Analytical Technologies in Biotechnology
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Module - 6
Spectroscopic Techniques
Lecture - 2
UV-Visible Spectroscopy

In previous lecture, we discussed about the basic concepts of Spectroscopy Techniques. If you could recall, spectroscopy is mainly the interaction between the matter and the electromagnetic radiation. And electromagnetic radiation in terms of say frequency or wavelength carries particular energy, it is a form of energy, which is derived from matter only.

And electromagnetic radiation molecules, absorb or emit light, they could absorb the light or electromagnetic radiation or they could emit the electromagnetic radiation, as well as they can scattered also, different types of electromagnetic radiation and particles like say electrons or neutrons. So, these atoms or we can say electrons, they reside in discrete energy labels, that is it will have a ground state, it will have the first excited state in electronic label and to excite from one state to another state, they require very specific amount of energy, that is the difference of the energy between the 2 energy states.

And once they absorb that particular amount of energy, they could be excited to higher energy label. And that particular information, where either it could be absorption or emission could be recorded. Likewise, there are spectroscopic techniques, which have developed, which are based on scattering of the particles or electromagnetic radiations by the matter, the wavelength at which maximum absorption, takes place is called lambda max.

And this particular property that is the wavelength of light is specific for each transition that is transition, as we were talking about movement of the atoms or the electrons as we can say to higher labels, this is called transition actually. And this transition is a specific in terms of energy, which is related to the wavelength of that particular radiation, type of radiation. So, we also introduced the techniques, which we are going to discuss in this section, which included UV visible spectroscopy, IR spectroscopy, circular dichroism florescence spectroscopy, atomic absorption and flame emission spectroscopy. And x-ray

diffraction or x-ray spectroscopy, mostly we will be discussing about x-ray scattering and then nuclear magnetic resonance spectroscopy and mass spectrometry.

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Absorption Spectroscopy

In this lecture, we will be discussing about, one of the techniques, which is comes under absorption spectroscopy and that is UV visible spectroscopy. Now, here in UV visible spectroscopy, this is dependent or this is derived from electronic transition that is the type of transition here and from electronic transition a standard absorption spectroscopy is derived, for analyzing small and large molecules.

So, absorption spectroscopy, if we say it basically, refers to a number of spectroscopic techniques that will analyze the absorption of the energy of the radiation by a sample, the absorption by a sample may be defined as the fraction of the radiation absorbed by the sample, from the pool of incident radiation falling on it. Now, the absorption is basically determined by the atomic and molecular composition of the material. So, the absorption will occur at frequency that has the energy equivalent to the difference between the 2 quantum mechanical energy states.

The energy absorbed causes the electrons to jump from one energy label to the other and absorption brings about, quantum mechanical changes, which could be say, as we said electronic label, it could be vibrational, like in IR spectroscopy, rotational could be there, but that we are not going to discuss. So, this could be different kinds of transition could occur in these molecules or atoms and which will give you a lot of information for

analysis.

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Let us start with the first technique that is UV visible spectroscopy; now this branch of spectroscopy deals with the interaction of matter with radiation in the ultraviolet and visible region. And the transition involved in this form of spectroscopy are primarily, electronic transition, as we have discussed, if you could recall UV visible, a part of little bit of infrared and x-ray comes under electronic transition. It refers to, so here it, refers to absorption spectroscopy or also it could be reflectance spectroscopy, in the ultraviolet visible spectra region.

This means that, it uses light in the visible and adjacent near UV and infrared ranges that is both sides of the visible range actually, the absorbance or reflectance, in the visible range, directly affects the perceived color of the chemical involved. And this technique is complimentary to say to florescence a spectroscopy, where florescence deals with transitions from the excited state to the ground state.

And while absorption means transition from the ground state to the excited state, so both are like complementary to each other and a florescence there is a light emitted, so that could be seen actually mostly, it is emitted in visible region. Now, in this particular like as we were talking about electronic transition, molecules containing say sigma or pi or non bonding electrons, can absorb the energy in the form of ultraviolet or visible light to excite, these electrons to higher anti bonding molecular orbits. And like I said the energy

should be equivalent to the difference in the in the energy of 2 states types of electrons and their possible transition could be as shown in here.

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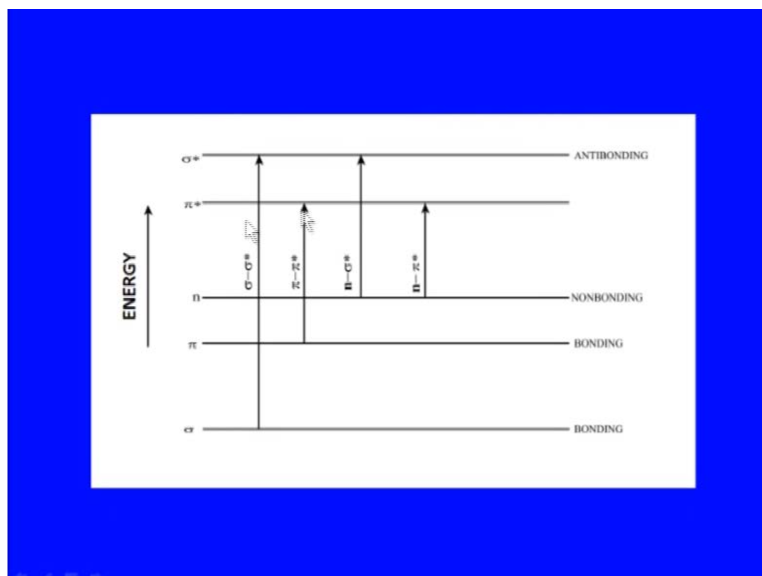

If you see in this figure, then you can see, there could be different types of transitions, so one is sigma bonding, which is main bonding actually, then sigma bonding the transition is only from sigma to antibonding orbital that is here. There could be other transitions, like from pi electrons or pi bonding to the antibonding or pi antibonding orbital.

Then there could be other transitions, that is from nonbonding electrons, that is n to sigma or n to pi antibonding. So, these are few transitions, those are sigma to sigma antibonding then pi bonding to pi antibonding then n that is nonbonding to sigma antibonding and from nonbonding to pi antibonding. These could be different types of transitions, which could occur while, absorption of the energy in the form of ultraviolet or visible region.

Now, to take a few examples here, from sigma to sigma bonding to sigma antibonding transitions, this is for compounds with sigma bonds only and it requires high, it is it has a high delta energy or difference in the energy of the 2 states here. So, it requires a wavelength, which is smaller wavelength or high frequency, so this will be around less than 200 nanometer wavelengths are usefully does, but it is not, it occurs only in saturated hydrocarbons with sigma orbitals like cyclopropene to take a few examples here.

So, this is like kind of not, so common actually, the second transition, which we are talking about, was from nonbonding orbit to the pi antibonding transition. Now, the excitation of electrons, from nonbonding orbital such as unshared paired of electrons, on say nitrogen, sulfur, oxygen or any other atom to an antibonding pi orbital, it usually occurs in double bonds, at where heteroatom's like for example, C O or C double bond O C double bond S or N double bond O are there.

Now, this is mostly, asymmetric and forbidden and it is a low intensity transition, it occurs only saturated aldehydes and ketones and lambda max is around 185 to 300 nanometer. Then there could be from bonding pi to antibonding pi transitions, which occur in compounds containing double, triple or aromatic rings. A pi electron is excited to an antibonding pi orbital and this is a like symmetrical and allowed and it is a high intensity transition, it is if it there is a extended conjugation will it will lower the difference in a energy and will increase the lambda max.

Then, there is transition of nonbonding electrons to sigma, which is sigma antibonding transition, excitation for nonbonding to antibonding orbital is also like, it could be occurring in only, few like say C H 3 O H or others. Now, so these are a few transitions, which could occur between different kinds of in atoms or molecules, if we consider macromolecules like say, proteins or DNA, which are vital quite studied in biotechnology.

In protein dominant chromophore is the amide group, which has n that is nonbonding to antibonding sigma transition at about 220 nanometer and has an intense pi to pi star or you can say bonding pi to antibonding pi transition at about 195 nanometer. Electronic transition are most site change, occur below 200 nanometer and it is overlapped by the previous, pi bonding pi to and antibonding pi transition of amide group.

Except for certain amino acids like, phenylalanine tyrosine or tryptophan and also cystine methionine and disulphide groups, which will have their electronic transitions below 300 nanometer. And as we know that phenylalanine tyrosine and tryptophan has pi electrons systems and they have this bonding pi to antibonding pi transitions, in nucleic acids basis are the chromophores, which have extensive pi electron systems and which give raise to pi bonding to pi antibonding or pi pi star transitions.

In proteins like mostly absorption occurs, protein absorbs at 280 nanometer and as we have seen 2 to around 220 nanometer, because of amides and from 280 nanometer, because of these aromatic residues actually or aromatic side chains. Likewise, the lambda max for nucleic acids is around 260 nanometer and you to the basses here. So, these word, this was about little bit about different times of transitions, which occur, in molecules or atoms and particularly as we have seen proteins and DNA.

Now, UV visible is spectroscopy, as we see it is primarily governed by, the beers lamberts law as we see here, molecules absorb light and as the molecules absorb light and interact with electromagnetic radiation. In this form, this absorption can give information about, a structure or chemical composition or concentration of the of the absorber and is a very powerful technique, for analysis, for a small and large molecules.

So, here beers, lamberts law, which is a combination or 2 laws, as we know, one is beers law and another is lamberts law, one deals with concentration of the absorber, another with the path length. So, both of these laws are dealing separately with the absorption of light, one related to concentration of the absorber that is the substance responsible for absorbing the light, that is called absorber and the path length or thickness of the layer that is what is the path length of the absorber, it is present.

So, it is states that the absorption of a solution is directly proportional to the concentration of the absorbing species and the path length. Thus for a fixed path length, most of the time cuvettes are of a particular size, so they have a fix path length say of one centimeter or so for a fixed path length UV visible spectroscopy can be used to determine the concentration of the absorber in a solution.

So, it states that, the concentration of a substance in solution is directly proportional to the absorbance of the solution, remember like the instruments, which measures this like a spectrophotometer. There will be a limit to this linear or direct proportional region actually, but higher very high concentration this linearity will be lost. So, this has to be remembered that, at a particular concentrations, these measurements has to be taken.

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Expressed mathematically as,

$$A = \log_{10}(I_0/I) = \varepsilon.c.l$$

$$A = \varepsilon_\lambda cl$$

Where,
A = Absorbance,
I_0= Intensity of incident light at a given wavelength
I = Intensity of the transmitted light
ε_λ = molar absorption coefficient
And l = length of the sample,
For each species the ε refers to the molar absorptivity or the molar extinction coefficient and is the characteristic of the substance in a particular solvent.

So, if you express mathematically, the beers law it will be like absorbance, like I said it is proportional and it is expressed in terms of here that is molar absorption coefficient or extinction molar extinction coefficient, as we can call it. As concentration and l is the path length here, for each species or molecule, the molar absorption coefficient or molar extinction coefficient, refers to the particular, it is a characteristics of that particular substance, in a particular solvent. And also it at a particular wavelength actually, like it is clear written it is for particular wavelength.

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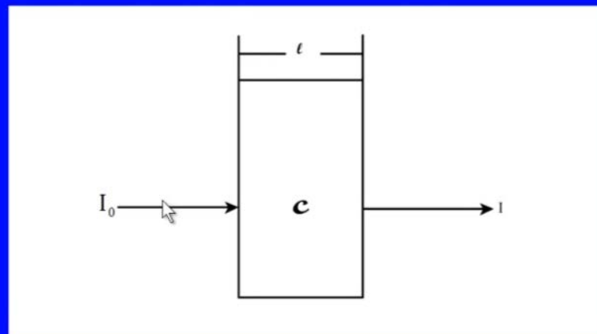
Absorbance A = constant (molar absorbance coefficient) x concentration x cell length
$= \varepsilon_\lambda c\, l$



So, if you see here, this is how this is a cuvette, where you have particular absorber, in a solution, it has a the absorber has particular of a set concentration and there is a path length here. As the intense light, that is initial light enters, it is absorb to different extent at varying extent depending upon the concentration and type of molecule in here or absorber here. And there will be, so this is incidental light and this is the transmitted light, which comes out after certain absorption, which occurs in here.

So, when sample molecules are expose to light, having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorb and as the electron is promoted to higher energy orbital. And this will be recorded by a spectrophotometer and it will record the wavelength, at which this absorption occurs, together with the degree of absorption that is height of the peak you can say, at each wavelength molecule, that show increase in degree of conjugation.

Acquires less energy for excitation and as a result absorb radiation of higher wavelength, so depending on what kind of molecule is in there, it could be at higher wavelength or lower wavelength, but it is typical of a characteristics of a particular kind of molecule. Now, as we were discussing, it is a at particular wavelength, where absorption is maximum is called lambda max. And if we consider in simple terms there should be spectral lines, which should be obtained at that particular transition, where it is occurring.

But, most of the time it is quite complex and there are a set of spectral lines as I have shown you in energy diagram, there are vibration labels and others. So, to understand simply, it is like set of spectral lines, which are there. And so you do not really get to see this spectral lines, when the output is shown rather, what you see is a peak actually, small peak depending on the concentration, it will have a particular area and that peak is shown in form of absorbance on the read out.

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Considerations of Beer-Lambert's law

There are lot of things to be considered for beer's Lambert's law, the band width utilized for the analysis should be sufficiently low or it might alter the absorption features of the sample. The absorption should be taken at the wavelength, where the absorption should be the highest at the variation might lead to alteration, in the measurements, the light that is used should be of a monochromatic wavelength.

Then concentration of sample should not be too high, as it leads to saturation of the absorption signal and might result in false might get false results here. So, remember the molar absorption coefficient will alter or the beer's law will not will be violated, when you have a very large concentration or different aspects needs to be met, so that beer's law is not violated. Like here, absorption should be taken at particular wavelength, many times a molecule can have multiple lambda maxes for different properties chemical properties of the absorber. So, those things also needs to be taken into account actually, so when we are considering this things, in terms of lambda or lambda max or in terms of like, I said the spectral lines are obtained, but like let me show you that, on a screen here.

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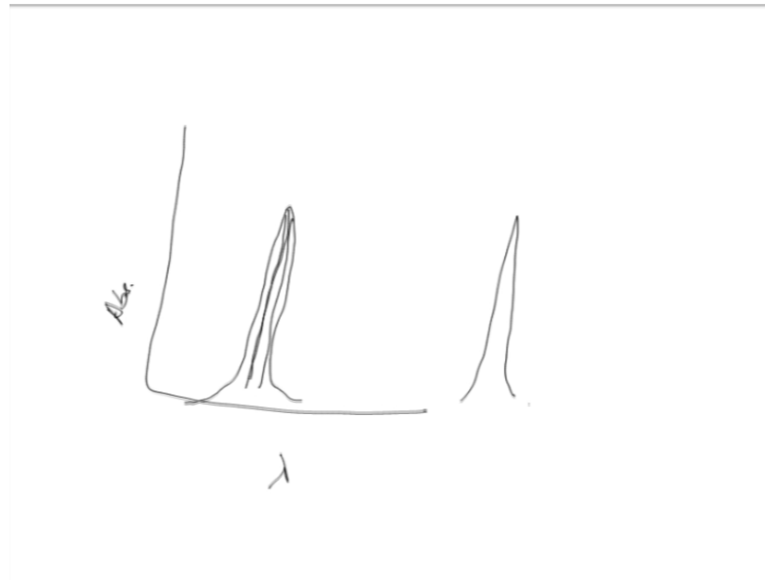

So, particular transition should be obtained in terms of, if I say in terms of spectral lines actually, but like I said, there are much more complex, because there are lot of vibrational labels and how it goes from like, it is not it is like, it will absorb at and transition takes place, which is much complex. So, you do not get spectral lines as such,, but you get series of spectral lines, which are closely spaced in here.

So, what you get in real terms you get a peak actually, which does not show or to be very clear it get a peak here which is which does not show these spectral lines,, but there are closely related or closely spaced spectral lines in here. The absorbance, if I say or optical density, as we say as the read out comes out it is or a spectrophotometer is essence reading the absorbance at a particular wavelengths. So, if you plot absorbance versus wavelength, then you will get these peaks at a particular wavelength and that is lambda max in here.

So, that has to be understood, that as we were talking about beer's law, one is concentration it too, if it is too high then also it may be violated then at proper wavelength it has to be done. So, that beer's law hold and lot of other precautions has to be taken. So, that this particular analysis could be done without any problems or without any spurious results actually.

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Instrumentation

Let’s discuss little bit about the instrumentation, as we have discussed about principal of the UV spectroscopy that is based on beer’s law or beer’s Lambert’s law and how this is an electronic transition here, let us discuss about the instruments, which are utilized for measuring this absorption. Now, if a particular instrument is only based on like wavelengths in visible region that is colored region, then colorimeters could be used, but more widely used and sophisticated instrument is spectrophotometer.

So, the UV visible spectrophotometers are widely used and they measure the intensity of light passing though a sample and they will compare it to the intensity of light before, it passes through the sample. So, as I have shown you earlier, this is like as light enters at a incident light and when, it passes through, it is a transmitted light. So, the ratio here is called the transmittance and the transmittance will be expressed as a percentage that is, if there is no absorption, it is the 100 percent transmittance and if there is complete absorption, there is a 0 transmittance, that is an object is totally opaque.

There could be UV visible spectrophotometer and could be configured to measure reflectance also, in this case the spectrophotometer measures the intensity of light reflected from a sample. And it compares to the intensity of light, reflected from a say reference material and the ratio will be called the reflectance and usually expressed as a percentage reflectance. So, this is how either transmittance or reflectance could be measured, now the basic parts of a spectrophotometer are essentially a light source.

Then a holder for the sample that is cuvette or where, you can put in your sample, then a diffraction rating, in a monochromator or a prism to separate the different wavelengths of light and finally, a detector. So, let us discuss each of these, a light source could be or the radiation source is often a tungsten filament, which is from 300 to 2500 nanometer mostly towards visible reason actually and it is cutoff is around 350, you can say then for UV visible.

So, visible if you take tungsten filament then for UV a deuterium or arc lamp is it required, which is a continuous over the ultraviolet reason from 190 to 400 nanometer. Both lamps could be are switched on, when it is working or either could be switched on, then xenon arc lamp, which has a continuous range from 160 to 2000 nanometer could be utilized.

In place of above 2 lamps, that is the tungsten and deuterium or more recently, there are light emitting diodes for the visible wavelengths, which are also utilized. Since the emitted light consists of many different wavelengths here, a monochromator consisting of either a prism or a rotating metal grid of high precession called grating is placed between the light source and the sample. So, that is also required to separate different wavelengths of light, the detector is typically, a photomultiplier tube or it could be photodiode photodiode array or chart coupled device.

A single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light, so that only light of a single wavelength reaches, the detector at one time. The scanning monochromator moves the diffraction gradient to step through each wavelength, so that it is intensity may be measured, as a function of wavelength fixed monochromators are used with C C D or charged coupled devices and photodiode arrays, as both of these devices consists of many detectors grouped into 1 or 2 dimensional arrays.

They are able to collect light of different wavelengths on different pixels or groups of pixels, simultaneously, there could be various arrangements here, in these sophisticated instruments. Now, spectrophotometer can be, there could be specialized spectrophotometers, which have various options, like one is spectrophotometers can be either single beam or a double beam spectrophotometer. So, the single beam spectrophotometer focuses, all light on the sample and you have to do reference

separately.

In a double beam spectrophotometer light is split into 2, before it reaches the sample and one of the light paths is directed to the reference, where the other goes to the sample. So, there is like it is divided into 2, 1 goes to the sample, other goes to the reference cell to be analyzed and the intensity ratio between the 2 is measured. So, mostly what is done when you have a single beam spectrophotometer, baseline correction is done with the reference, that is 0 absorbance is taken at the reference.

And then all you can say it is 0 and then the sample is absorbance is taken in double beam the reference automatically like sets the baseline. So, in double beam spectrophotometer, therefore the light is split into 2 beams, before it reaches the sample and like one is going to reference, other is going to the sample. The reference beam intensity is taken as 100 percent transmittance or we say 0 absorbance, that is it is zeroed actually. So, that the peak starts from the very baseline in terms of if it is passing through the sample.

So, the measurement is displayed at a ratio of the 2 beam intensities, some double beam instruments have 2 detector photodiodes and the sample and reference beams are measured at the same time. In terms of sample holder, samples are typically, placed in a transparent cell and these transparent cells are known as cuvettes, to cuvettes are typically rectangular in shape. And they come in different sizes and different path lengths, most of the time very commonly used one has a internal width of 1 centimeter.

The most widely applicable cuvettes are made of high quality silica or quartz glass particularly, for UV reason and because these are very transparent throughout the UV and visible region. But, for visible region, you can use glass and plastic cuvettes could also be utilized are very common although, glass and most plastic will absorbed in the UV regions, so they are not utilized there. So, for UV region you require, say quartz or silica cuvettes whereas, for visible region either could be utilized, that is plastic or quartz cuvette.

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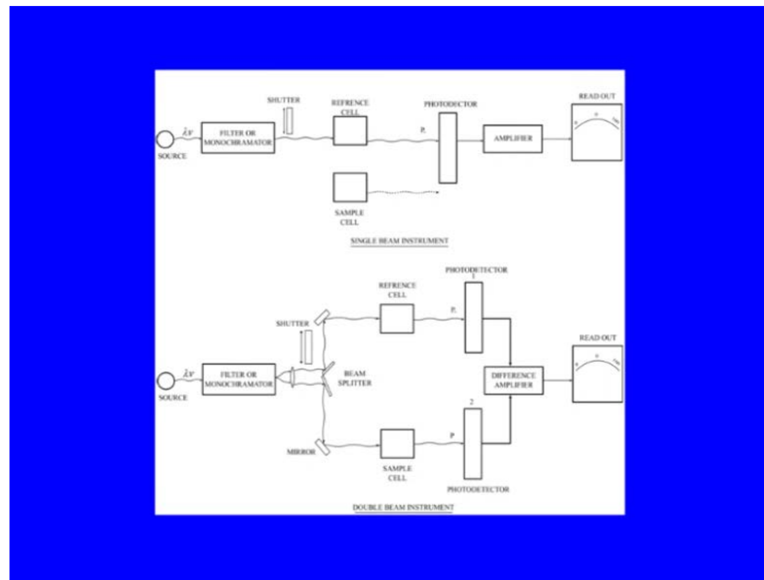

This is a schematic of spectrophotometer, these are 2 here, if you can go through, these are light source, as we have discussed this could be different light source. There is a filter or monochromator, filter is mostly utilized, for again filtering the wavelengths in particularly, in the it is utilizing calorimeter for a different color filters are available. But, in spectrophotometers, mostly monochromators are used, then there is a reference cell there is sample cell and like, I said there could be 2 kinds, 1 is single beam or double beam spectrophotometers.

There is a photodetector amplifier and then finally, read out in the form of peaks will be shown that is absorbance, more is the absorbance bigger will be the peal at a particular wavelength. Then here it is a double beam spectrophotometer, you can see, it is a same, but light is here, divided an into 2, through a beam splitter and 1 goes through 1 beam goes to the reference cell, another goes to the sample cell. And then finally, difference amplifier is there and read out is taken like, it is for reference cell, it is 100 percent transmittance and 0 absorbance is taken.

So, this is a typical single beam and double beam spectrophotometers, they could be different like alterations in modern spectrophotometers or various applications, there could be attachments for say very small cuvettes like, replicable cuvette holders are there. They could be provision for taking absorbance at very low temperature, so that thermal motions are avoided, there could be provisions for stop flow kinetics, as it

should be lot of different types of analysis could be done in these spectrophotometers. So, there are lot of advancement, which has taken in here.

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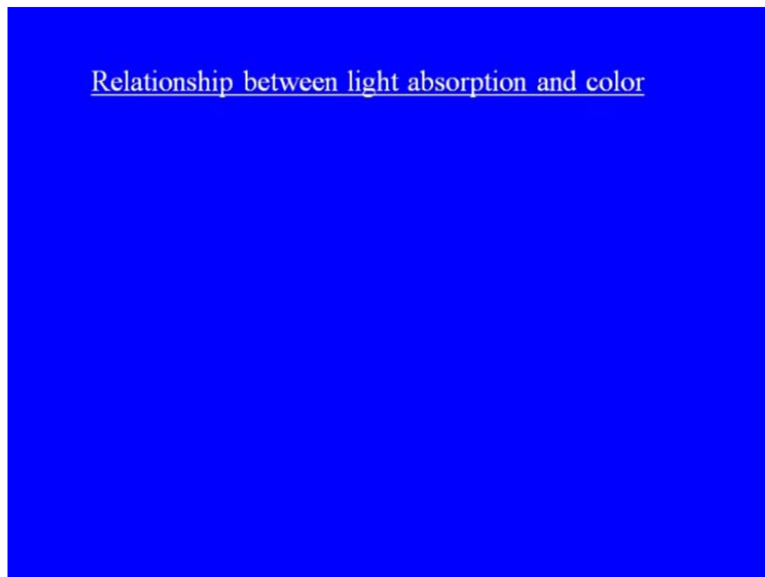

Now, if you consider, as far as visible region relationship between light absorption and color, so white light, when falls upon a sample the light may be totally reflected, in which case the substance appears white. If the light is totally absorbed, I think it will be as we all know, in which case the substance will appear black. If however, only the portion of the light is absorbed and a balance is reflected the color of the sample is determine by the reflected light or reflected wavelength, that say violet is absorbed the sample appears yellow green. And if yellow is absorbed, sample appears blue likewise, so colors described as complimentary here, however, many substances, which appears colorless to have absorption spectra. And in this instance absorption will take place in the infrared or ultraviolet region, as we have discussed.

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COLOR ABSORBED	COLOR OBSERVED	ABSORBED RADIATION (nm)
VIOLET	YELLOW- GREEN	400 - 435
BLUE	YELLOW	435 - 480
GREEN- BLUE	ORANGE	480 - 490
BLUE- GREEN	RED	490 - 500
GREEN	PURPLE	500 - 560
BLUE- GREEN	RED	490 - 500
GREEN	PURPLE	500 - 560
YELLOW- GREEN	VIOLET	560 - 580
YELLOW	BLUE	580 - 592
ORANGE	GREEN- BLUE	595 - 605
RED	BLUE- GREEN	605 - 750

Now, this is a table here, which we can see that, they are different color absorb, the color if there is particular color absorbed, you see a particular color observed here and which are absorbance wavelength is given in here, for various different colors of different wavelengths in visible region actually. So, this only depicts about, if the particular say for violet is absorbed, then yellow green will be seen.

And this is the range, which is absorbed actually likewise, the whole range different situations are given in here. So, this was about the spectrophotometer, which is now modern instrument with various modifications and advancement. And for so that lot of different arrangements or different accessories could be put in and lot of applications could be done on this spectrophotometers.

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Applications

There are lot of applications of UV visible spectroscopy and will be discussing a few here, like we said could be utilized for analysis of small and big molecules and for various applications. So, UV visible spectroscopy is routinely used in analytical chemistry, for the quantitative determination of different analytes, of examples say, in say transition metal ions, highly conjugated organic compounds, biological macromolecules could be routinely checked info.

Most commonly this type of spectroscopy is used for quantification of biological samples, either directly or by choloromatric acids, there are whole lot of different kinds of choloromatric acids, which are available for proteins and DNA. In many cases proteins can be quantified directly using their intrinsic chromophores, like tyrosine and tryptophan, as we have discussed tyrosine and tryptophan absorbs and protein, they have for absorbs at 280 nanometer and DNA or nucleic acids absorbs at 260 nanometers.

Spectroscopic analysis is commonly carried out in solutions, but solids and gases may also be studied, solutions of transition metal ions can be colored, they absorb visible light, because of deelectrons within the metal atoms and can be excited from one electronic state to another. And the color of the metal ion solution is strongly affected by the presence of other species say sort an annoyance or ligands, for example, the color of dilute solution of copper sulphate is very light blue.

But, if you add ammonium ammonia, it will intensify the color and change the wavelength maxima or lambda max, which is maximum absorption. Organic compounds especially, those with a high degree of conjugation for example, DNA R N A or protein will absorb light in the UV or visible region of the electromagnetic spectrum. The solvents for these determinations are of an water, for water soluble compounds or could be certain organic solvents.

Organic solvents may have significant UV absorption and so not all, solvents are suitable for UV spectroscopy, like for example, ethanol absorbs very weakly, so that could be 1. Solvent polarity and PH also affect the absorption spectrum of an of a particular molecule, for example, tyrosine for example, increase in absorption maxima and molar extinction coefficient, when PH increases from 6 to 13 or when solvent polarity decreases, let me show you that on your screen. So, these things like, I said PH polarity or orientation play a major role in here for example.

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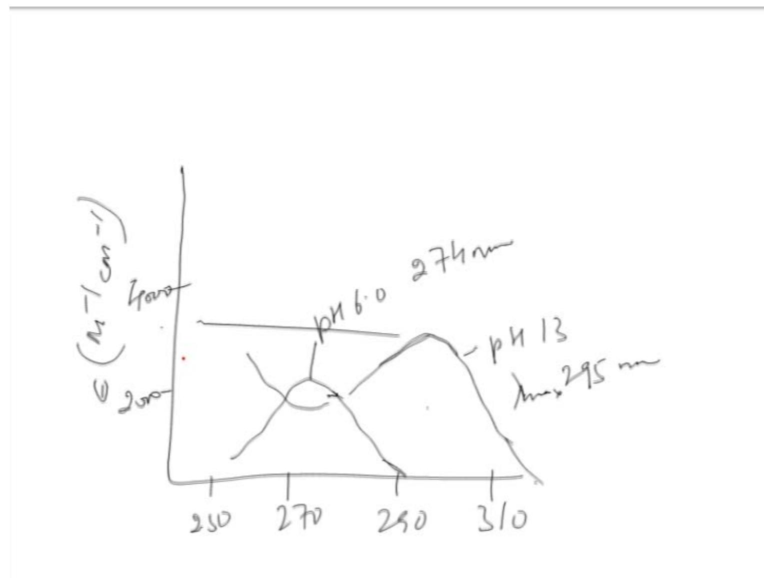

If I say PH in terms of PH actually, if we are having say molar absorption coefficient in here, epsilon, which is in terms of particular units and if say just to show you here and if there is particular wavelengths here. Now, if I consider that, the 2 PH, which is PH 6 and PH 6 and PH 13, as I was talking about, then there is a particular difference actually here, you can see that, this is a tyrosine spectra, absorption spectra of tyrosine. This is as PH 6 and this is a PH 13 here, the absorption maxima is around 274 nanometer and here, the

lambda max is around 295 nanometer, somewhere around that.

So, what you see is and also, there is a increase in the absorption coefficient, so when it is done a 2 different PH affects the lambda max, as well as absorption molar absorption coefficient or extinction coefficient likewise it also affects. What you call a polarity or what you call orientations will also affect the ... ((Refer Time: 40:34)).

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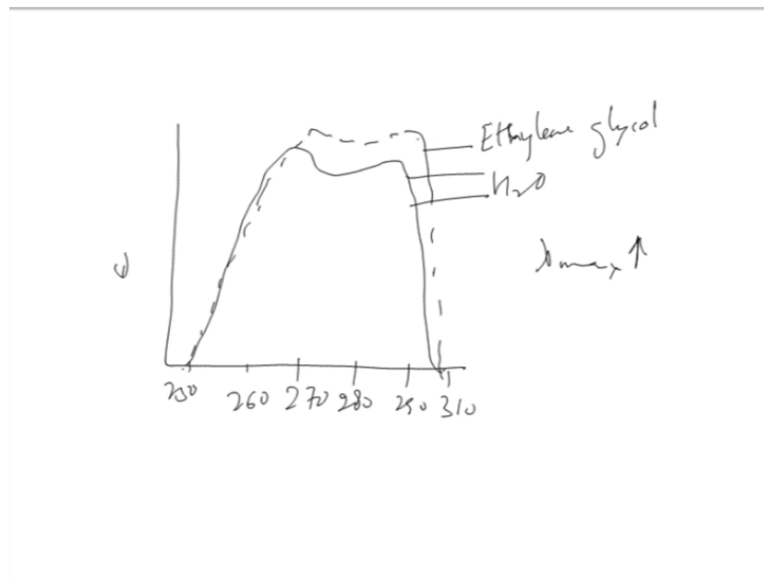

For example, if say one needs to do solvent polarity here, if solvent polarity has to be looked into, let see halt affects the absorption, so in one case, it will be supposing, this is the these are not very exact figures, but you can at least relay on them, for understanding. So, these are 2 spectra in here, one is at ethylene glycol, which is non polar and another is in water actually. So, you have 2 of these spectra here, you can see, now one spectra is polar and another is in non polar solvent, you can see there is a lambda max increase in here the lambda max is increased in case of the ethylene, in case of less polar solvent. So, polarity will affect certainly the lambda max and also molar absorption coefficient and third part, we were talking about is the orientation effects.

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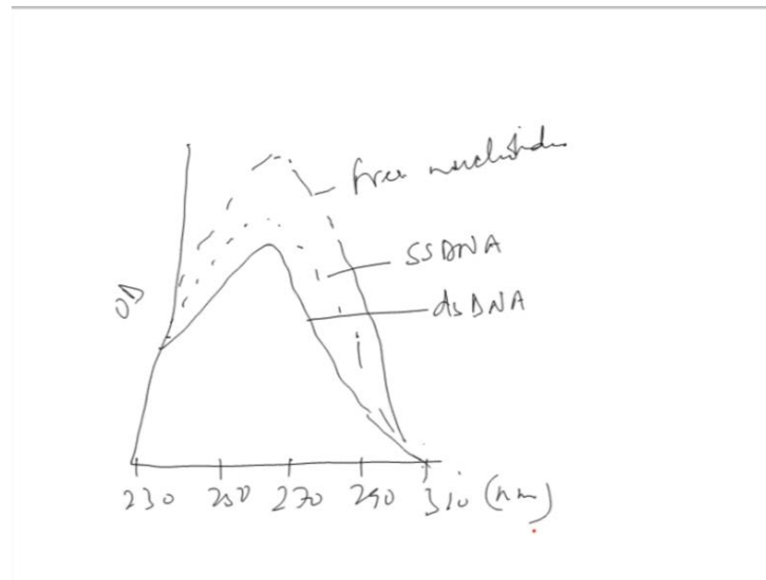

So, orientation effects, that is how a molecule is present in situation, if we take optical density in here and say and this example, we are taking is of DNA and I think all of you know that, DNA is present in different forms. So, they are 3 curve, I have made, now you take this one, this is double stranded DNA that is arranged quite well and this is singular stranded DNA and this is free nucleotides.

So, you can see here, that as these are the arrangement changes, like in double stranded DNA, they are well arranged and you will have a particular spectra and as this arrangement is loosened, like an single stranded DNA or free nucleotides. There is increase in absorments. So, these are few examples, where you can see that effect of PH polarity or orientation on different spectra.

So, as we have seen solvent polarity PH and orientation of a particular molecule in different forms can affect absorption spectrum of these compounds. UV visible spectrophotometers, as a they could be utilized for recording the absorption patterns of different molecules. They could have be attached to other instruments, like H P L C, where of the presence of the analyte could be found out or a response, assume to be proportional to the concentration could be recorded, for accurate results the instruments response to the analyte.

In the unknown should be compare with the response to a standard and this is very similar to the use of calibration curves actually. So, this spectrophotometer could be

arranged with a purification instrument, like H P L C or F P L C, for analysis of different types of purified analyze. The wavelength of absorption peaks can be correlated with the type of bonds in a given molecule and are valuable and determining the functional group within a molecule.

The nature of the solvent, the PH of the solution temperature high electro light concentrations and presence of interfering substances can influence the absorption spectrum. So, the experimental variations, such as the now select with that is a effective band width of the spectrophotometer will also, alter the spectrum to apply UV is spectroscopy to analysis these variables must be controlled or accounted for in order to identify the substances present. So, lot of procedural things has to be perform to get accurate results actually.

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So, finally, UV V is spectroscopy utilized for lot of different types of analysis, there are lot of different applications, to summarize, it could be utilized to determine concentration of an analyte by seen the absorption patterns and comparing it to the standards nonstandards and through calibration curve. The concentration of unknown sample could be calculated, it could be utilized for exchange a chemical reaction, for to observe the chemical reaction or S A enzymatic S A or other S A, which are going on in a chemical reaction.

As one substrate changes to a particular product, which could be colored or absorbs at a particular wavelength, that could be that processes could be monitor. They could be structural studies of DNA could be perform, like for example, denaturation and renaturation studies could be perform, where melting curves could be drawn as a function of temperature. And depending on that one could drive idea of about G C content of DNA and depending upon G C content, there will be a particular melting temperature that could be derive from these studies.

So, lot of structural studies could also be performed like, as we have shown, depending on the orientation or depending on the particular form of the of the macromolecule, they will have different absorption. This UV Vis spectroscopy can also be used for studies of proteins and structural studies is certain structural, now her when we say structure does not need 3 dimensional structure.

But, certain signature certain strong indicators are there to understand, like for example, at different PH titrations or by difference spectroscopy, they could be certain indications about, structural changes could be observed for both DNA and proteins. So, they could be lot of different applications of UV Vis spectroscopy and lot of different kinds of analysis could be performed, on this as per requirement.

To summarize UV Vis spectroscopy is kind of absorption spectroscopy, it is utilizes the ultraviolet and visible reason of the electromagnetic spectrum and as per the absorbance of the molecules in the solution. Lot of different kinds of analysis could be done, it follows like, here it is governed by the beers Lamberts law and the instrument, which utilized, it is spectrophotometer, which have been modified and advanced quite a lot for different kinds of analysis.

And lot of different accessories have been included to do experiments say at different temperature or in different conditions. So, this is one very useful analytical techniques in the field, in the area of biotechnology and it is utilized for analysis of different compounds including proteins and DNA. In the next lecture, we are going to discuss few more techniques, which are based on absorption or emission or other different phenomena’s.

Thank you for your attention.