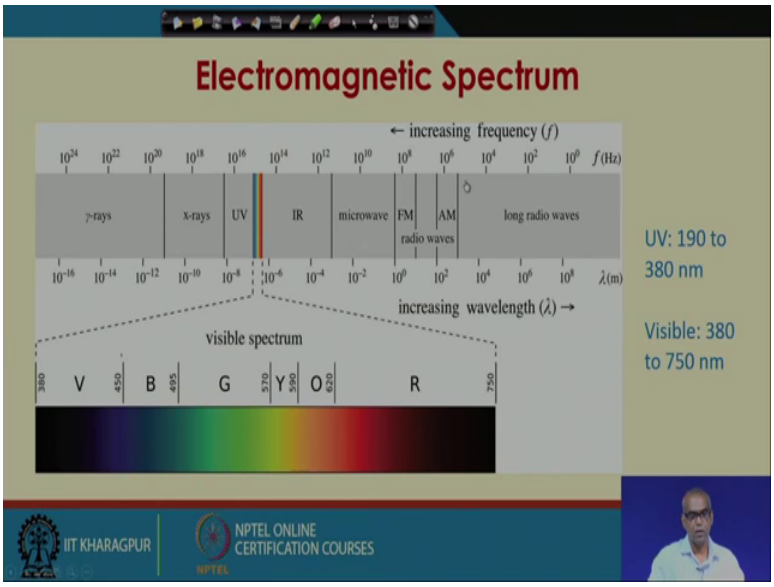

Let us first look at an electromagnetic spectrum. The UV region is typically from 190 to 380 nanometre, and the visible region is typically from 380 to 750 nanometre.

So, here the visible spectrum is shown, and next to that is the UV region. Note that the wavelength increases in this direction. So, UV has shorter of length corresponding to visible lights. So, the UV spectrophotometer or UV spectroscopy will make use of light, whose wavelength falls in the region of UV or ultraviolet and visible region.

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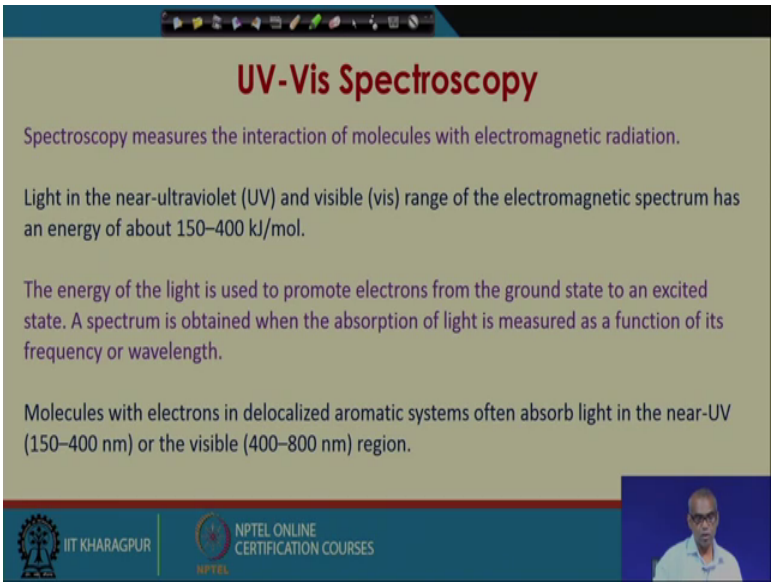

Spectroscopy measures the interaction of molecules with electromagnetic radiation. Light in the near ultraviolet and visible range of the electromagnetic spectrum has an energy of about 150 to 400 kilojoules per mole. The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of it is frequency or wavelength.

Molecules with electrons in delocalized aromatic systems of an absorb light in the near UV or in the visible region; that means, in the wavelength range of 150 to 400 nanometre or 400 to 800 nanometre.

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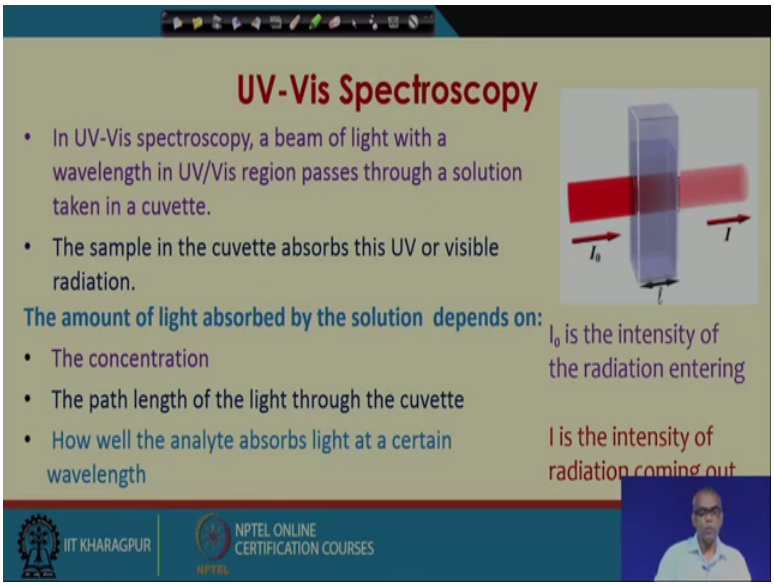

In UVV spectroscopy a beam of light with a wavelength in UV-Vis region passes through a solution taken in a cuvette; so, this is an image of cuvette. The sample in the cuvette absorbs this UV or visible radiation. So, cuvette is nothing but a sample holder, it is transparent. So, in cuvette or in the sample holder, you take the sample which you are going to study.

So, the sample is taken in a clear transparent solvent. Then, a beam of light who is a beam of light with a wavelength in the UV-Visible region is passed through it. Let us consider that I 0 is the intensity of the light that enters the cuvette or the sample and the eye is the intensity of the light that comes out from the cuvette; that means, the intensity of the light that leaves the sample. If this sample absorbs light in UV-Vis region, the intensity I will be less the intense than the intensity by 0. Now the amount of light that is absorbed by the solution will depend on certain factors. This factors are the concentration of the solution the path length, the path length of the light through the cuvette and how well the analyte absorbs light at a certain wavelength.

So, there are 3 factors on which the amount of light absorbed by the solution depends their concentration of the solution, the path length of the light through the cuvette, and how well the analyte absorbs light in a certain wavelength. Since the amount of light absorbed depends on the concentration of the solution by measuring the absorption, I can find out the concentration of the solution.

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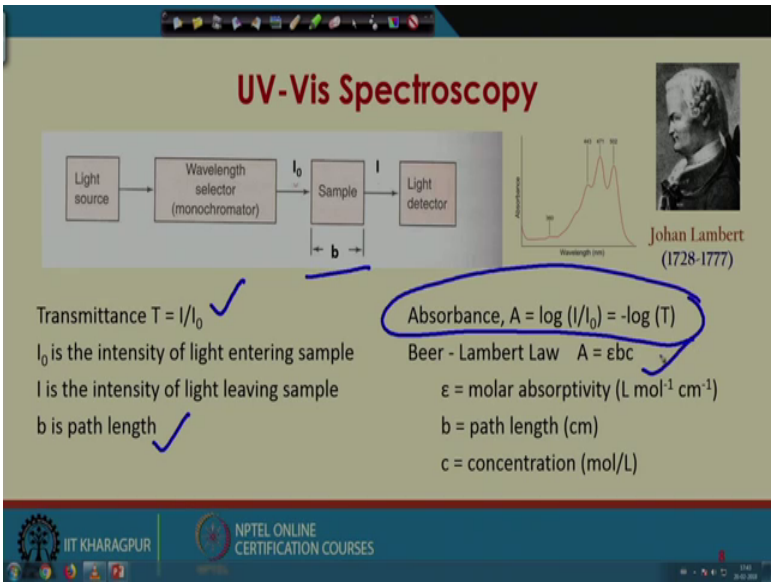

What you see is, a very simplified description of UVV spectroscopy. The basic components are you have a light source which produces light in the UV-Vis region, then you have a monochromator or wavelength selector. Means, the wavelength you choose a single wavelength light from this light source. So, you have a light source for generating UV light and visible light, and then wavelength selector or monochromator is choosing a light of single wavelength.

Now, this light passes through the sample, the sample has path length b, the intensity of the light that enter sample let it be I 0. And the intensity of the light that leaves the sample is I. This light goes to a light detector, it goes to a detector. So, the detector measures the intensity of the light. And then it can calculate the absorbance, because the detector calc detector detects the intensity of the light I, and detector also has information about I 0 will see later how.

Now, from the knowledge of the absorbance, the detector can plot how the observations absorbance changes with the different wavelength, because you can generate different wavelength using this wavelength selector. So, if you do that you get a curve like this. So, you get a plot of absorbance versus wavelength, which is called UV-Vis spectroscopy. The instrument is known as UV-Vis spectrophotometer.

Now, the relationship between this absorbance the path length and the concentration of the solution is given by beer lamberts law. Let us define transmittance as I by I 0. So, this

is the ratio of the intensity of the light that leaves the sample to the intensity of the light that enters the sample. So, I 0 is the intensity of the light entering sample, I is the intensity of the light leaving sample. B is the path length, then absorbance is defined as log of I by I 0; which is also the transmittance. So, the beer lamberts law is absorbance equal to epsilon into path length into concentration; where epsilon is molar absorptivity and it has a unit of litre per mole per centimetre. B is path length; let us say as a unit of centimetre and the concentration has the unit of mole per litre.

So, if the beer lamberts law holds true for the absorption, I can find out the concentration of the sample by knowing absorption. We of course, we should know the molar absorptivity of the solution. The path length is known for the UV-Vis spectrophotometer. Now we will talk about various components of a UV-Vis spectrophotometer. The components are sources of light, then we have wavelength selector we have sample containers or sample holders or cuvette and we have detectors. So, these are important components of any vs dr photometer. They are sources of light, light sources that generates light in the UV region and the visible region. Then we have wavelength selectors, then we have sample containers or cuvette, and then we have detectors.

So, we will now talk about these various components in some detail. Sources used in the UV-Vis spectrophotometer are continuous sources. Continuous sources emit radiation or of all wavelengths within the spectral region for which they are to be used. Sources of radiation should be stable and of high intensity. The commonly used visible light sources tungsten filament lamp, and the commonly used ultraviolet sources deuterium discharge lamp.

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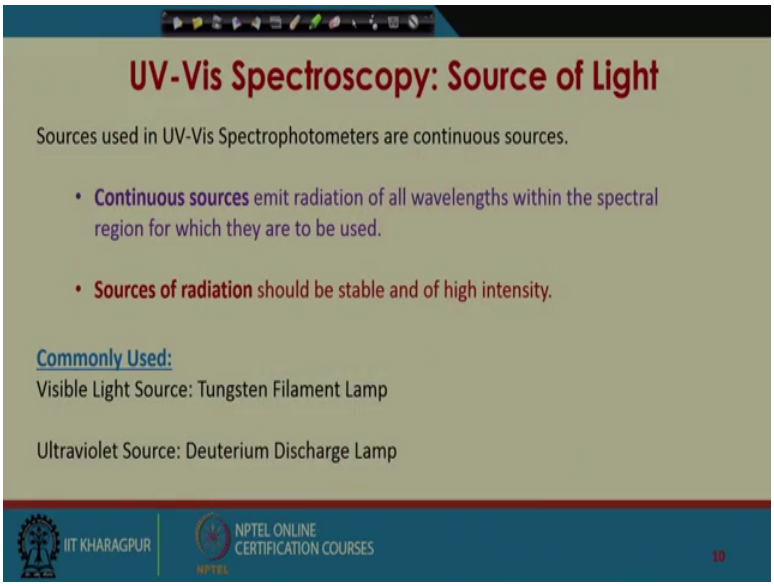

Ideally the output of a wavelength selector should be a radiation of a single wavelength.

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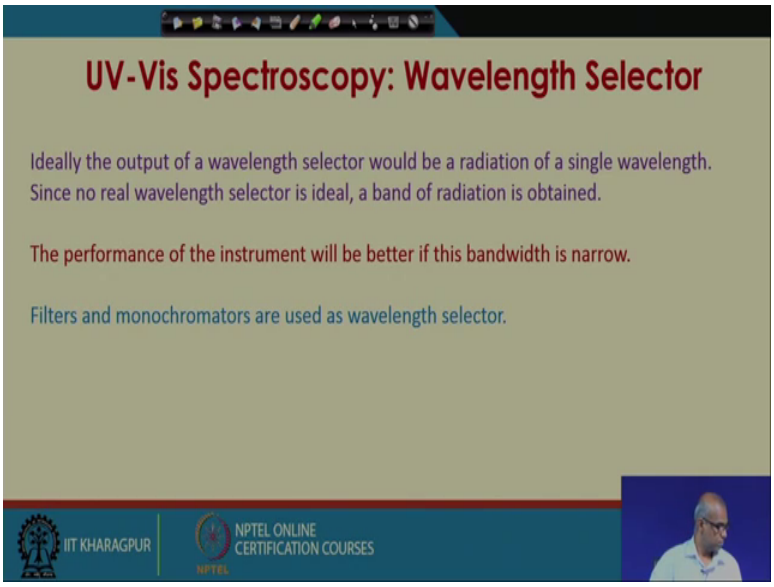

So, wavelength selector is supposed to choose a radiation of single wavelength. So, ideally speaking the output of a wavelength selector would be a radiation of a single wavelength. Since, no real wavelength selected is ideal, a band of radiation is always obtained. The performance of the instrument will be better if this bandwidth is narrow.

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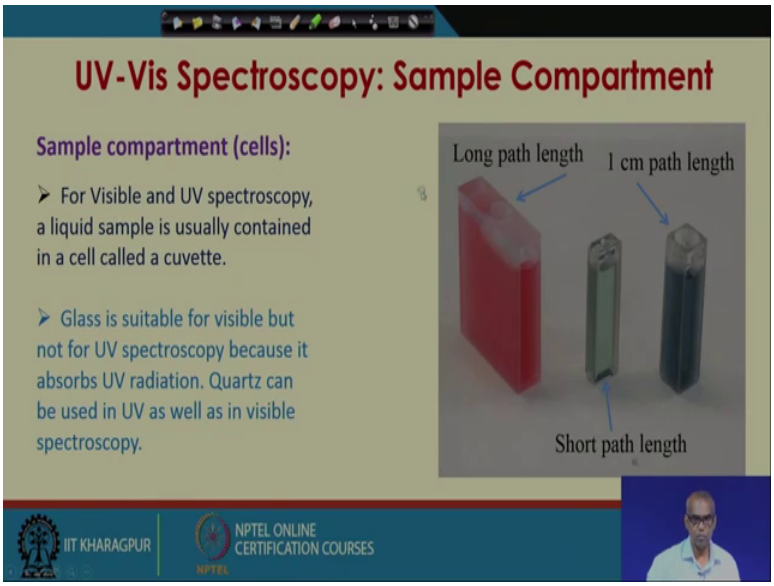

Filters and monochromators are used as wavelength selector. Sample compared compartment or sample container or cells or cuvettes are basically sample holders to perform UV-Vis spectroscophy. For visible and UV spectroscopy, a liquid sample is usually contained in a cell called cuvette. So, sample compartment or cells or cuvettes they are all synonymous, they are all sample holders for performing UV-Vis spectroscopy. Glass is suitable for visible, but not for UV spectroscopy. Because glass absorbs UV radiation, quartz can be used in UV spectroscopy, as well as in visible spectroscopy.

So, basically the cuvette or cells made of quartz are used for UV-Vis spectroscopy. You see images of cuvettes of various paths length. You have cuvette of long path length, you have cuvette of short path length, and you have cuvette of one-centimetre path length.

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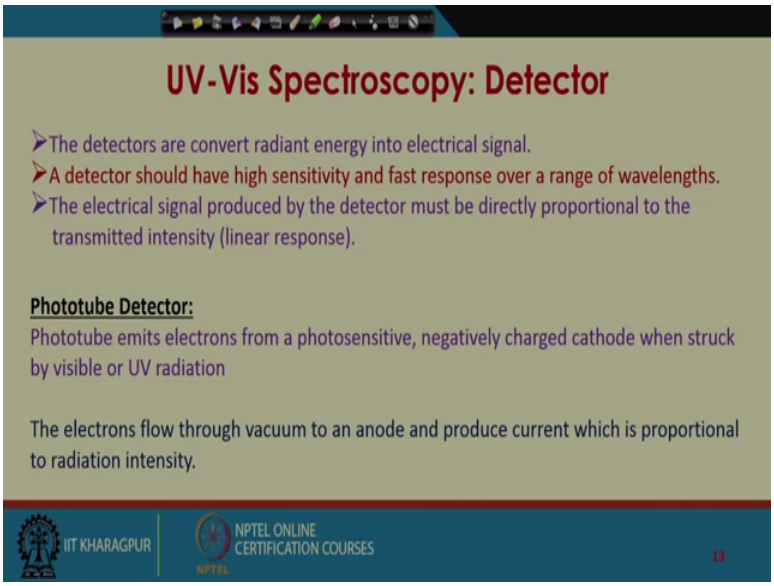

Detector is one of the most important component of UV-Vis spectrophotometer. The detectors convert radiant energy into electrical signal. Our detector should have high sensitivity, and fast response over a range of wavelengths. The electrical signal produced by the detector must be directly proportional to the transmitted intensity. This is necessary so, that we get linear response. Phototube detector emits electrons from a photosensitive negatively charged cathode when stuck by visible or UV radiation.

The electrons flow through vacuum to an anode and produce current which is proportional to radiation intensity. Here will talk about 2 types of detectors, the first is photo tube detector. The photo tube emits electrons from a photosensitive negatively charged cathode when stuck by visible or UV radiation. The electrons flow through a vacuum to an anode and produce current which is proportional to radiation intensity.

Another detector that will talk about is photomultiplier tube.

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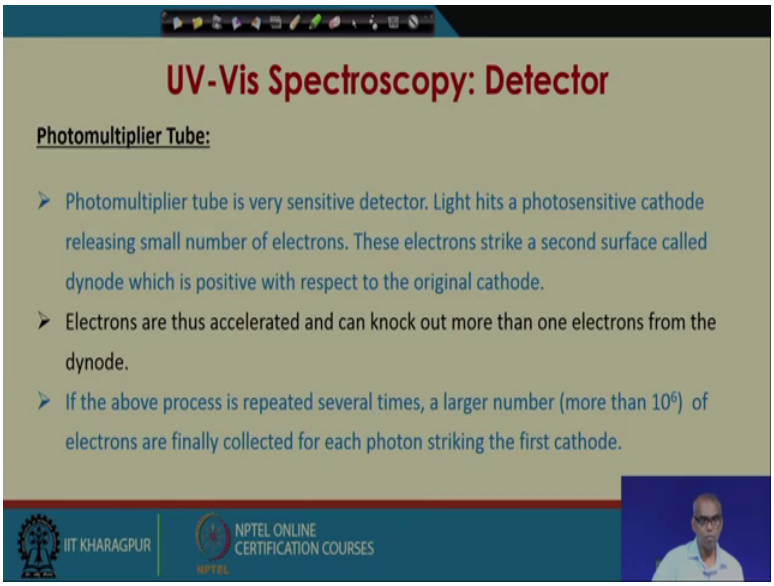

Photomultiplier tube is very sensitive detector, light hits the photosensitive cathode releasing small number of electrons. These electrons strike a second surface called diode which is positive with respect to the original cathode. Electrons are thus accelerated and can knock out more than one electron from the dynode. If the above process is repeated several times, a large number of electrons are finally, collected for each photon striking the first cathode the number of electrons that can be produced or as large as 10 to the power 6.

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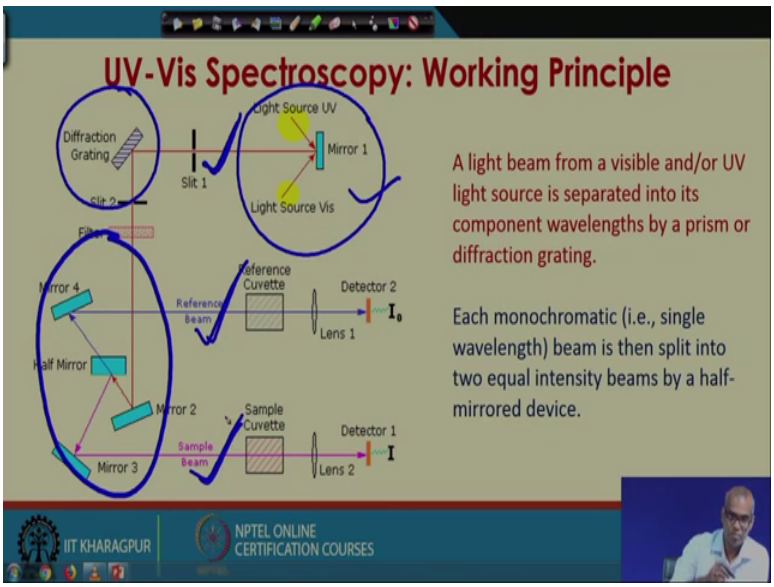

Now, let us look at the working principle of UV-Vis spectroscopy in some more detail. What you see on the screen is a schematic of a UV spectrophotometer. A light beam from a visible and or UV light source is separated into it is component wavelengths by a prism or deflecting diffraction grating. So, you have the light source, and the beam of light is separated into it is component wavelengths by a prism or diffraction grating. So, these diffraction grating, separates this beam of light into it is component wavelengths. So, you have now several beam of lights with single wave lengths. Each monochromatic that is single wavelength beam is then split into 2 equal intensity beams by half mirror device.

So, each monochromatic beam is then split into 2 equal intensity beams by a half-mirrored device. So, this is one beam, this is another beam. So, the light source sends a light beam which this diffraction grating or you can also use a prism separates into individual component wavelengths; that means, monochromatic beams are produced each monochromatic beam is split into 2 equal intensity beam, one is this, another is this.

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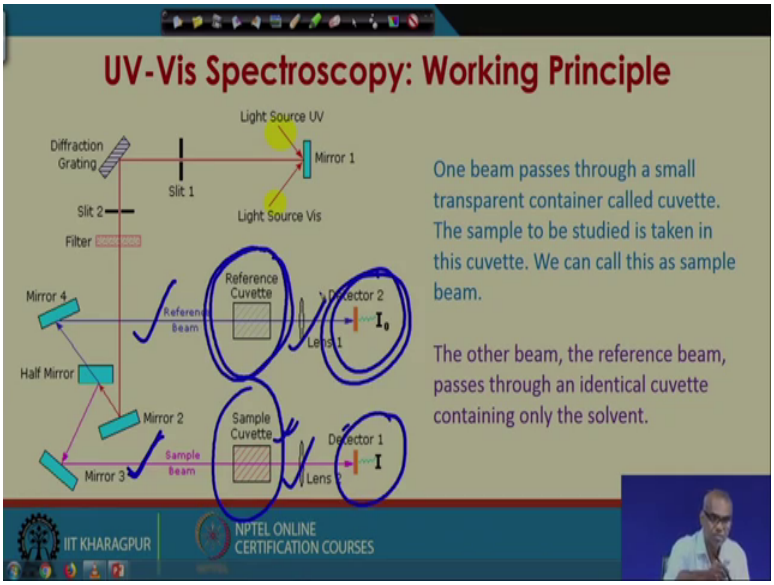

One beam passes through a small transparent container called cuvette. So, this is the cuvette, where you have taken sample. So, one beam passes through the cuvette the other beam passes through an identical cuvette, but this cuvette contains only this solution. So, one beam passes through the cuvette, where I have taken the sample which needs to be studied. So, the sample is taken in some transparent solvent in the cuvette. The other beam, passes through an identical cuvette, but instead of taking the solution to be

investigated, I take the solvent the same solvent, which was there in this cuvette. The lights that comes out of both the cuvettes goes to detectors. So, here the detector measures how much of light is absorbed, because here the light is passed through the solution. Since here the light is passed only through the solvent, there is no solvent there is no sample which I am investigating here it measures I 0.

So, we call one as sample beam the other one can be called as reference beam.

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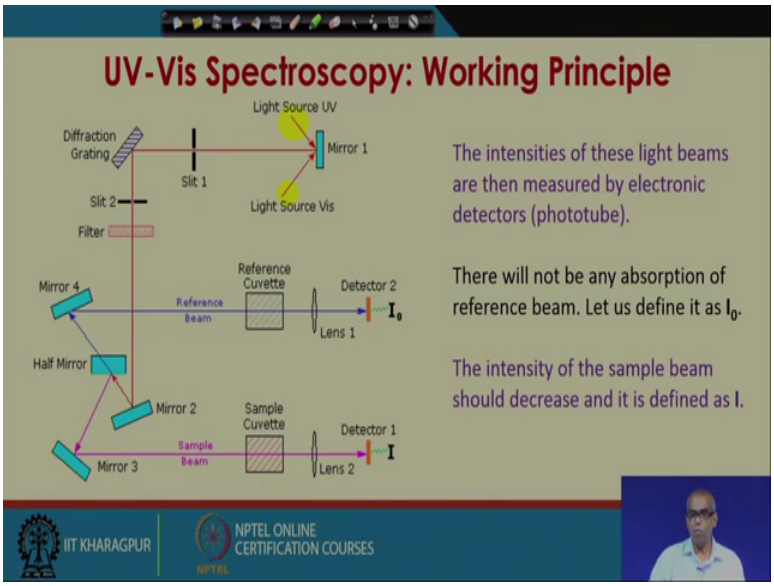

The intensity of these light beams are then measured by electronic detectors, phototubes that we have talked about previously. There will be, there will not be any absorption of reference beam we call it I 0 the intensity of the sample beams should decrease and it is defined as let us say I.

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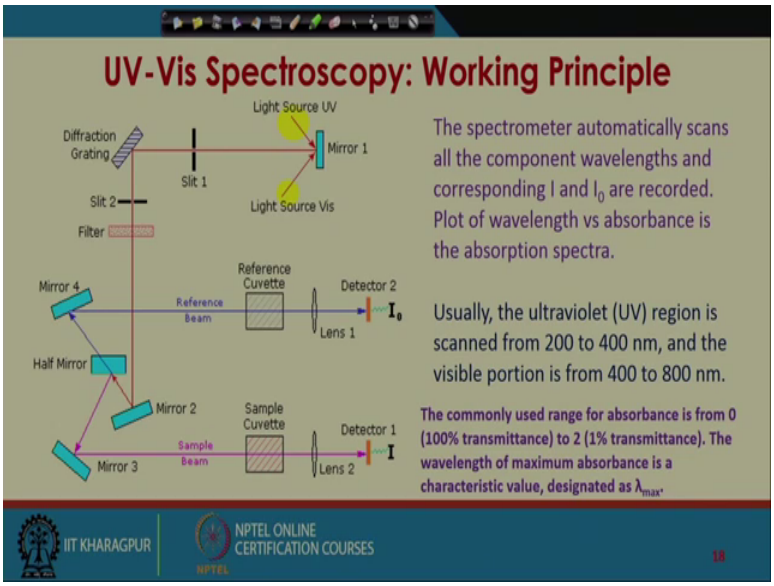

The spectrophotometer automatically scans all the component wavelengths, and corresponding I and I 0 are recorded. From the values of I and I 0, I can find out absorbance and the detector of the UV-Vis spectrophotometer generates a plot of wavelength vs absorbance. This is the absorbance absorption spectra.

Usually the ultraviolet region is scanned from 200 to 400 nanometre, and the visible portion is scanned from 400 to 800 nanometre. The commonly used range for absorbance is from 0 to 2, 0 corresponds to 100 percent transmittance, no absorbance means 100 percent transmittance. And 2 corresponds to one percent transmittance. The wavelength of maximum absorbance is the characteristic value of the sample, and it is designated as lambda max. So, the lambda max is the wavelength at which maximum absorbance occurs.

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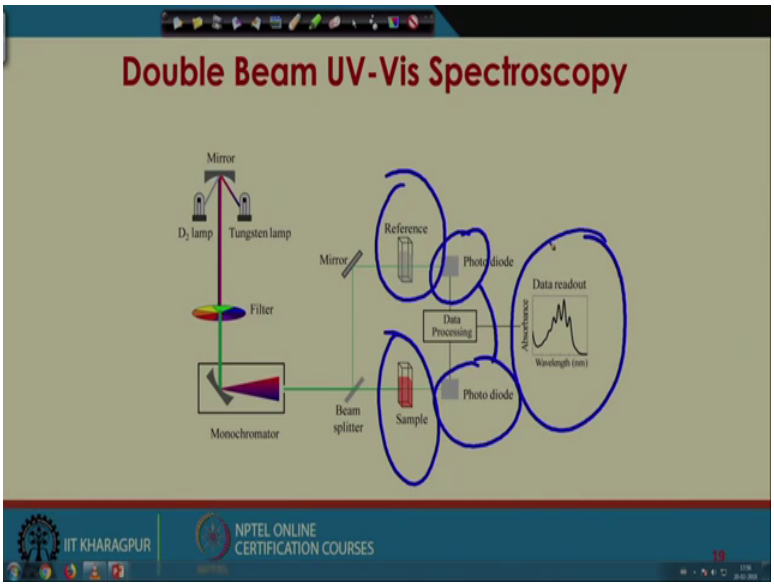

In case of double beam UV-Vis spectroscopy or double beam UV-Vis spectrophotometer that are commercially available, a beam alternately passes through the reference cuvette and the sample cuvette. These are passed through the detectors, and then these detectors process data; that means, absorbance versus wavelength, and absorbance versus wavelength is plotted as spectroscopy. So, the basically the light beam alternately passes through the reference cuvette and the sample cuvette. When the light beam passes through the sample cuvette, it measures I when the light beam passes through a reference cuvette, it basically measures I 0.

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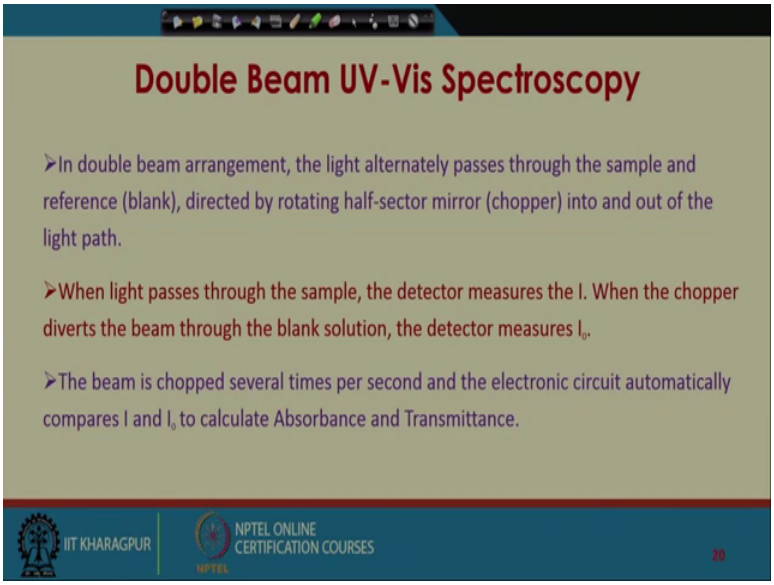

In double beam arrangement the light alternately passes through the sample and reference, directed by rotating half sector mirror or chopper into and out of the light path. When light passes through the sample the detector measures I, when the chopper diverts the beam through the blank solution the detector measures I 0. The beam is chopped several times per second and the electronic circuit automatically compares I and I 0 to calculate absorbance and transmittance.

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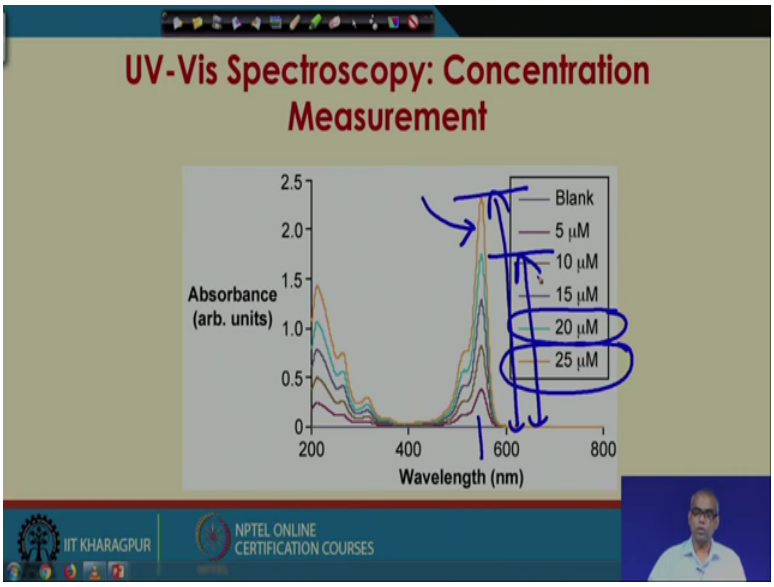

So now let us see how we can measure concentration using UV-Vis spectrophotometer. What you see is an absorption spectra, for say a particular compound dissolved in a transparent solvent. And I have prepared samples of various concentration; say, the concentrations are 5 micro molar, 10 micromolar, 15, 20, 25 so on and so forth. So, I also prepared a blank sample; that means, I take only the solvent, but no sample is there.

So, if you look at the absorption spectra, you see that corresponding to this lambda, there are some peaks and corresponding to this lambda again there are some peaks. As the concentration increases, the peak height increases. Look at here let us say this is the lambda at which I get maximum absorbance. So, if we focus you are at your attention at this wavelength, this solution has this peak height, whereas the solution with 20 micro molar has below this. So, what it means is, that I can relate the peak height at a particular wavelength, and I choose that wavelength at which maximum absorbance occurs. Let us

call that as lambda max, at that lambda max the peak height can be related to the concentration of the solution.

So, this information can be used to set up a calibration curve.

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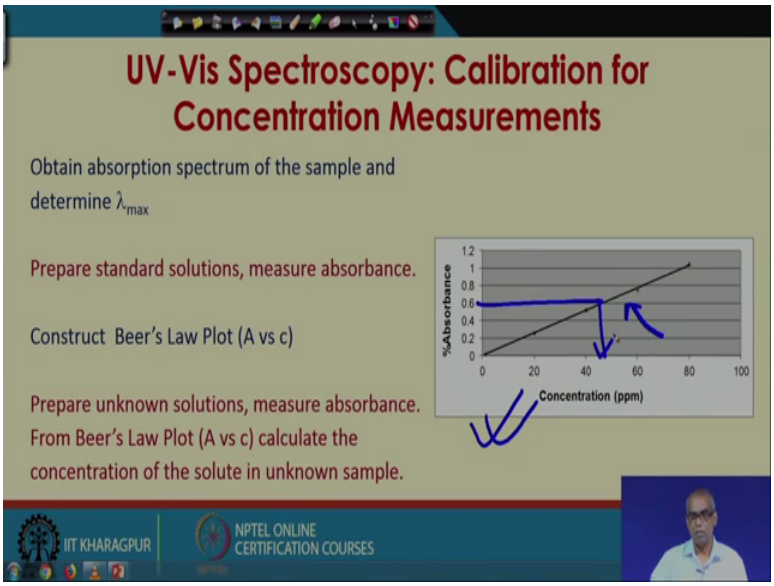

So, to set up a calibration for concentration measurement. We first need to know the lambda max so, obtain absorption spectrum of the sample and determine lambda max. Now prepare standard solutions measure absorbance construct beer slope lot; that means, absorbance versus concentration. So, first let us know the lambda max, then prepare standard solutions of known concentrations of that sample. Look at that take the absorption spectra, look at the peak height or the absorbed percentage absorbance corresponding to that lambda max, and make a plot of absorbance versus concentration that we have done here; so, this is the calibration plot.

Now, when I want to know the concentration of the unknown sample, I take the absorption spectra find the absorbance. So, if I get the 6 my absorption as absorbance as 0.6 from this plot I know that the concentration is somewhere around let us say close to 50 ppm. Note that these analysis are best carried out at dilute solutions.

So, this is how you can prepare a calibration curve, and from the calibration curve you can know the unknown samples concentration. What I showed is for the case when you have a single component in this solution. Suppose you have a mixture, I have 2

component let us say x and y. I can still extend this for multi component mixture as follows.

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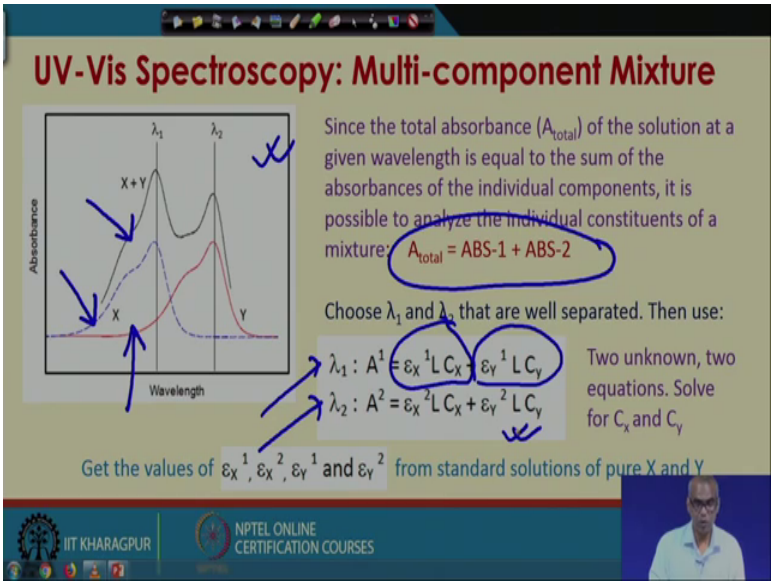

Now, let us say this is the absorption spectra for pure x and this is absorption spectra for pure y. So, when you have a mixture of x and y, you get these as absorption spectra. Since the total absorbance of the solution at a given wavelength is equal to the sum of the absorbance of the individual components. It is possible to analyze the individual constituents of a mixture. Because we know that a absorbed total absorbance is equal to sum of the individual absorbance.

So, what we will do is, we will first choose 2 wavelengths let us say lambda one lambda 2 that are well separated as shown in the figure. Then write this equations for 2 lambdas. We know that absorbance is molar absorptivity into path length into concentration. So, the absorbance at lambda one is the sum of absorbance for x and for y. So, I write molar absorptivity for x into path length into concentration of x, plus, molar absorptivity of y into path length into concentration of y. The same equation I write for lambda 2. Now look at these equations, you have 2 equations and 2 unknowns, unknowns are Cx and Cy.

So, you can solve these 2 equations to find out the concentration Cx and Cy. Of course, we need to know the x the molar absorptivity. Molar absorptivity at lambda one for x and y and molar absorptivity for x and y at lambda 2. We can find out these values of the molar absorptivity of x and y at lambda one lambda 2 from standard solutions of pure x

and pure y. So, this is how I can calculate I can find out the concentrations of x multi component mixtures. I have shown it here for 2 component mixture, but this can also be extended for more than 2 component mixtures.

So, we will stop our discussion on concentration measurement by UV-Vis spectrophotometer here.