**Advance Analytical Course**

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**Lecture No. # 31**

In UV spectroscopy, 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.

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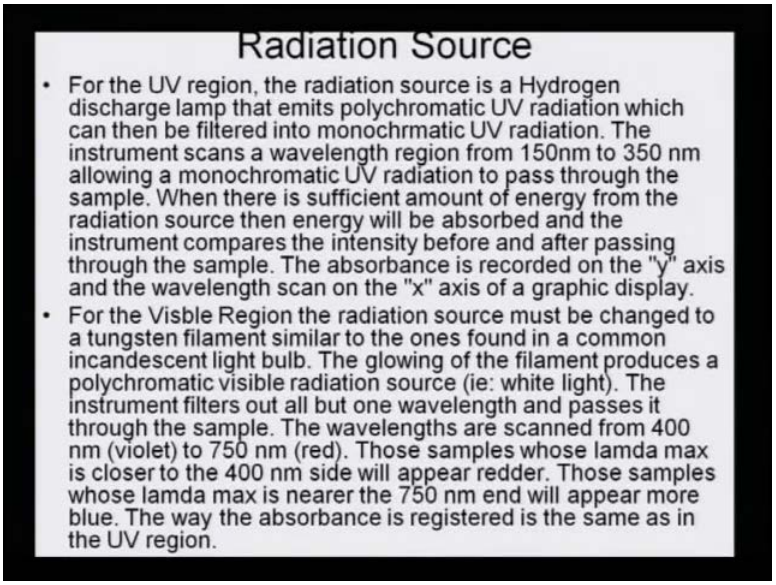


The instrument scans a wavelength region from 150 nanometers to 350 nanometers, or sometimes, 190 to 400 nanometers allowing a monochromatic UV radiation to pass through the sample. When there is sufficient amount of energy from the radiation source, then energy will be absorbed and the instrument compares the intensity before and after passing through the sample. The absorbance is recorded on the y-axis and the wavelength scan on the x-axis of the graphical display.

So, when we try to look at the UV absorption spectroscopy, the first thing that must be remembered is that the source of radiation for UV light is a Hydrogen discharge lamp. Second thing is that the lamp actually gives out polychromatic radiation, but it needs to

be filtered and only monochromatic radiation is allowed to pass through the sample. And then, that radiation before and the transmitted light are compared on the x and y-axis.

If the molecule has chromophoric groups, as what we discussed a while ago, then it will definitely absorb, and that is an indication, that there are presence of chromophoric groups or there is at least one chromaphore in the molecule. For visible region, that is for colored compounds, the visible region has to be taken into account and when visible light has to be shown on the sample, it is necessary to change the lamp to a tungsten filament or an ordinary incandescent bulb.

Now, the glowing of the bulb again gives polychromatic light, but this polychromatic light is then filtered through our filter and monochromatic light is passed through the sample, but this monochromatic light is from the visible region. The glowing of the filter produces polychromatic visible radiation, that is, the white light. The instrument filters out all but one wavelength and passes it through the sample. The wave lengths are scanned from 400 nanometers which is a violet region, to 750 nanometers which is the red region. That means in that VIBGYOR, the violet and the red regions are demarcated by 400 nanometers and 750 nanometers, respectively. Those samples whose lambda max is closer to the 400 nanometers side will appear redder. Those samples whose lambda max is nearer the 750 nanometers end will appear more blue; that is because we see the complimentary color through the eyes.

The way the absorbance is registered is same as the UV region. So, the absorption part that is the light status before and after the absorption is actually compared and whatever is absorbed is considered to be absorbed due to the presence of chromophoric group in the molecule; so, that part remains the same.

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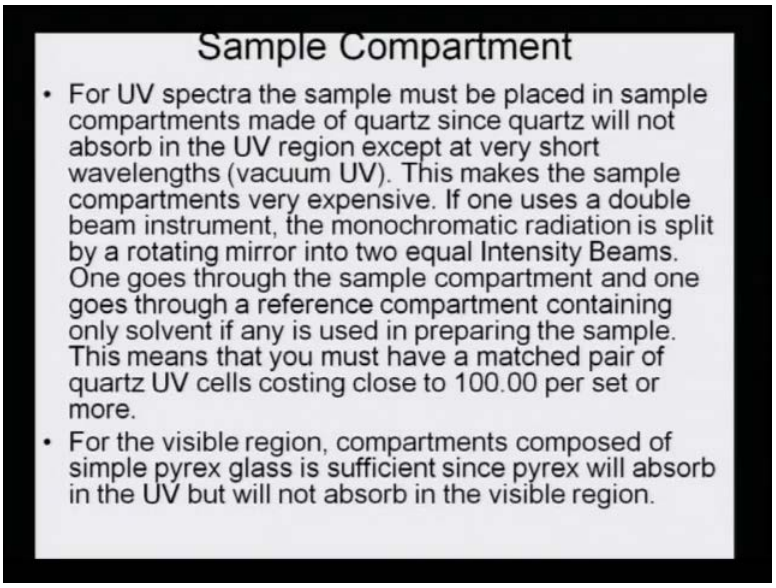


That sample compartment is also very special. For UV spectra, the sample must be placed in a sample compartment made of Quartz, since Quartz will not absorb in the UV region except at very short wavelengths that is vacuum UV, which we normally do not use. So, it needs to be made out of a material; the sample compartment or the sample cell must be made out of Quartz. Why? Because Quartz does not absorb in the normal UV region; it is only under a very special vacuum UV wavelength or very short wavelength that it absorbs. And that is not the region of study. Anyway, this makes the sample compartments very expensive. If one uses a double beam instrument, the monochromatic radiation is split by a rotating mirror into two equal intensity beams: one goes through the sample compartment and one goes through a reference compartment containing only solvent, if any is used in preparing the sample. This means that you must have a matched pair of Quartz UV cell costing close to 1000 or more dollars per set.

For visible region, that compartment is composed of simple pyrex glass and that is sufficient, because pyrex does not absorb in the visible region, but it absorbs in the UV region. So, one cannot use it for the analysis of UV region, but if we take Quartz, we can use it for both UV region and visible region. So, it is better to use one cell compartment which can suffice the need for both, but there is a possibility that pyrex cells can be used in the visible region, as well.

Now, I want to make it very clear, that this light which is split into two beams - one is passing through the sample and the other one is going through only the solvent. It is to nullify the effect of the solvent, if at all it has absorbed any, because this we called as auto zero or nullifying the solvent effect.

Now, if both the lights are passing, then if there is a chromaphore present in the sample chamber or sample compartment, then it will absorb. So, the one which only has solvent will not absorb and the one which holds the sample will absorb, and so this difference will also give us an indication that the analyte thus possess chromophoric group, whether it is in the UV region or the visible region, it holds good.

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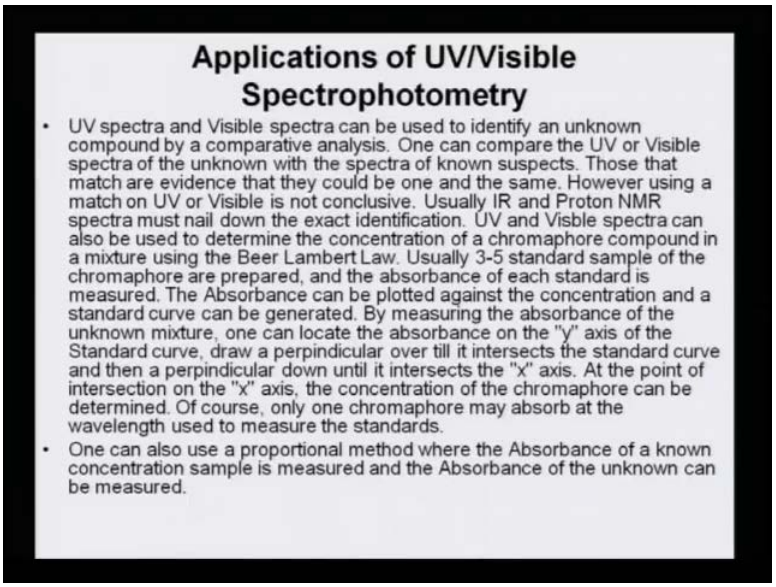


Application of UV visible spectrometry - it has a huge application; UV visible, UV spectra, and visible spectra can be used to identify an unknown compound by a comparative analysis. One can compare the UV or visible spectra of unknown with the spectra of known suspects. Those that match are evidence that they could be one and the same. However, using a match on UV or visible is not conclusive, which means that we can compare the spectra of known and the unknown. And if they have similar peaks, we can say that it possesses or it may have the same molecular structure, but it is not just one method is not enough that it is conclusively the same molecule.

Usually IR and proton NMR spectra must nail down the exact identification. UV and visible spectra can also be used to determine concentration of chromaphore compound in a mixture using Beer Lambert's law.

Now, if we want to find out, whether a particular substance is present, we can use the lambda max identification method. But if we want to quantify how much of that substrate is present, that means the concentration, as what was earlier mentioned to you, that beer Lambert's law states that absorption is directly proportional to the concentration of the analyte, if the solutions are dilute.

So, that criteria, if it meets, it can be used for quantitative analysis, as well. Usually 3 to 5 standard samples of the chromaphore are prepared, and the absorbance of each standard is measured. The absorbance can be plotted against the concentration and a standard curve can be generated. By measuring the absorbance of the unknown mixture, one can locate the absorbance on the y-axis of the standard curve, draw a perpendicular over till it intersects the standard curve, and then a perpendicular down till it intersects the x-axis.

At the point of intersection of the x-axis, the concentration of the chromaphore can be determined. Of course, only one chromaphore may be absorbed at the wave length used to measure the standards. So, the usual calibration method, which has been described just now to you, must be adapted in order to quantify. That is how we analyze Chromium 6 in a solution. Chromium 6 standards are prepared and usually 5 standards are prepared like: 1 ppm 2 ppm 3 ppm 4 ppm and 5 ppm, and their concentrations are recorded, and then the concentrations are plotted on a graph; if it goes through the x and y, intersect of the x and y, then that shows that the method has linearity. And then, an unknown concentration can be evaluated by the method that was described to you, a while ago.

One can also use a proportional method where the absorbance of the known concentration sample is measured and the absorbance of the unknown can be measured. So, one can If there is only one type of compound, even the absorbance method can be used, and therefore, it is important to understand that, both for quantitative as well as qualitative analysis, UV visible spectroscopy can give a lot of information; although, not the conclusive and full information.

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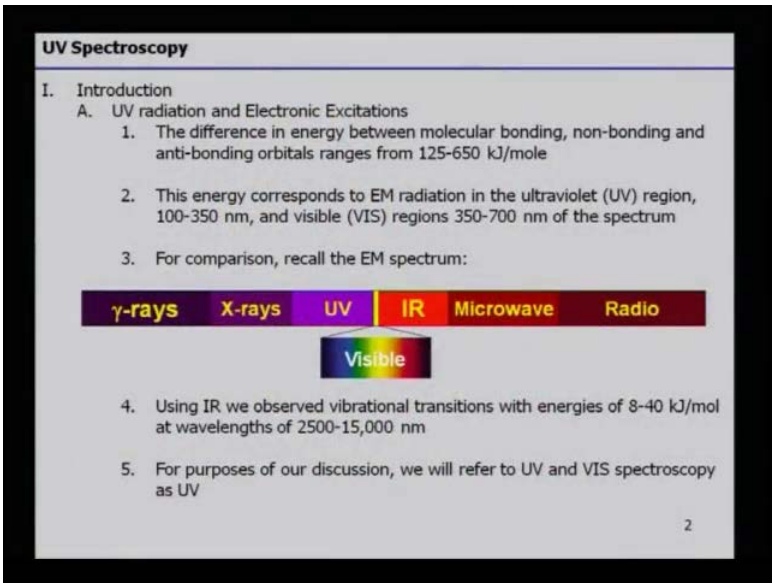


We will now, take a more serious look at UV visible spectroscopy from the analyst point of view. A few slides may be repetitive, but this is just to recap your information. If we look at the introduction of UV visible radiation and the electronic excitation, the difference in energy between molecular bonding, nonbonding and anti-bonding, ranges from 125 to 650 kilojoule per mole. So, that is the kind of energy that is required for pi to pi star, or n to pi star; it is in the region of this. And then only, if the radiation shown on the analyte or chromaphore is having energy range of this, then the molecular orbital excitations take place; otherwise, it will not take place. This energy corresponds to electromagnetic radiation in the ultra violet region that is from 100 to 350 nanometers and in the visible region which is 350 to 700 nanometers of the spectrum.

So, for comparison, I am just giving you a outlook that gamma rays, x-rays, UV, IR, microwave and radio. And in between UV and IR lies the visible region. Using IR, observe the vibrational transition with energies which are very low in their energy content; only 8 to 40 kilojoule per mole at wavelengths between 2500 to 15000 nanometer, or we in IR, we actually express in centimeters per inverse. So, this particular, you know, gap one is 125 to 650 kilojoule permole in UV visible region; that is why the electronic excitations are possible. Whereas, in IR, the energy contents are very low - between 8 and 40 kilojoule; that is why, there is only vibration, rotation, and so on and so forth.

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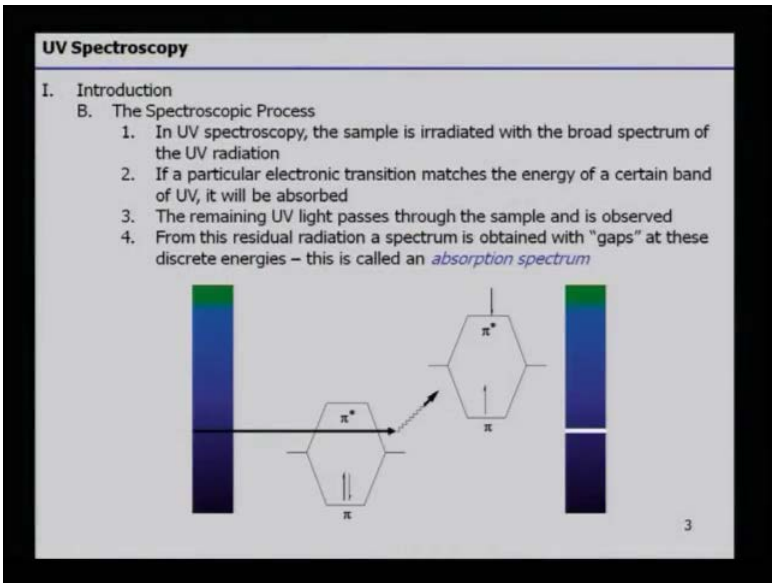


For purposes of our discussion, we will refer only to UV visible as UV. Now, what exactly happens? Now, we have discussed this, but the spectroscopic process, what is the spectroscopic process? In UV spectroscopy, the sample is irradiated with the broad spectrum of UV radiation; if a particular electronic transmission matches the energy of a certain bond of UV, or rather band of UV, it will be absorbed; the remaining UV light passes through the sample and is not observed; from this residual radiation, a spectrum is obtained with gaps at these discrete energies and this is called an absorption spectrum.

So, first thing is that, it is an example of absorption spectroscopy and the spectrum generated is absorption spectrum. And only when the radiation band matches with the energy and electronic transmission in the molecule or analyte or sample, then only it will take place; like the pi to pi transition will not occur with all the radiations that are shown on to the molecule. So, only the precisely matching one will create the excitation. So, this should be understood, very clearly.

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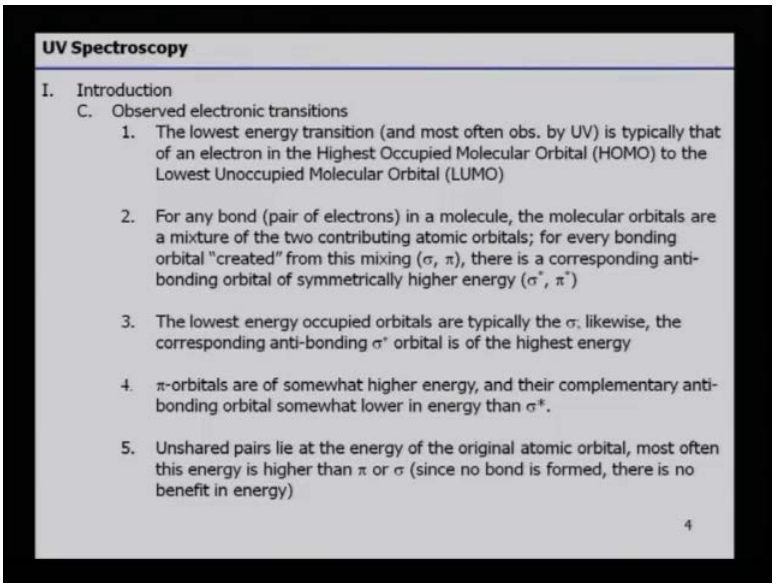


Now, the observed electronic transitions: What are the absorbed electronic transitions in the UV spectroscopy? The lowest energy transition and most often observed by UV is typically that of an electron in the Highest Occupied Molecular Orbital that is the HOMO, to the Lowest Unoccupied Molecular Orbital that is the LUMO. We just talked about anti-bonding orbitals and non anti nonbonding orbitals, but now, we will get into a little more serious discussion about, what is the Highest Occupied Molecular Orbital HOMO and what is the Lowest Unoccupied Molecular Orbital LUMO? It is a transition from the HOMO to the LUMO; that should be made clear, but now, the nomenclature or the way we call these orbitals has slightly changed because a little more advanced information needs to be given to you.

For any bond, pair of electrons, in a molecule, the molecular orbitals are a mixture of two contributing atomic orbitals; for every bonding atomic orbital created from this mixing of the sigma and pi, there is a corresponding anti-bonding orbital of symmetrically high energy which is called sigma star and pi star. The lowest energy occupied orbitals are typically the sigma; likewise, the corresponding anti-bonding orbital is sigma star and it has the highest energy. pi orbitals are somewhat higher energy, and their complimentary anti-bonding orbital are somewhat lower in energy than sigma star.

Unshared pairs lie at the energy of the original atomic orbital; most often this energy is higher than pi and sigma. Since no bond is formed, then there is no benefit in energy. So,

this pattern should be very clearly understood. The gap between sigma to sigma star is the maximum; the gap between pi to pi star is relatively less. And that is why the transition falls in the region of 125to 650 kilojoule permole and that is what causes the transition.

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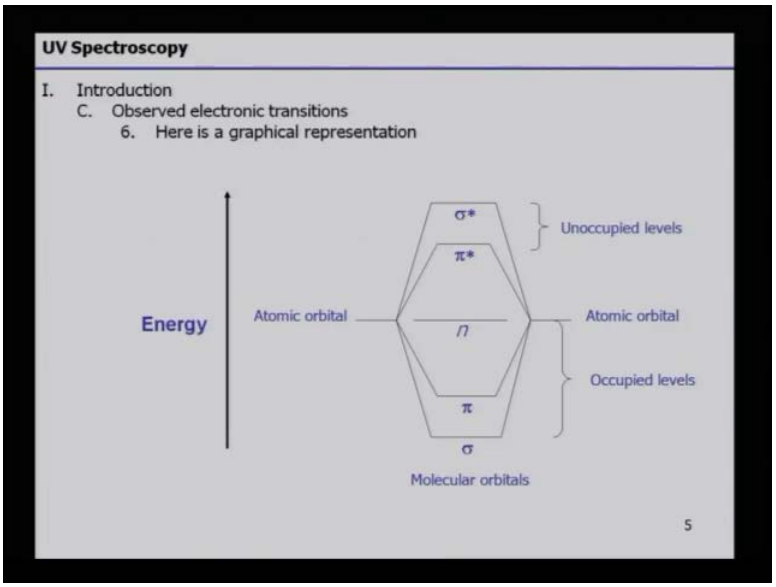


This is how the orbitals can be represented graphically. Now, you see this; the way the energy rises. The maximum differences between sigma to sigma star, then comes difference between pi to pi star, and the third or the minimum difference comes between n to pi star, when we look at the occupied levels and the unoccupied levels of molecular orbital.

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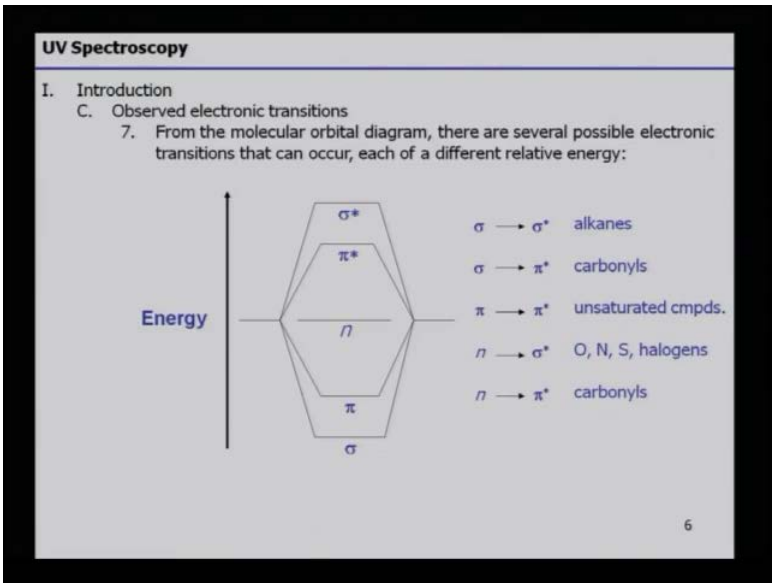


Observing these - what are allowed, what do they represent, let us try to look at the molecular orbital diagram and understand the possible electronic transitions that can occur or that may be allowed, at a different relative energy. If we try to look at sigma to sigma star, it means it is all consisting of single bond which represents alkane.

If we try to look at sigma to pi star, we must understand that, this is a kind of a heteroatom containing bond; that is the Carbonyls. If we try to look at pi to pi star transitions, it means that it has levels of un-saturations. And then, if we try to look at n to sigma star, it could be consisting of: Oxygen, Nitrogen, Sulphur, or Halogens, and if we try to look at n to pi star, again, it represents the possibility of Carbonyls.

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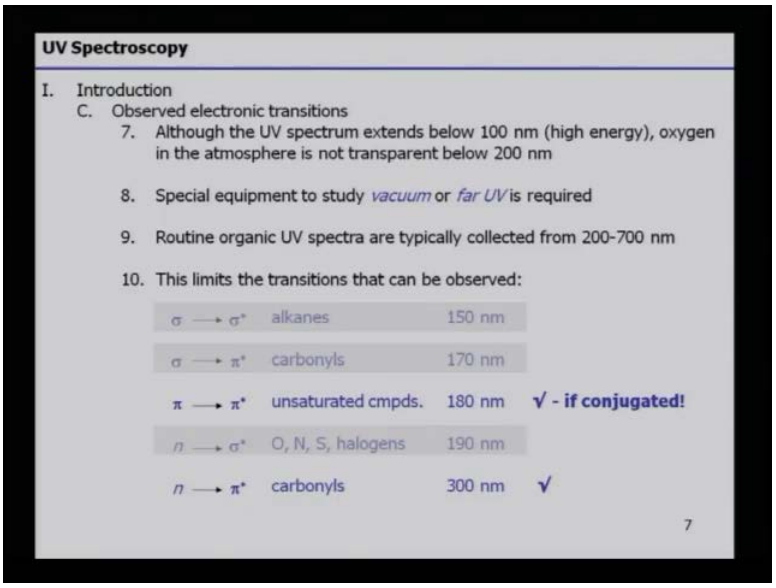


Now, although UV spectrum extends below 100 nanometers that is at very high energy, Oxygen in the atmosphere is not transparent below 200 nanometers. Special equipment to study vacuum or far UV is required. Routine organic UV spectra are typically collected or recorded or analyzed between 200 to 700 nanometers.

So, it should be remembered very clearly, that under very special conditions, the vacuum UV or the far UV is actually practiced. The more popular or the routine UV spectroscopy deals with the region between 190 or sometimes 200 to 700 nanometer. This limits the transition that can be observed that we cannot now consider pi to pi star that is the alkane which is in the region of 150, or sigma to pi star Carbonyls which are in the region of 170 nanometers are out because we are scaling it from 200; so, naturally, it will not analyze which is anything below 200; so, we that is ruled out.

For routine, we just try to look at, what are the possible analytical zones and chromaphores that will be targeted or analyzed by UV visible, and that is pi to pi star because it is in the region of unsaturated compounds that is 180, and I said it can go up to 190, so that it is allowed. And if it is further conjugated, it will come up to 200.

The n to pi star which consists of Oxygen, Nitrogen, Sulphur, Halogens, also can be analyzed to some extent, and n to pi star that is corresponding to Carbonyls which are above 300 nanometers can also definitely be analyzed in UV spectroscopy.

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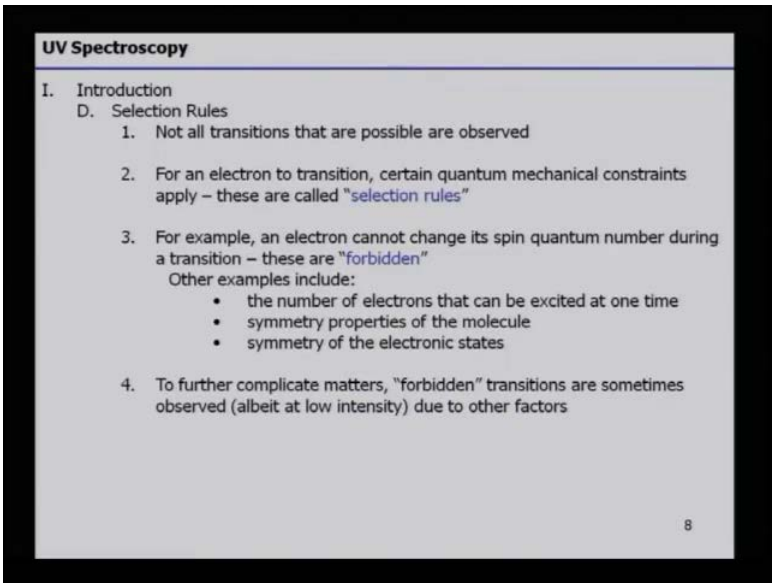


Now, there are certain selection rules. We are getting into a little… First, I gave you an overview, but now, we are trying to get into a little more intricate information and a more detailed information of UV spectroscopy because one should understand it thoroughly.

Not all transitions are possible as we have observed. For an electron to transition you know to take to transit, certain quantum mechanical constraints apply, and these are called selection rules. So, that means that electron cannot jump from any orbital to any orbital; the HOMO LUMO rule must be followed. For example, an electron cannot change its spin quantum number during a transition. These are forbidden. Other examples include: the number of electrons that can be excited at one time; symmetry properties of the molecule; symmetry of the electronic transitions. All these have to be kept in mind, when we are looking at the possible allowed transitions of the electron from the bonding molecular orbital to anti-bonding molecular orbitals. To further complicate matters, forbidden transitions are sometimes observed due to other factors, but sometimes, some of the forbidden factors can also take place, and these are under very special conditions; not normally.

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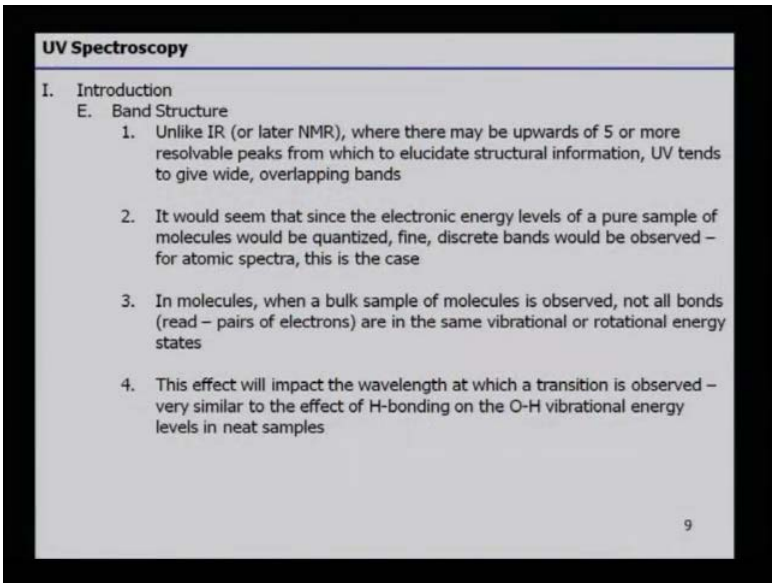


Now, the Band Structure: Unlike IR or later NMR, which we will study, where there maybe upwards of 5 or more resolvable peaks from which to elucidate structural information, UV tends to give wide, overlapping bands. Now, it is possible that there are too many chromaphores in a group; then the band structure can start; you know, there will be many lambda maxes on the spectrum. It would seem that since the electronic energy levels of pure sample to molecules would be quanta, fine, discrete bands would be observed from atomic spectra.

In molecules, when a bulk of sample of molecules are observed, not all bonds, which can be read as a pair of electrons, are the in the same vibration or rotational energy states. This effect will impact the wave length at which a transition is observed - very similar to the effect of Hydrogen bonding and so on, as what we observe in IR, in the O-H vibrational energy enable levels of a neat sample.

So, you see, there are certain very minute points also which needs to be considered. It is not that every jump is allowed - first in the foremost thing is that. Then, if there are too many chromaphores, there is a possibility that these jumps may have peaks which are overlapping with each other, and therefore, it is important to understand, how to do it and when to do it.

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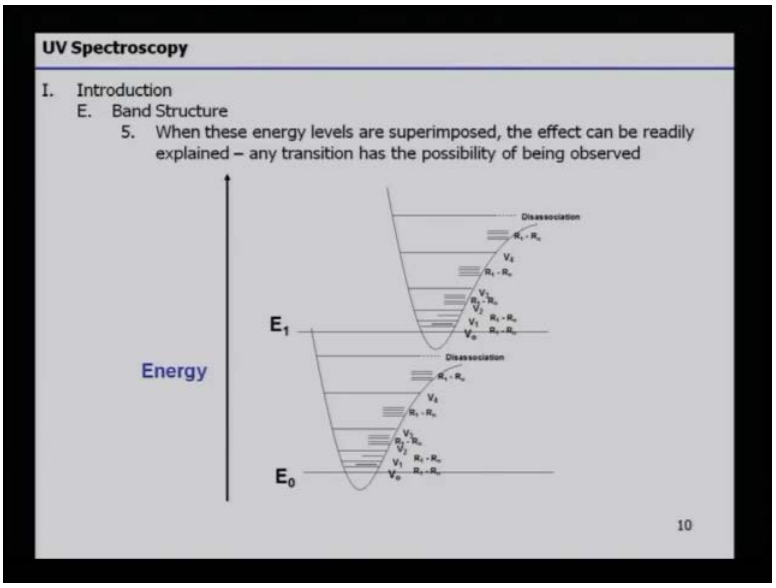


So, band structures could have a lot of this kind of superimposing effect on each other. When these energy levels are superimposed, the effect can be readily explained; any transition has a possibility of being observed; so, you see that, there are several possibilities and any one of them can take place because the energy radiation of the energy of the radiation is actually matching with the transitions.

Now, how does the instrument look like, the instrumentation and then the spectra? How is the outcome or the graphical representation of this data that is collected by the machine called UV visible spectrophotometer?

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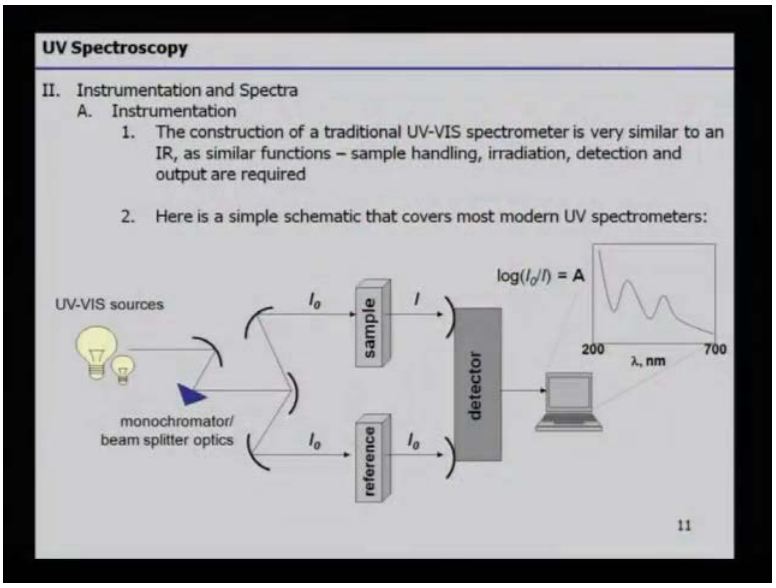


The construction of a traditional UV visible spectrum meter is very similar to an IR and I have almost having similar functions - sample handling, irradiation, detection and output are required. So, there will be a source of light, and we now know that, two different types of lights are required for UV region as well as the visible region, and then these lights are actually polychromatic lights which are cracked down to mono chromatic and then through the monochromatic chromator or being beam splitter optics and then it is passed through reference and sample, and then detected. The change in them will give the value.

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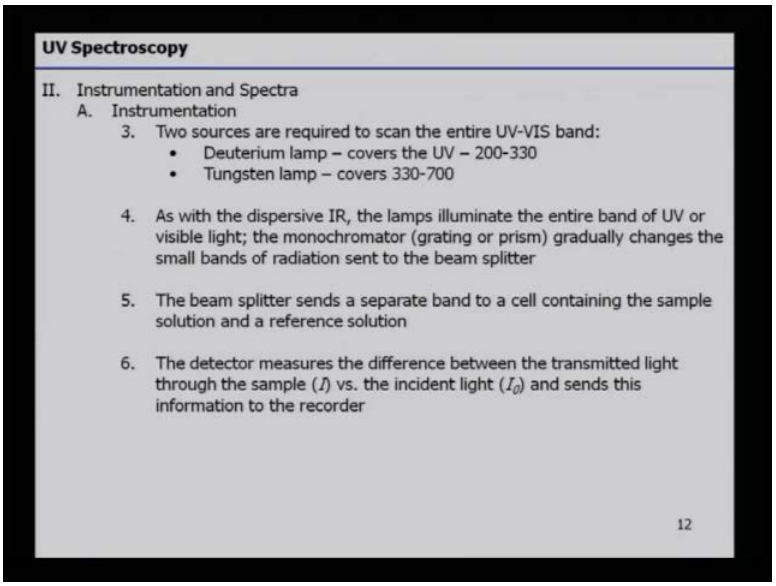

l o remains l o in the reference, but l o becomes l in the sample, and that is what is recorded and you see there are peaks. Now, these peaks actually represent the lambda max for different chromaphores.

Two sources are required to scan the entire UV visible band, as what I told you, Deuterium lamp which is the Hydrogen discharge lamp, which I told you, cover the UV region between 200 to 330 or 350 nanometers, and a Tungsten or Incandescent lamp which covers from 350 to 700 nanometers. As with the dispersive IR, the lamps eliminate the entire band of UV or visible light; the monochromater or the grating prism gradually changes the smaller bands of radiation sent to the beam splitter. So, what it means? That this these polychromatic lights must be changed to the mono chromatic light with these help of beam splitter.

The beam splitter sends a separate band to a cell containing the sample solution and the same light is then passed through the reference solution. The detector measures the difference between the transmitted light through the sample and the incident light that is sent to the recorder to record, the differences recorded by the recorder.

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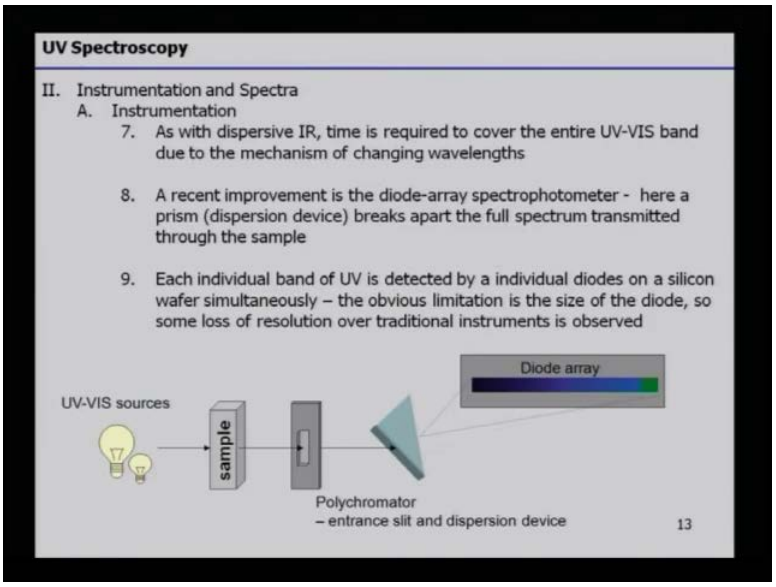


So, if we try to look at the UV machine, it is quite similar to the IR machine. As with dispersive IR, time is required to cover the entire UV visible band due to the mechanism of changing wave length. So, slowly, each wavelength is kind of shown on the sample. A recent improvement in diode-array spectrophotometer; here, the prism, a dispersion

device breaks apart the full spectrum transmitted through the sample. So, the beauty of diode-array detector is that, all the wave lengths are scanned at the same time and therefore the time required for analysis is shortened and entire wave length is covered. Each individual band of UV is detected by an individual diode on a silicon wafer, simultaneously.

The obvious limitation is the size of the diode. So, some loss of resolution over traditional instrument is observed; however, it still does the purpose because you see only under very special conditions, we need to have very accurate results, but this is more used as a qualitative instrumentation for finding out whether a chromaphore is present or absent.

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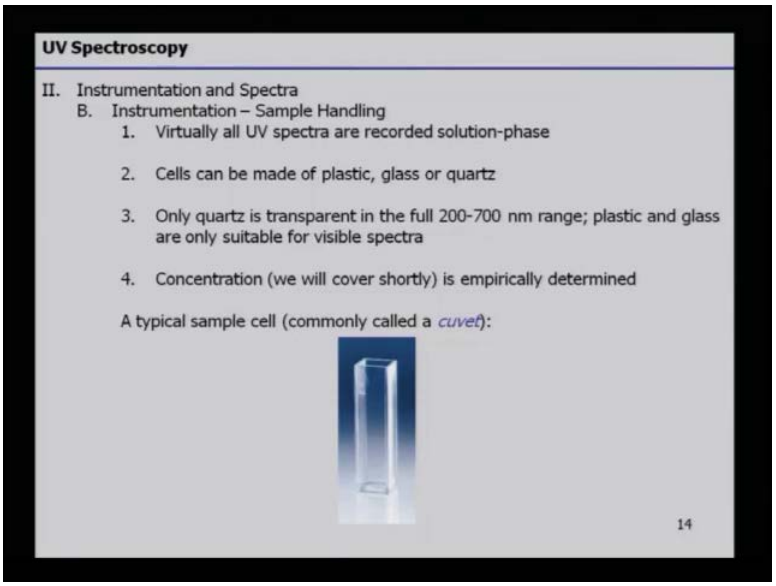


When we try to look at the sample chamber, we just understood that it should have a Quartz cuvet. How does the Quartz cuvet look like? Virtually, all UV spectra are recorded in solution phase; cells can be made out of plastic glass of Quartz, but it should be remembered that these materials should not absorb in the UV region. Only Quartz is transparent in the full 200 to 700 nanometer range; plastic and glasses are suitable only for visible spectra. we Just a while ago, I told that this is how the sample chamber should be or sample compartment needs to be only preferably made out of Quartz.

Concentration, we can cover shortly, and empirical determination by finding out what is the initial concentration and what is the final concentration because the absorption of the

material is only related to the concentration of the analyte. A typical sample cell or cuvet looks like this.

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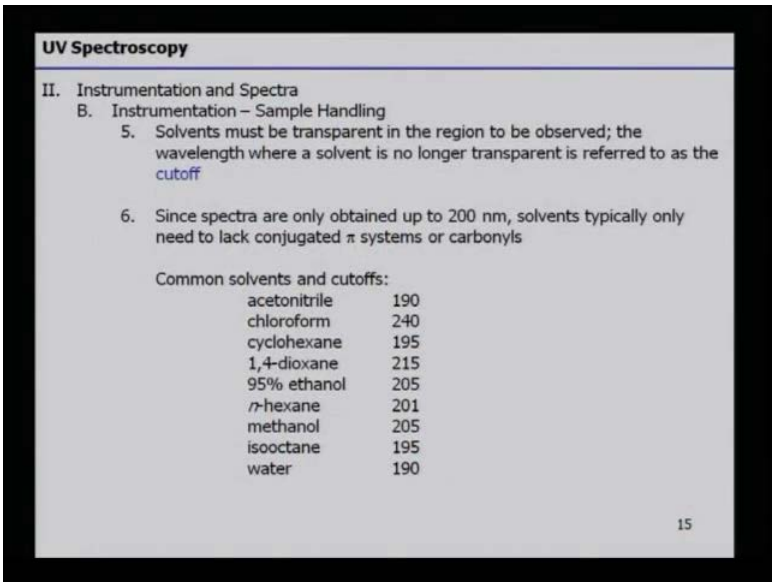


When solvents are being used, solvents need to be chosen in a manner that it should be transparent in the region where the observation needs to be done. So, the wavelength where a solvent is no longer transparent is referred to as the cutoff. Since spectra are only obtained up to 200 nanometers, solvents typically only need to lack conjugated pi systems or Carbonyls. So, such solvents must be used which are far below 200 so that they do not interfere; that is acetonitrile which is 190, chloroform which is just 240, and so on and so forth. So, you will see the region that Cyclohexane, 1, 4-dioxane, ethanol - all these are in the regions between 190 to max to max 240, but chloroform is not one of the solvents of choice; it is normally taken; even water is 190; so, one can use water as the solvent for analyzing UV spectra.

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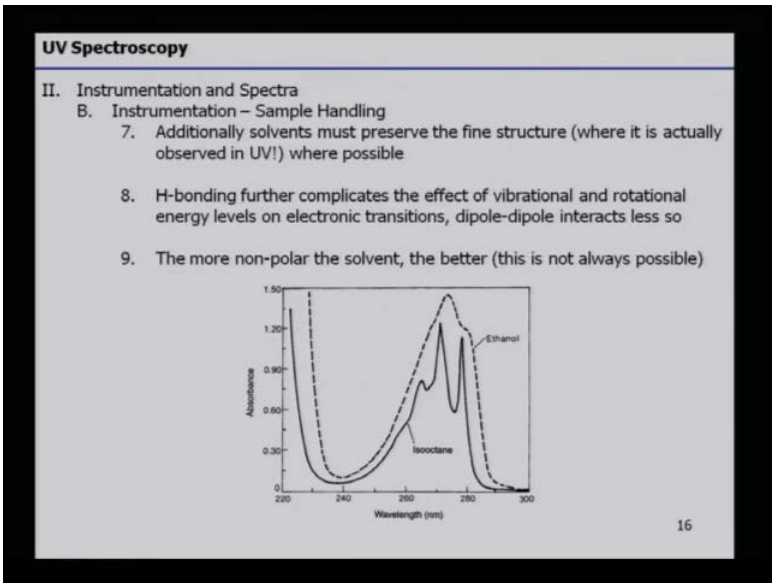


Additionally, solvents must preserve the fine structure where it is actually observed in UV where ever possible. Hydrogen bonding further complicates the effect of vibrational and rotational energy levels on electronic transitions because of the dipole-dipole interaction and so on in the case of IR. However, a more non-polar the solvent, the better it is because it will not create any kind of Hydrogen bonding effect is also witnessed in the case of UV spectrum.

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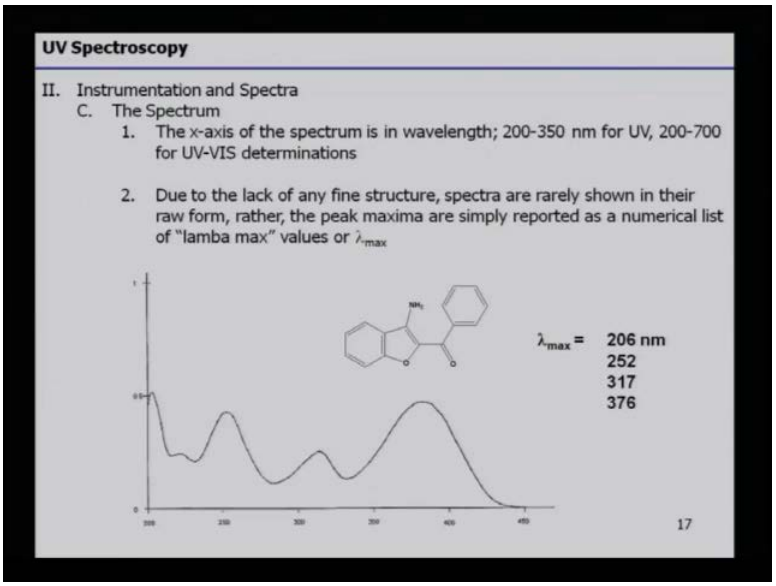

Now, a typical spectrum: The x-axis of the spectrum is the wavelength that is between 200 to 350 nanometers for UV and 200 to 700 nanometers for the entire UV visible determination. Due to the lack of any fine structures, spectra are rarely shown in their raw form, rather than peak; that is the lambda max are simply reported as numerical list and they are written with the subscript of max; a lambda sigh with the subscript of max. And this is the UV spectra of a molecule which is shown above and it shows lambda max at 206 nanometers, 252 nanometers, 317 nanometers, and 376 nanometers.

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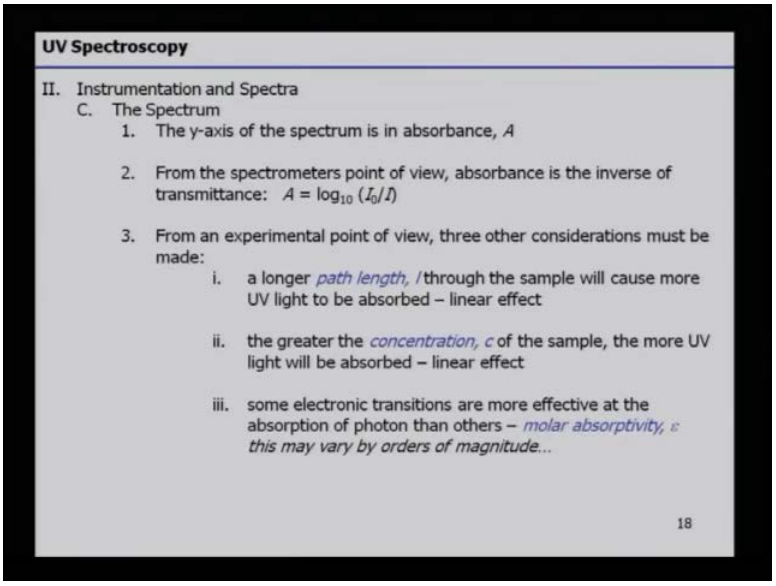


The y-axis of the spectrum shows the absorbance. For the spectrophotometer point of view, absorbance is the inverse of transmittance. So, either we can talk in terms of absorbance or it is a reversal or reciprocal which is called transmittance. For an experimental point of view, three other considerations must be made: a longer path length, through the sample will cause more UV light to be absorbed and will have a linear effect; the greater the concentration of the sample, the more UV light will be absorbed; some electronic transitions are more effective at the absorption of photon than others - molar absorptivity, this may vary by orders of magnitude.

So, there, three things that must be kept in mind: one is the cell path length; cell path length plays a very important role and therefore, because it will affect the linearity of the calibration; so we need to have a standard cell path length and one centimeter cuvet is actually utilized in most of the times. Then the samples should not be highly

concentrated because if they are highly concentrated, then what will happen? The Beer Lambert's law will not hold good and it will start affecting the linearity again.

So, therefore, the molar absorptivity that is the absorption per mole of an analyte or a chromaphore will get affected. So, these things have to be kept in mind when one is utilizing or making use of this instrumentation called UV spectroscopy.

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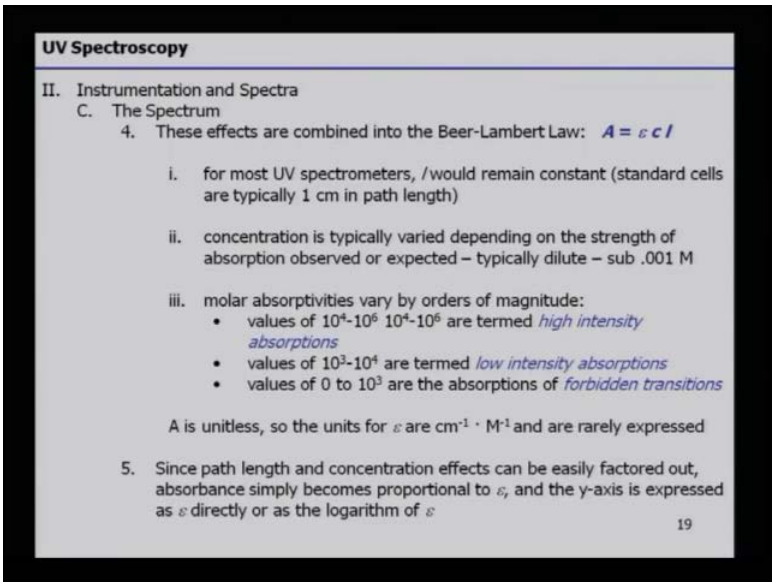


These effects are combined into Beer Lambert's law: A is equal to moral absorptivity into concentration into path length. For most UV spectrometers would remain constant, standard cells have a typical path length of 1 centimeter. Just a while ago, I told you, concentration is typically varied depending on the strength of the absorption and its typical dilution should be 0.001 molar; not concentrated than that.

Molar absoptivity vary by orders of magnitude: Values of 10 to the power of 4 to 10 to the power of 6 are termed high-intensity absorption, values of 10 to the power of 3 to 10 to the power of 4 are termed as low intensity absorption, and any values between 0 to 10 to the power of 3 are absorptions of forbidden transitions. A is unitless because absorption is what has taken place. it does not… it is not represented by any unit, but the molar absorptivity that is the epsilon is actually represented, has a unit called centimeter inverse and molar inverse, and that is rarely actually expressed. We just write molar absorptivity and then the number.

Since path length and concentration effects can be easily factored are out. Absorbance simply becomes proportional to molar absorptivity and the y-axis is expressed as epsilon directly or as the algorithm or logarithm of epsilon.