Advance Analytical Course

Prof. Padma Vankar

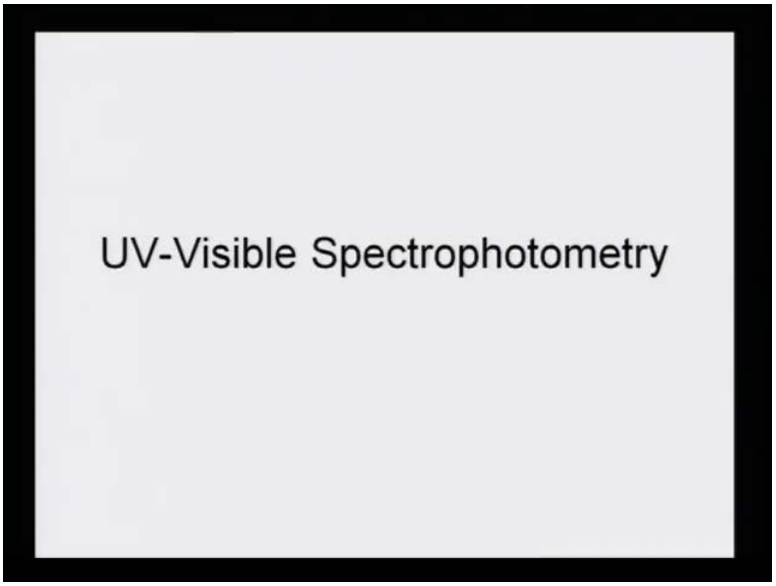
Department of Chemistry

Indian Institute of Technology, Kanpur

Lecture No. # 30

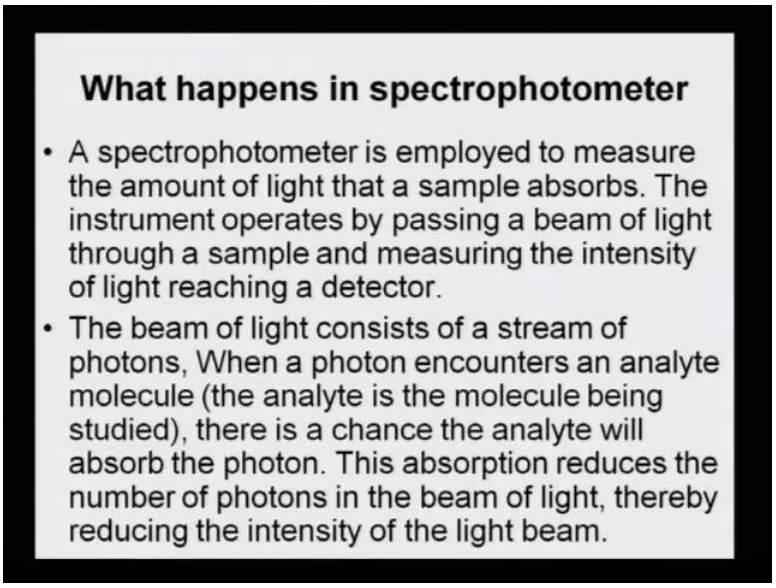
Among the other methods of detection of organic and inorganic compound, one major spectroscopic method we have fleetingly talked about is the UV visible spectrometry.

(Refer Slide Time: 00:56)



We also call it UV visible spectrophotometry, because there is a spectrophotometer, the machine has relation with the electromagnetic radiation, and that is why it is called UV visible spectrophotometer or UV visible spectrophotometry.

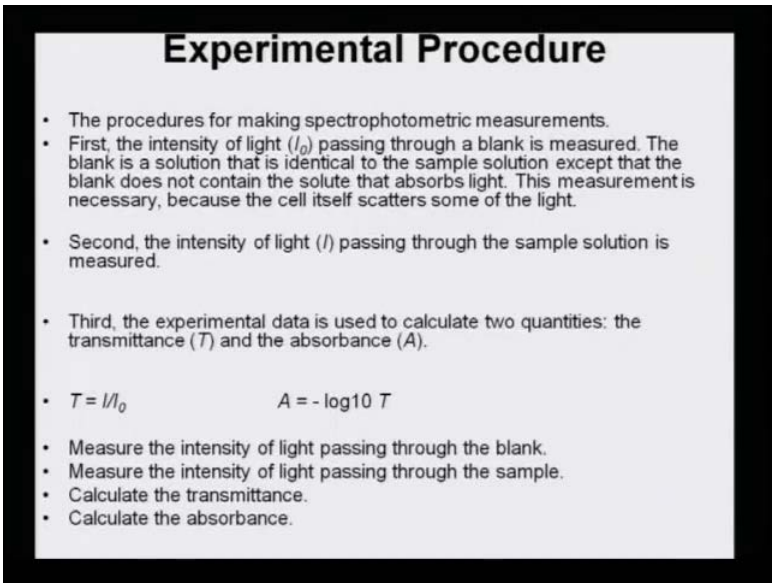
(Refer Slide Time: 01:02)



What happens in a spectrophotometer? A spectrophotometer is employed to measure the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector. So, as what I mentioned, if the incident light has a UV active component, will absorb certain portion of the light. And so, the beam of light, when it passes through the sample, the intensity of the transmitted light will be reduced if some energy has been absorbed.

The beam of light consists of stream of photons. When a photon encounters an analyte molecule, the analyte is the molecule being studied, there is chance that the analyte will absorb the photon. This absorption reduces the number of photons in the beam of light, thereby reducing the intensity of light beam. So, that is what actually happens. But, this only happen if there are compatible groups, which can absorb UV light, not otherwise. So, the compound must have certain portions in the molecule, which can get excited.

(Refer Slide Time: 02:44)

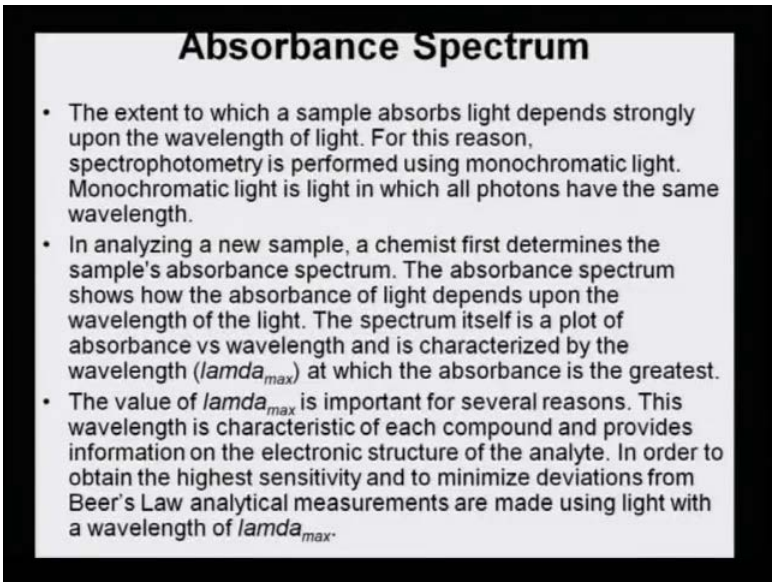


The experimental procedure is very simple. The procedure for making spectrophotometric measurement is, first, the intensity of light (l o) passing through a blank is measured. The blank is a solution that is identical to the sample solution except that the blank does not contain the analyte or the solute that absorbs light. So, the blank is just the solvent whatever be the solvent of measurement, and the sample will have the solvent and the analyte. This measurement is necessary, because the cell itself scatters some of the light. So, it is only for zero corrections that anything which is absorbed by the solvent should not create any interference, and therefore, the solvents are kept the same. Secondly, the intensity of light (l) passing through the sample solution is then measured. And, the third thing that is done is that the experimental data used to calculate two quantities: the transmittance or the absorbance; so, it is either of them. So, transmittance is equal to l by l 0. That means whatever is the intensity of light after passing the sample through the sample and l 0 is the intensity of light passing through the blank; or, it can be expressed in terms of absorbance, which is equal to minus log 10 T.

Measure the intensity of light passing through the blank. Measure the intensity of light passing through the sample. Calculate the transmittance. And, calculate the absorbance. So, the equations are very simple; the activity of this machine is also very simple. Light of electromagnetic spectrum of the UV visible region is the incident light, but it is so much higher in energy that it can excite electrons, which are present as double bond CC

double bond, that is, the pi electrons, or the electrons, which are present as a coordinate electrons.

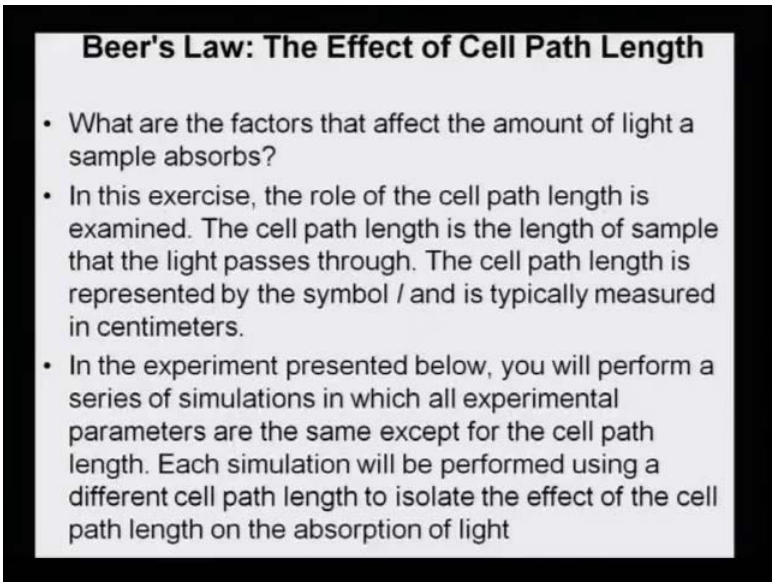
(Refer Slide Time: 05:15)



Absorbance spectrum – the extent to which a sample absorbs light depends strongly upon the wavelength of light. For this reason, a spectrophotometry is performed using monochromatic light. Monochromatic light is light in which all photons have the same wavelength. So, it is a simplified module or machine, which uses the simple idea of using monochromatic light. As the name suggests, monochroma, that means there is only one wavelength incident on the sample at a time. In analyzing a new sample, a chemist first determines the sample's absorbance spectrum. The absorbance spectrum shows how the absorbance of light depends upon the wavelength of light. So, when absorbance spectrum is taken for the analyte, it is simply qualitative information, whether or not any functional group or chromophore is present which can get excited by this incident light. The spectrum itself is a plot of absorbance versus wavelength and is characterized by the wavelength (lambda max) at which the absorbance is the greatest. So, what happens, that when the light is incident and the analyte has a matching chromophore or excitation moieties within the molecule, those portions will get excited, and the absorbance is directly proportional to the concentration, and the wavelength of a characteristic compound remains the same. Only if we change the solvent, then there may be some variation.

The value of lambda max is very important for several reasons. This wavelength is characteristic of each compound and provides information on the electronic structure of the analyte. In order to obtain the highest sensitivity and to minimize deviations from Beer's-Lambert's law, analytical measurements are made using light with a wavelength of lambda max. So, what happens is that, this value is of great significance. Every compound because of its perfect structure, the structure of a compound does not change. So, the positioning of the chromophore in the molecule remains the same, and therefore, for that reason, the wavelength is a characteristic of every compound. And that, if the compound has to follow this UV visible spectrum analysis, then it should follow the Beer's-Lambert's law, which states that absorbance is directly proportional to the concentration of the material.

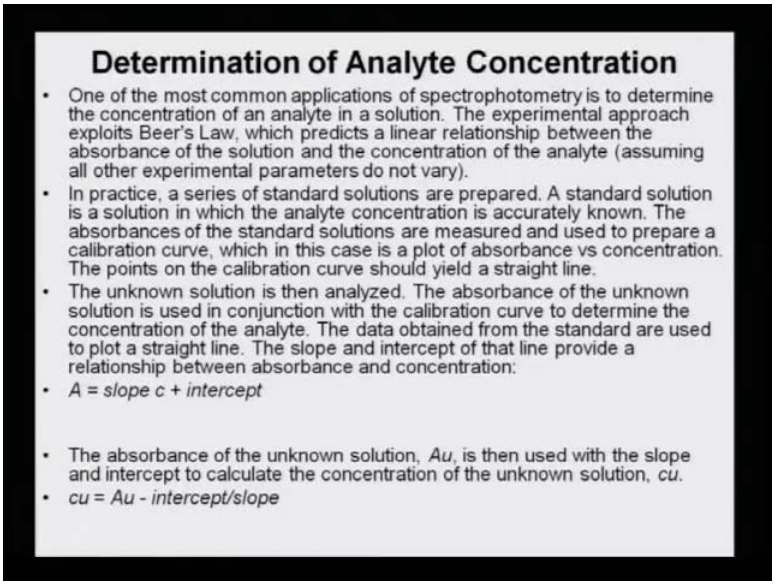
(Refer Slide Time: 08:42)



Beer's law – the effect of cell path length – What are the factors that affect the amount of light a sample absorbs? In this exercise or in this lecture, the role of cell path length is examined; the cell path length is the length of sample that the light passes through. The cell path length is represented by the symbol l and is typically measured in centimeters. So, the cell path length is very important, because the amount of light that will be passing if the cell path of the sample and the analyte are varied, the results cannot be compared. It cannot be a validation or calibration method if the cell path is not kept constant.

In the experiment presented below, there will be a performance of series of simulation in which all experimental parameters are the same except the cell path length. Each simulation will be performed using a different cell path length to isolate the effect of cell path length on the absorption of light. Therefore, the cell path length is of great importance; why, because the if the cell path length is varied, if the cell is made small or larger or very large, the amount of light that will pass through will also be variable, and therefore, it cannot be compared.

(Refer Slide Time: 10:35)



Determination of analyte concentration – a little while ago, I did mention that the absorbance is related to the concentrate of the analyte. One of the most common applications of spectrophotometry is to determine the concentration of an analyte in a solution. The experimental approach exploits Beer's-Lambert's law, which predicts a linear relationship between the absorption of the solution and the concentration of the analyte, assuming that all experimental parameters do not vary. That under identical condition, the sample or the analyte will show a direct relationship between the absorbance and the concentration, provided the experimental parameters do not change or are not changed.

In practice, a series of standard solutions are prepared. A standard solution is a solution in which the analyte concentration is accurately known. The absorbances of the standard solutions are measured and used to prepare a calibration curve, which in this case is a

plot of absorbance versus concentration. The points on the calibration curve should yield a straight line. Now, in order to find out whether there is validity between the absorbance and the concentration, the best method to validate this process is to prepare a calibration curve. And, for the purpose of calibration curve, several standard solutions are prepared. Now, these standard solutions will have a known concentration, that is, 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm standards will be prepared. And, their absorbances will be plotted against the concentration. If the concentrations are correct, if the sample has been prepared correctly, and if it follows Beer's-Lambert's law, then it will be giving a straight line passing through the axis. And, that kind of calibration curve, we say that it is the perfect calibration curve and can be used for further unknown samples.

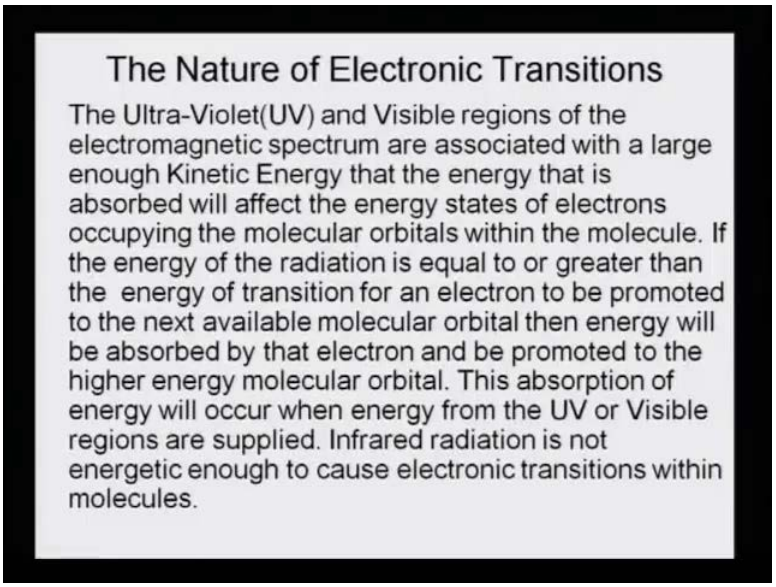
The unknown solution is then analyzed. The absorbance of the unknown solution is used in conjunction with the calibration curve to determine the concentration of the analyte. The data obtained from the standard are used to plot a straight line. The slope and the intercept of that line provide a relationship between the absorbance and the concentration; or, we can say that A is equal to slope c plus intercept. What does this mean that we have made a calibration curve of 1, 2, 3, 4, 5 ppm. Now, if the sample is ranging between these 1 to 5 ppm solution, it will fall somewhere on this line. If the concentration is very high, 10 or above ppm or if the concentration is very low, 0.01 ppm, then this calibration curve will not hold for the particular dilution of the sample. So, that means that the sample and the unknown sample and the calibration curve must be of equal competence; there should not be too much of diversity.

If we are dealing with 1, 2, 3, 4, 5 ppm, the unknown should fall somewhere between this range; otherwise, we will need to make other calibration standards for able to fit into this calibration range; otherwise, we can dilute the solution. Suppose if it is 10 ppm unknown is having something above 10 ppm, then definitely it cannot be calibrated in a range of 1 to 5. Then, the solution must be diluted. And, let me tell you that the art of doing an excellent UV absorption concentration analysis lies when the solutions are very dilute. That is the time the Beer's-Lambert's law completely follows and is adapted best. If the solutions are concentrated, then the adaption does not take place, there are aggregates that are formed, then the absorbance is directly not proportional to the concentration and problem arises, and the results that are available or not the correct results. Hence, for unknown samples, two things have to be kept in mind: first thing, the

dilution should be good, and second thing is that it should fall into the calibration range of the calibration standard that is being used to identify the concentration of the analyte.

The absorbance of the unknown solution, Au, is then used for the slope and the intercept to calculate the concentration of the unknown solution, cu. So, cu is equal to Au minus intercept upon slope. That is how it is calculated. So, it is a simple analysis and yet it has a very great utility. Sometime back when I was talking about chromium and its analysis from water samples, the best method to use is a UV visible spectrophotometer. And, the concentrations of chromium 6 are analyzed by this machine, and they are found to be very accurate and very correct.

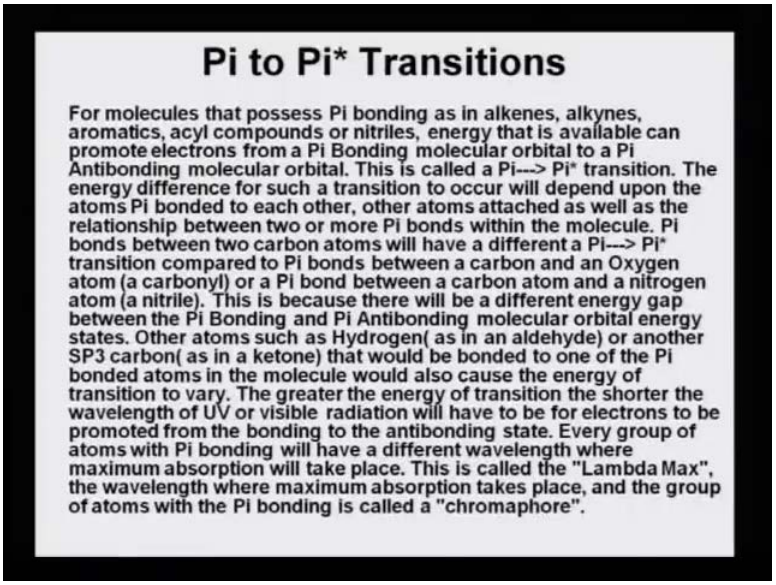
(Refer Slide Time: 16:54)



What exactly happens in UV visible spectrophotometry? The ultra-violet and the visible regions of the electromagnetic spectrum are associated with a large enough kinetic energy, and that the energy that is absorbed will affect the energy states of the electrons occupying the molecular orbitals within the molecule, which means that there are bonds, unsaturated bonds; means there are pi bonds and sigma bonds. The pi electrons, which are more labile or loosely held up in the molecular orbitals. And, it is these loosely held electrons, which are then excited by the absorption of UV and visible light to more excited state. So, there is enough kinetic energy for the excitation of these electrons from the bonding molecular orbitals to the anti-bonding molecular orbitals.

If the energy of the radiation is equal to or greater than the energy of the transition for an electron to be promoted to the next available molecular orbital, then energy will be absorbed by that electron and be promoted to the higher energy molecular orbital. This absorption of energy will occur when energy from the UV or visible regions are supplied. Infrared radiation is not energetically enough to cause electronic transitions within the molecules. We just learned about IR and we saw that IR, the radiation energy content is much lower. So, what it does? It only kind of shakes the bond, but in this UV visible spectrophotometry, the electrons are excited, and they are excited and going to the next molecular orbital. Therefore, the electronic transactions actually take place in this case. Whereas, in IR's case, the bonds only simply oscillate or bend or rotate, and that is the only thing that can happened to a bond; there is no electronic excitation; only the bond excitation, because they are in lower in energy content.

(Refer Slide Time: 19:50)



The most fissile transition of electrons getting promoted to antibonding molecular orbital is the Pi to Pi star transition. Time and again, I have fleetingly mentioned, but now is the time to look into the details of UV visible spectrophotometry. For molecules that possess Pi bonding, such as alkenes, alkynes, aromatics, acyl compounds or nitriles, energy that is available can promote electrons from a Pi bonding molecular orbital to a Pi antibonding molecular orbital. So, you see, from a bonding, it goes to an empty antibonding molecular orbital, and this transaction can only take place by the absorption of UV or visible light. This is called the Pi to Pi star transition. So, the energy difference

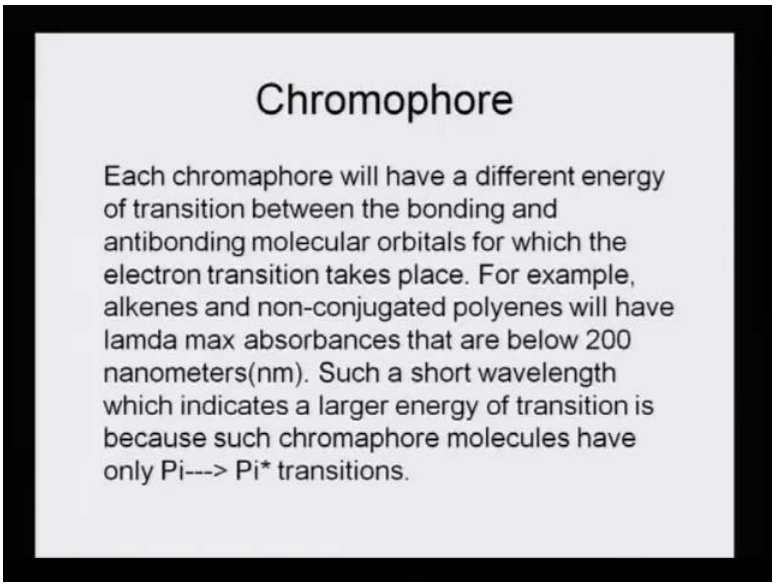
for such a transition to occur will depend upon the atoms Pi bonded to each other, other atoms attached as well as relationship between two or more Pi bonds within a molecule. So, if it is an isolated bond, then the Pi bonding, one single bond, which is a double bond or a triple bond, or it is an array of double bond, single bond, double bond, or the double bond is alternating with a carbonyl group or a nitrile group. Such situations can give rise to Pi to Pi star transition.

Now, if one has to look at various possibilities, the Pi bonds between two carbons will have a different Pi to Pi star transition compared to Pi bonds between a carbon and an oxygen atom. Obviously, the electronic environment of carbon-carbon double bond and an electronic environment of carbon-oxygen double bond are bound to be different. So, the Pi to Pi star transitions also will be very different. So, one should not generalize that all Pi to Pi star transitions are the same; why, because when there is a hetero atom, there is a Pi to Pi star transition possible and there is an n to pi star transition also possible, particularly in the case of hetero atoms like oxygen and nitrogen. This is because there will be different energy gaps between the Pi bonding and the Pi antibonding molecular orbital energies and their states.

Other atoms, such as hydrogen as in the case of aldehyde or another SP3 carbon as in the case of ketone that would be bonded to one another Pi bonded atoms in the molecule would also cause the energy of the transition to vary. So, the changes are brought because of the immediate environment, where this Pi bond is located. What is the next group? Whether it is contributing or it is not contributing into these transactions will also make a difference. The greater the energy of transition, the shorter the wavelength of UV or visible radiation will have to be for electrons to be promoted from the bonding to the antibonding state. Obviously, if the gap between the antibonding and the bonding is small, the electron has to jump from here to here (Refer Slide Time: 23:20). If the gap is large, the electron has to jump from here to here. So, obviously, the energies that would be required would be dependent on the positioning of the available antibonding molecular orbitals. Every group of atoms with Pi bonding will have a different wavelength, where lambda max or the absorption maximum will take place. This is called the lambda max. A range of wavelength of light is falling, but at one particular wavelength, the absorption is maximum. And, that point at which the absorption is

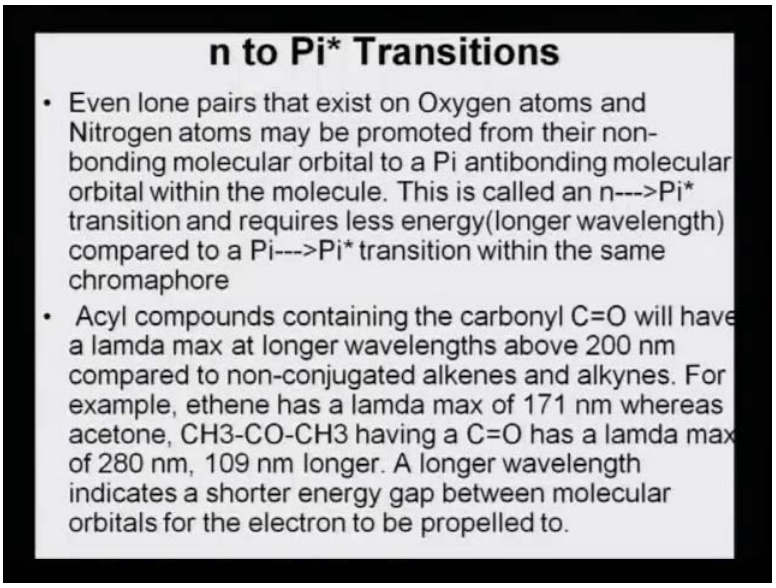
maximum is called the lambda max as the name suggests. And therefore, the group or the chromophore will have a certain lambda max always remaining the same.

(Refer Slide Time: 24:46)



The group of atoms with Pi bonding is called the chromophore; or, the functional group part or the most sensitive part of the molecule, which causes these electronic excitations are the points, which are referred as chromophore. So, let us look at the definition of chromophore. Each chromophore will have a different energy of transition between bonding and the antibonding molecular orbital for which the electron transition takes place. For example, alkene and non-conjugated polyenes will have lambda max absorbances that are below 200 nanometers. Such a short wavelength which indicates a large energy of transition is because such chromophore molecules have only Pi to Pi star transitions. So, what does it mean? That each chromophore definitely has different energy content, because of its chemical environment between the bonding and the antibonding molecular orbitals for which the electrons transition can take place. For example, if we take an alkene, a simple example ethane, which is non-conjugated; it has nothing attached to it; it is just a two carbon system with just one double bond. It will have a lambda max, which is approximately 200 nanometers, because this short wavelength means it will take more energy for its excitation to go to the Pi star antibonding molecular orbital.

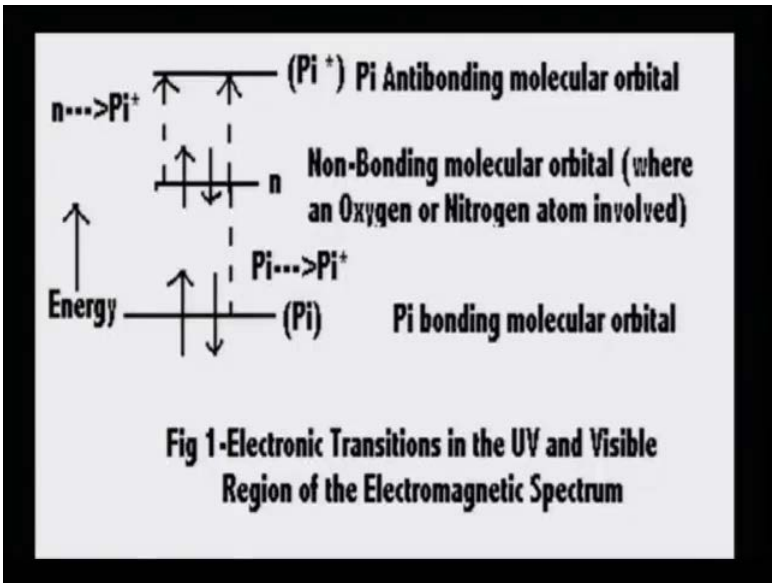
(Refer Slide Time: 26:33)



Then, there are these n to Pi star. n to Pi star transitions occur in the chromophore only when there are lone pairs, and the lone pairs are always available with the hetero atoms like oxygen, nitrogen. And, these may be promoted from their non-bonding molecular orbitals to the Pi antibonding molecular orbitals within the same molecule. So, now, it is the non-bonding electrons, which are getting excited to the pi antibonding molecular orbital. This is called the n to Pi star transition; and, requires lesser energy or longer wavelength compared to Pi to Pi star transition within the same chromaphore. So, Pi to Pi star requires more energy; whereas, n to Pi star… because the gap is small. I just explained to you that if the gap is small, n to Pi star is like this (Refer Slide Time: 27:15) and Pi to Pi star like this. So, obviously, more gap, more energy; less gap, less energy.

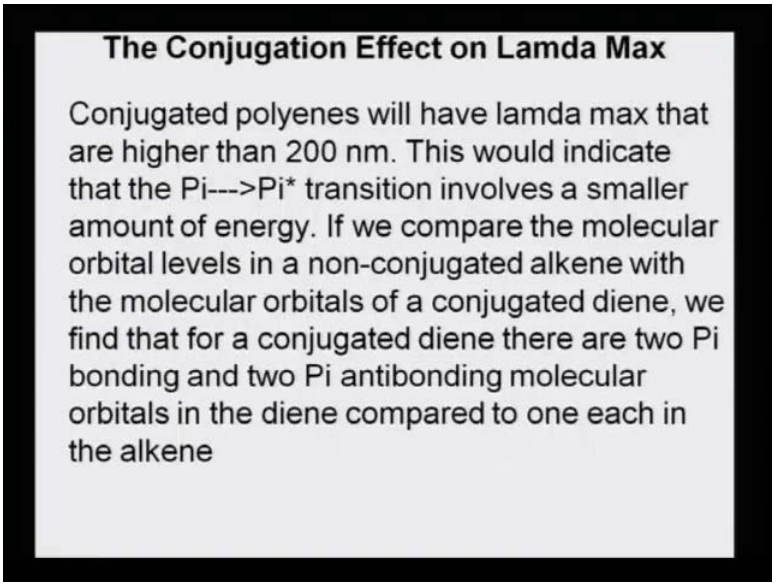
Acyl compounds containing the carbonyl CO will have a lambda max at longer wavelengths above 200 nanometers compared to non-conjugated alkenes and alkynes. For example, ethene has a lambda max of 171 nanometers; whereas, acetone, which is CH3-CO-CH3 having a CO has a lambda max 280 nanometers. So, there is a difference of 109 nanometers and it is longer by 109. A longer wavelength indicates a shorter energy gap between the molecular orbitals for the electrons to be propelled to. So, I just explained to you that n to Pi star is like this (Refer Slide Time: 28:13) with the smaller gap, and Pi to Pi star is like this with the longer gap. So, the shorter the gap, the shorter energy will be required; the longer the gap, the larger energy will be required for transition.

(Refer Slide Time: 28:32)



Now, looking at this slide, which is a very important pictorial description of the energy levels of where the Pi electron is, where the Pi star molecular orbitals are, and where is the gap. This is the n electron, and from here to here, the jump is very small. So, electronic transitions in the UV and visible region of electromagnetic spectrum can be easily understood by this pictorial description.

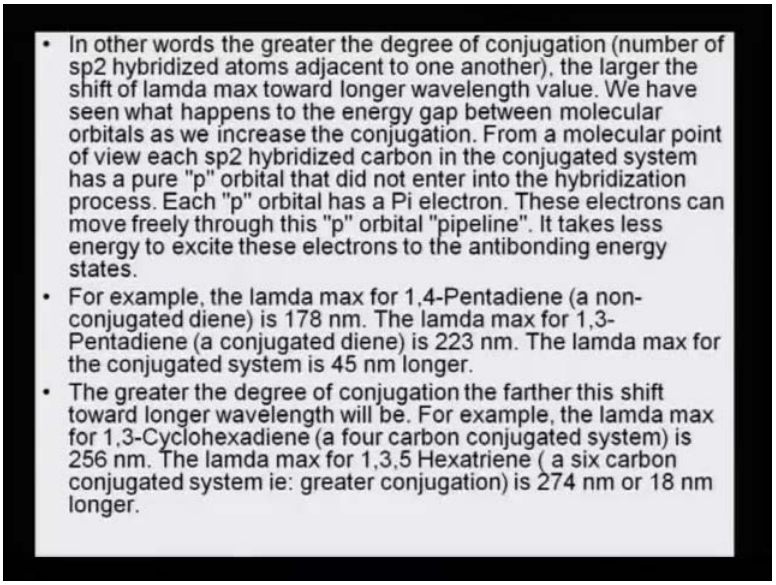
(Refer Slide Time: 29:03)



Now, what is the conjugation effect on lambda max? Conjugated polyenes will have lambda max that are higher than 200 nanometers. This would indicate that the Pi to Pi

star transition involves a smaller amount of energy. If we compare the molecular orbital levels in a non-conjugated alkene with the molecular orbitals of a conjugated diene, we find that a conjugated diene there are two Pi bonding and two Pi antibonding molecular orbitals in the diene in compared to one each in the alkene. Now, what if there are conjugations? That means double bond, single bond, double bond, single bond. And, if one compares what is the scenario of these transitions, Pi to Pi star transition has to take place. But, now, it will be more number of times in conjugated polyenes as compared to just one time in case of ethene. So, that is how it is compared.

(Refer Slide Time: 30:17)

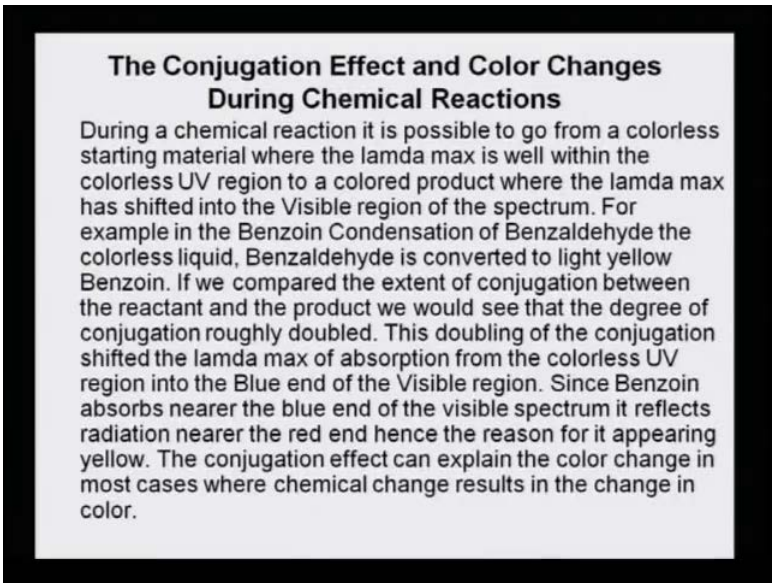


In other words, the greater the degree of conjugation, which means, the number of sp2 hybridized atoms adjacent to one another, the larger will be the shift of lambda max. Obviously, more and more and more excitations; so, the lambda max becomes larger, which requires lesser energy. We have seen what happens to the energy gap between the molecular orbitals as we increase the conjugation. From a molecular point of view, each sp2 hybridized carbon in the conjugated system has a pure p orbital that did not enter into the hybridization process. Each p orbital has a Pi electron. This electron can move freely through this p orbital pipeline. It takes less energy to excite these electrons to the antibonding energy state. So, it is the unreactive p orbital, which is now participating. And, because of this alternating double bond, single bond, double bond, the excitement of these electrons is more fissile, and therefore, lesser amount of energy is required and longer wavelength will be attained.

For example, the lambda max of 1, 4-Pentadiene, which is not a conjugated diene, which the one double bond is at one end and the other double bond is at the other end, and they are separated by two single bonds, the lambda max is 178 nanometers. The lambda max of 1, 3-Pentadiene, which is a conjugated diene, the lambda max becomes 223 nanometers. So, the lambda max for the conjugated system is about 45 nanometers longer. So, more the number of conjugation, the higher will be the wavelength, and therefore, lower will be the energy.

The greater the degree of conjugation, the farther the shift toward longer wavelength, and therefore, for example, the lambda max of 1, 3-Cyclohexadiene, a four carbon conjugated system, is 256 nanometers; whereas, the lambda max for 1, 3, 5 Hexatriene, a six carbon conjugated system, greater conjugation, is at 274 nanometers, which is about 18 nanometers longer. So, the more again and again I am emphasizing on the fact, that if we have more than one double bond situation, the wavelength or the lambda max goes on increasing in the sequential manner.

(Refer Slide Time: 33:30)

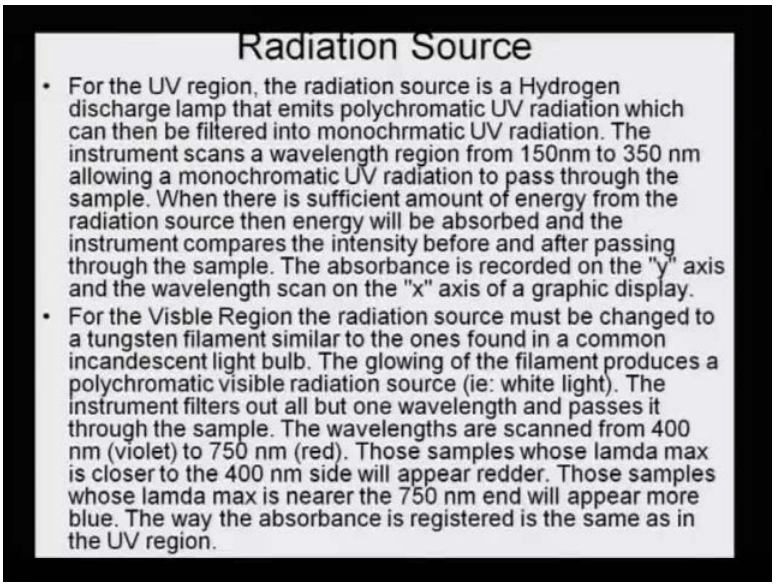


Conjugation effect and color changes during chemical reactions – it is the beauty that both visible as well as UV regions are ascertained by the same machine. So, during a chemical reaction, it is possible to go from a colorless starting material, where the lambda max is well known well within the colorless UV region to a colored product, where the lambda max has shifted to the visible region of the spectrum. If you had

remembered, the UV region was followed by visible, was followed by IR region. Therefore, one can see that if a reactant to begin with is colorless and if the product of those reactants is a colored compound, even they can be analyzing on UV visible spectrum. For example, in the Benzoin Condensation of Benzaldehyde, the colorless liquid, Benzaldehyde is converted to light yellow Benzoin.

If we compare the extent of conjugation between the reactant and the product, we would see that the degree of conjugation roughly doubled. This doubling of the conjugation shifted the lambda max of absorption from the colorless UV region into the blue end of the visible region. Since Benzoin absorbs nearer the blue end of the visible spectrum, it reflects radiation nearer to the red end; hence, the reason for it to be appearing yellow. The conjugation effect can explain the color change in most cases where chemical change results in the change in color. So, it is the beauty of chemistry and the reactions and the molecules that are formed that from Benzaldehyde, which was the starting material, which was colorless, colored compound Benzoin, light yellow compound, was synthesized by the Benzoin condensation of Benzaldehyde. And now, because it was having more number of conjugations, the colorless compound shifted from UV region to visible region for getting analyzed.

(Refer Slide Time: 35:42)



Radiation source – for the UV region, the radiation source is a hydrogen discharge lamp that emits polychromatic UV radiation, which can then be filtered into monochromatic

UV radiation. The instrument scans a wavelength region from 150 nanometers to 350 nanometers, allowing a monochromatic UV radiation to pass through the sample. When there is sufficient amount of energy from the radiation source, then energy will be absorbed and the instrument compares the intensity before and after passing through the sample. The absorbance is recorded on the y-axis and the wavelength scan is taken on the x-axis of the graphic display. So, in a spectrum of UV or visible spectrum, the x-axis is the wavelength scan, which is expressed in nanometers, and the y axis shows the absorbance. Now, it is very simple information and correlation that hydrogen discharge lamp is the source of polychromatic UV light, but polychromatic light is not actually passed through the sample. It is filtered and only monochromatic light is passed through the sample. And, this sample or the light source gives radiation from 150 nanometers to 350 nanometers.

For the visible region, the radiation source must be changed to a tungsten filament similar to the ones found in the common incandescent light bulb. The glowing of the filament produces a polychromatic visible radiation source, that is, the white light. The instrument filters out all, but one wavelength and passes it through the sample. The wavelengths are scanned from 400 nanometers to 750 nanometers. Those samples whose lambda max is closer to the 400 nanometers side will appear redder. Those samples whose lambda max is nearer to 750 nanometers end will appear more blue. The way the absorbance is registered is same as that of the UV region. So, you see that for two different sources or for the two different regions, that is, the UV region and the visible region, radiation sources are different. In the case of UV, the use of hydrogen discharge lamp is made and in the case of visible, it is the common tungsten filament lamp, which is called incandescent light bulb. So, these have to be kept in mind that although the spectrophotometer can be used for UV and visible, both spectrum, but the light sources are different.