

Structural Biology
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Lecture – 31
Introduction to Spectroscopy

Hi everyone, welcome again to the course of structural biology. We are going through structural Biology techniques. We have already discussed high resolution techniques like crystallography in details. High resolution techniques like NMR spectroscopy and from NMR we have also introduced Spectroscopy, but today it is a new module. We are continuing from the NMR spectroscopy to introduce other spectroscopy's feature giving us low-resolution structure. Today is the introductory class of the module Spectroscopy.

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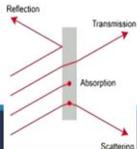
Introduction to Spectroscopy:

The science of spectroscopy grew out of studies of the interaction of electromagnetic energy with matter

When light shines on an object, for example, we know that part of the light is scattered and part is absorbed

Of the initial part that is absorbed, some is later emitted as light of a different color or wavelength

Spectroscopy is that science which attempts to determine what specific energies and amounts of incident light are absorbed by specific substances, and what specific energies and amounts are later re-emitted



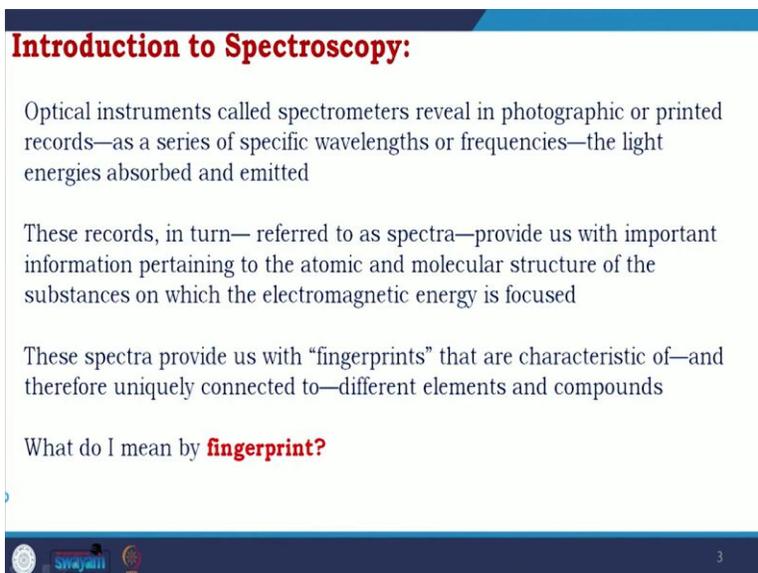
The diagram shows a vertical grey bar representing an object. Several red arrows representing light rays are incident on the bar from the left. One arrow is reflected upwards and to the left, labeled 'Reflection'. One arrow passes through the bar, labeled 'Transmission'. One arrow is absorbed by the bar, labeled 'Absorption'. One arrow is scattered downwards and to the right, labeled 'Scattering'.

So, the science of Spectroscopy grew out of studies of the interaction of electromagnetic energy with matter. So it is the interaction how the light rays come and interact. As you see this picture this Interaction could be reflection, transmission, absorption, scattering. There are number of Spectroscopy's, and we will discuss about them. When light shines on an object means light interact on objects, we know that part of the light is scattered and that part is absorbed.

The initial part that is absorbed some is later emitted as light of a different colour or wavelength. Spectroscopy is that science which attempts to determine what specific energy and amount of

incident light are absorbed by specific substance and what specific energy and amounts are later re-emitted.

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Introduction to Spectroscopy:

Optical instruments called spectrometers reveal in photographic or printed records—as a series of specific wavelengths or frequencies—the light energies absorbed and emitted

These records, in turn—referred to as spectra—provide us with important information pertaining to the atomic and molecular structure of the substances on which the electromagnetic energy is focused

These spectra provide us with “fingerprints” that are characteristic of—and therefore uniquely connected to—different elements and compounds

What do I mean by **fingerprint?**

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Optical instrument call spectrometer reveal in photographic of printed records as a series of specific wavelength or frequencies the light energy is absorbed and emitted. These records in turn referred to spectra provide us with important information pertaining to the atomic and molecular structure of the substances on which the electromagnetic energy is focused.

What we want from the experiments? We find out if there is something called fingerprint. As I was talking about that are characteristic of it. Therefore it is a unique connection to the different element, compound to the fingerprint. Just imagine you have a molecule A and molecule B, if you put some ray on them and you get differentiated reaction. You could have record them and use that differentiation for recognition. That' is what we are doing in Spectroscopy so as I was talking about the term finger print. What is fingerprint?

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Line spectra for three distinct elements:

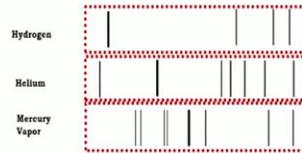
Let us consider three elements: Hydrogen, Helium and Mercury

Now if you want to differentiate between them—you need to take help of a “special instrument”

With the help of this technique you will get characteristic fingerprint—which will guide you to differentiate between the three elements

The process of this characterization could be applied in many things and broadly everything:

Very interestingly this gives rise to a complete field to study the characterization of different elements, atoms, atomic states, molecular interactions etc. and it's called **“Spectroscopy”**



(Adventures in Physics, Highsmith and Howard, 1972)



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Let us discuss that. So what we did we consider the three elements Hydrogen, Helium and mercury. Now if you want differentiate between them, you have to take the help of a special instrument. With the help of the special instrument you will get characteristic fingerprints which will guide you to differentiate between the three elements.

So this is Hydrogen, Helium and Mercury, as I told these are gone through an experimental process using a special instrument. You can see that there are lines and these lines are different for Hydrogen, Helium and mercury. The process of this characterization could be applied in anything and broadly everything.

That is why we are spending so much time to understand the general principles because if you understand what is the general principle of Spectroscopy then you go and you see that by changing energy, by changing different parameter newer Spectroscopy's are developed and studied. Very interestingly this gives rise to a complete field to study the characterization of different elements, atoms, atomic states, molecular interactions etc in a special instrumentation which we call Spectroscopy.

Before going into the Science as you know from my trend of classes we will take a look at the history of the Spectroscopy, how it is grown?

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History of Spectroscopy:

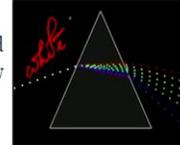
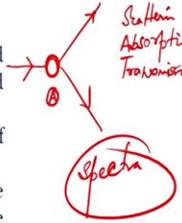
In ancient times, Egyptians and Greeks thought about light and color and considered light to be mostly "something" that emanated from the eyes

The great minds of Ptolemy, Plato, and Aristotle failed to perceive of possible applications that might involve light as we know it

Following the Middle Ages (400-1350 AD) and the Renaissance period (1350-1700 AD), ancient, classical ways of thinking gave way to more creative, academic analyses and crude optical instruments began to appear

Scientists like **Johann Kepler**, **Willebrord Snell**, and **Galileo Galilei** used combinations of lenses in telescopes to see distant objects

Sir Isaac Newton, in the latter half of the 17th century, showed how a prism "broke" white light passing through it into a rainbow of separate and distinct colors



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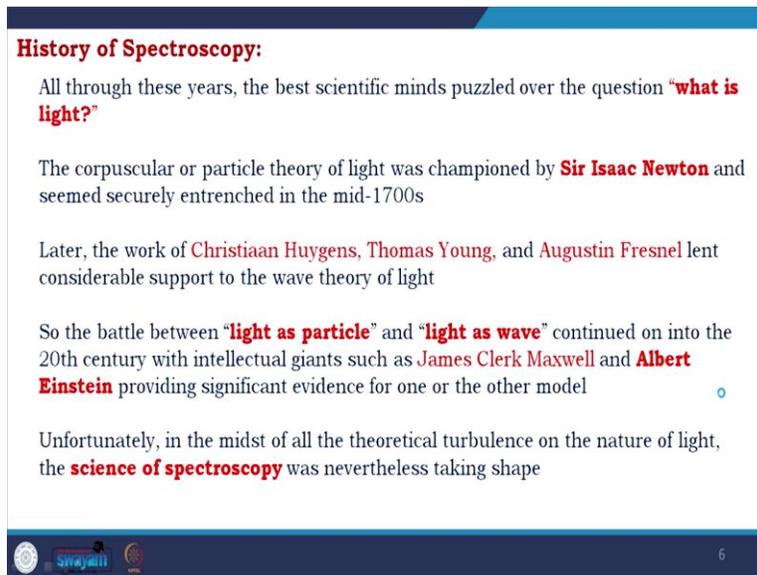
In ancient times Egyptians and Greeks thought about light and colour and considered light to be mostly something that emanated from the eyes. The great minds of Ptolemy, Plato and Aristotle failed to perceive of possible application that might involve light as we knew it now. Following the middle ages 400 to 1358 AD and the Renaissance period of 1350 to 1700 AD classical ways of thinking gave way to more creative academic analysis and crude optical instrument began to appear.

So if you stop here, just put your thinking what we want? We have material let us A. We want light to come here and do different things be it scattering, be it absorption, be it transmission anything, and this anything will create a change. The change would be detected and that is we called as spectra. So by spectra we mean characteristic spot or line, and by recording the changes you want to identify something that is what Spectroscopy is.

So in that time; in the Renaissance period optical instrument begin to appear, which very crude form of modern Spectroscopy. Scientist, like Johan Kepler, Willboards snell and Galileo Galilei use combination of lenses in telescope to see distant objects. Sir Isaac Newton in the latter half of the seventeenth century showed how a Prism broke white light passing through it into a rainbow of separate and distinct colour.

That is a innovation with gives him the title on entitle him to be the father of spectroscopy as you see here white light is coming here. It is breaking to 7 different lines.

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History of Spectroscopy:

All through these years, the best scientific minds puzzled over the question “**what is light?**”

The corpuscular or particle theory of light was championed by **Sir Isaac Newton** and seemed securely entrenched in the mid-1700s

Later, the work of **Christiaan Huygens**, **Thomas Young**, and **Augustin Fresnel** lent considerable support to the wave theory of light

So the battle between “**light as particle**” and “**light as wave**” continued on into the 20th century with intellectual giants such as **James Clerk Maxwell** and **Albert Einstein** providing significant evidence for one or the other model

Unfortunately, in the midst of all the theoretical turbulence on the nature of light, the **science of spectroscopy** was nevertheless taking shape

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All through these years the best scientific mind puzzled over the question what is light? The corpuscular or particle theory of light was championed by Sir Isaac Newton and seemed secularly entrenched in the mid 1700. Later the work of Christian Huygens, Thomas young and Augustine Fresnel, lent considerable support to the wave theory of light. So, there was a classical theory as we know mainly contributed by Sir Isaac Newton whereas wave theory of light is also coming. So the battle between light as a particle and light as a wave continued on in the twentieth century with intellectual Giants like James Clerk Maxwell and Albert Einstein providing significant evidence for one or the other model. But unfortunately in the midst of all the theoretical turbulence on the nature of light, the science of Spectroscopy was nevertheless taking shape it was slow down.

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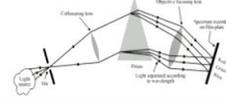
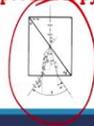
History of Spectroscopy:

In 1802 a physicist named W. H. Wollaston used a prism, lenses, and a narrow beam of light to produce an image of a **single wavelength of the light**

Following this work, with the help of a different light dispersing element—a **diffraction grating**—scientists produced similar **monochromatic** images of **“split light”**

The spectroscope as an instrument, became a practical laboratory instrument in the hands of German physicists such as **Josef Fraunhofer**, **G. R. Kirchoff**, and **Robert Bunsen**, during the first half of the 1800s.

With **Fraunhofer's study of solar energy** and the **discovery of narrow dark lines in the solar spectrum**, and with the ongoing analysis of light sources based on flames produced with Bunsen burners, there appeared **bright lines** as well as **dark lines**: There happens the launch of **“Science of Spectroscopy”**



In 1802 a physicist name W. H. Wollaston used a Prism, lenses and a narrow beam of light to produce an image of a single wavelength of the Light. So if you see this is the analysis of the experiment what Wollaston had done. Following this work with the help of a different light dispersing element works in diffraction grating Scientist produce similar monochromatic images split light.

The splitting of light is coming into the front and its applications. The spectroscope as an instrument became a practical laboratory instrument in the hands of German physicist such as Joseph Fraunhofer, J. R. Kirchoff and Robert Bunsen during the first half of the 1800. As I told Fraunhofer sounds very, very familiar because of the study of solar energy in the discovery of narrow dark lines in the solar spectrum the Fraunhofer lines which we know. And with the ongoing analysis of light sources based on films produced with Bunsen burners, there appeared bright lines as well as dark lines: there happens the launch of science of Spectroscopy.

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History of Spectroscopy:

Scientists understood then that the dark and bright lines seen in absorption and emission were uniquely characteristic of the internal makeup of chemical elements

They assumed, correctly, that the energy in light could somehow excite the internal motions of atoms and molecules, extracting energy from the light at certain wavelengths, thereby giving rise to the narrow absorption lines

Similarly, heat or electrical energy could excite internal motions in matter which would then radiate away the absorbed energy as light, accounting for the bright or emission lines in the spectra

In every instance, the energy that was directed onto the target substance—to excite the internal motions of the electrons, atoms, and molecules—could be described as a well-known part of the **electromagnetic spectrum**



Scientists understood then that the dark and the bright lines seen in absorption and emission were unique characteristics of the internal makeup of chemical elements. See when you are working on a noble field of science. Initially the dilemma is you do not know how to use it. That is why this understanding was very significant because when people understand that the dark and the bright lines characteristic of molecular structure, they understood that if we could start recording them, and if you could have gotten instrumentation to study them. It would be fundamental; it would be very informative to know about the detection of the molecular structure of the small molecules to many things. That time scientists assumed correctly that the energy in light could somehow excite the internal motion of atoms and molecules, extracting energy from the light at certain wavelengths, thereby giving rise to the narrow absorption lines. From there a very correct direction takes place towards modern Spectroscopy.

Similarly, heat or electrical energy could excite internal motion in matter, which would then radiate away the absorbed energy as light, accounting for the bright or emission lines in the spectra. In every instance, the energy that was directed onto the target substance to excite the internal motion of the electron, atoms and molecules could be described as a well-known part of the electromagnetic spectrum which is something we have to study in detail to understand Spectroscopy.

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Electromagnetic (EM) wave and the spectrum:

The electromagnetic (EM) spectrum is the range of all types of EM radiation

Radiation is energy that travels and spreads out as it goes – the visible light that comes from a light source and the radio waves that come from a radio station are two types of electromagnetic radiation

The other types of EM radiation that make up the electromagnetic spectrum are microwaves, infrared light, ultraviolet light, X-rays and gamma-rays



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So, electromagnetic wave and the spectrum: The electromagnetic spectrum is a range of all type of electromagnetic radiation. Radiation is energy the travels and spreads out as it goes, the visible light that comes from a light source and the radio waves that come from radio station are two type of electromagnetic radiation. So how many of them are there, what are their property, how the distribution all are depicted in electromagnetic spectrum.

If you could understand electromagnetic spectrum you understand a very critical part of Spectroscopy. The other types of electromagnetic radiation that make up the electromagnetic spectrum are microwaves, infrared light, ultraviolet light, X rays and gamma rays.

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Development of an Electromagnetic wave: So how to create electromagnetic wave. All the electromagnetic waves are created by accelerating electric charge. Thus the frequency wavelength and energy of electromagnetic waves all depend on charge acceleration and just how this acceleration changes with time.

Electromagnetic wave emitted by the accelerating charge is equal to the frequency f of the charge motion. If the charge oscillates back and forth with the frequency of three times per second it will emit a wave with the frequency of 3 cycle per which could be call as three hertz. To create light waves in the visible part of the electromagnetic spectrum wavelength range of 0.4 micrometre to 0.7 micrometre have to be electric charge must accelerate at a rate high enough to generate waves of lengths around 0.5×10^{-6} to 7×10^{-7} metres.

Now we know that V the velocity equal to f the frequency into λ the wavelength where V is the wave's speed in metre per second. It is the frequency in cycle per second or hertz as we have seen here. λ is the wavelength in metre. So now when we have all this information for light in free space where we know the V equal to 3×10^8 metre per second and for the mid visible region of the light around 0.5×10^{-6} to 7×10^{-7} metre.

The frequency from previously mentioned equation, you will get again f equal to V by λ which calculates out 6×10^{14} hertz. So you could understand this is an industry high-value number. So, clearly there are no ordinary mechanical motion of charged substances at our disposal that can attain such high frequency that for challenge. Only in region inside atoms and molecules where the electrons move very rapidly around the nucleus and where atoms vibrate and oscillate very rapidly in molecules can such high frequency of moving electric charges be realised.

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Development of an Electromagnetic (EM) wave:

Figure demonstrates the creation of a single wave by an oscillating charge

This figure shows how such charge made to oscillate along the arms of an antenna gives rise to EM waves moving outwardly in regions surrounding the antenna

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So if you see this; the system where the electric charge is accelerated and waves produced the frequency f and wavelength λ . So this figure demonstrates the creation of a single wave by an oscillating charge and then how to use it for experiment. There are three stage formations.

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The opposite terminals of an AC power supply, connected respectively to the upper and lower arms of the antenna, generate electrons e^- that accelerate up and down the two arms

In stage 1, the accelerating electrons are moving downward in both arms and create the outward-moving EM field with the E-field directed downward. As the applied AC voltage changes polarity, so does the direction of electron flow in the arms and so then the outward moving E-fields change directions in stage 2

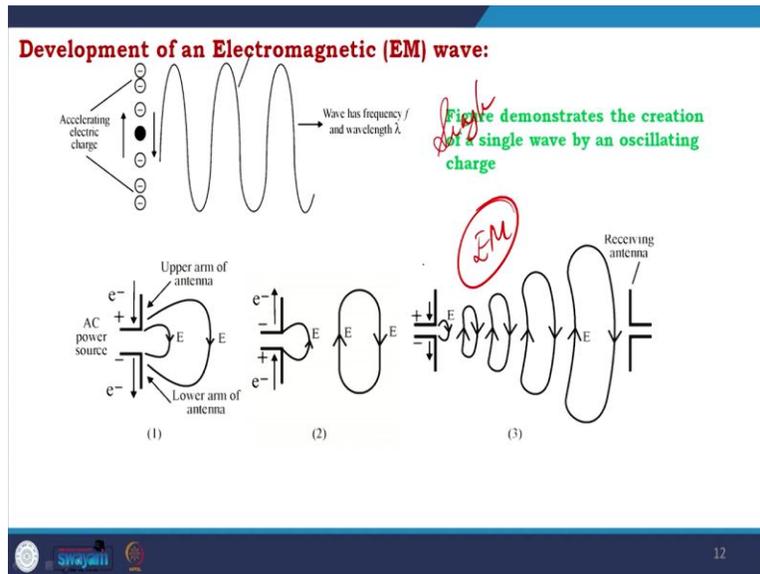
As the electron flow in the arms of the antenna continues to change directions, the newly produced electric fields are created next to the antenna and the previous fields are forced further outward as in stage 3, where finally they may be detected by a similar receiving antenna

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So you see there is a AC power source. The opposite terminal of an AC power supply connected respectively to the upper and lower arms of the antenna generate electron that accelerate up and down the two arms in the stage 1. In stage 1, the accelerating electrons are moving downward in both arms and create the outward moving electromagnetic field with the E-fields directed downward, as the applied AC voltage changes polarity, so does the direction of electron flow in the arm and so then the outward moving electron fields change direction in next stage. As the

electron flow in the arms of the antenna continues to change the directions. The newly produced electric fields are created next to the antenna and the previous fields are forced further outward as in the stage 3, where finally, they may be detected by a similar receiving antenna, there are series of that antenna.

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So, you see the number, how this big number is only possible to create inside atoms molecules, the single wave is generated here and then the actual electromagnetic wave which is going for experiment is created here.

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Electromagnetic Spectrum:

Now we know that accelerating charges produce electromagnetic waves

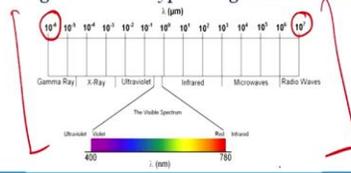
There are many levels in the structure of matter where moving (accelerating) charges exist

Some of the more obvious are electrons in an atom, freely-moving electrons in conducting metals, vibrating atoms in molecules, and charged particles in a nucleus
Thus, two factors result in the many different types of electromagnetic waves we observe

a) the source of the charge motions

b) the accelerations inherent in the motions

The many different types of EM waves are categorized according to their origins and their frequency/wavelength values. A typical organization of the electromagnetic is shown here:



Coming to the electromagnetic spectrum as I talked, if you start from in terms of wavelength, it is started from gamma ray where the wavelength is very low to radio waves where the wavelength is very high. In between there are x rays, ultraviolet, visible, infrared, microwave. So this is what we call electromagnetic spectrum. So, from our previous knowledge, now we know the accelerating charges produce electromagnetic waves. There are many levels in structure of matter where moving charges exist.

Some of the more obvious are electrons in an atom freely moving electrons in conducting metals, vibrating atoms in molecules and charged particle in a nucleus. Thus there will be two factors, which result in many, different type of electromagnetic waves which we see in general experiments. One the source of the Charge motion and second the acceleration inherent in the motion. In many different type of electromagnetic waves are categorized according to their origin and their frequency or wavelength values. A typical organization of the electromagnetic is shown here. This is the one which we call electromagnetic spectrum.

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Electromagnetic spectrum and different waves:

Here we are discussing the energies underlying these processes correspond to different regions in the electromagnetic spectrum

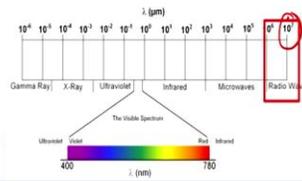
Radio: Your radio captures radio waves emitted by radio stations, bringing your favorite tunes

Radio waves are also emitted by stars and gases in space

Radiofrequency region has very low energies that correspond to the energy differences in the nuclear and electron spin states. These frequencies, therefore, find applications in nuclear magnetic resonance and electron paramagnetic resonance spectroscopy (MRI)

Microwave: Microwave radiation will cook your popcorn in just a few minutes, but is also used by astronomers to learn about the structure of nearby galaxies

Microwaves have energies between those of radiofrequency waves and infrared waves and find applications in rotational spectroscopy and electron paramagnetic resonance spectroscopy



Here we are discussing the energies underlying this processes correspond to different regions in the electromagnetic spectrum. If we start from radio, we know that we have radio which captures waves emitted by the radio station. So, radio waves are also admitted by stars and gases in space and as you see radio wave has very high wavelength, and so the energy is low because as you know energy is inversely proportional to wavelength. So the radio frequency region has very low energy that corresponds to the energy difference in nuclear and electron spin states. These frequencies therefore find application in Nuclear Magnetic resonance and Electron Paramagnetic Resonance Spectroscopy. Also, remember we have discussed this earlier because these NMR and EPR, both are operating at the higher wavelength which means low energy region. They are non destructive, non-invasive method. So you could plan instrumentation which could work directly on living organism. We have discussed about MRI using the nuclear Magnetic resonance Spectroscopy.

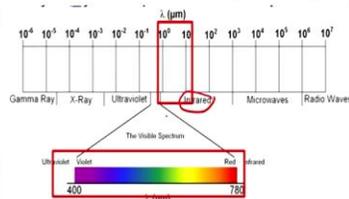
Coming to microwave: Microwave radiation will cook your popcorn in just a few minutes, but it is also used by astronomers to learn about structure of nearby galaxies. Microwave energy lies between those of radio wave and the infrared wave and find application in rotational Spectroscopy and electron paramagnetic resonance spectroscopy.

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Infrared: The energies associated with molecular vibrations fall in the infrared region of electromagnetic spectrum. Infrared spectroscopy is therefore also known as vibrational spectroscopy and is a very useful technique for functional group identification in organic compounds. Raman spectroscopy also appear in this region

Visible: Our eyes detect visible light
Fireflies, light bulbs, and stars all emit visible light
This are involved in electronic transition in the molecule

Ultraviolet: Ultraviolet radiation is emitted by the Sun and are the reason skin tans and burns. "Hot" objects in space emit UV radiation as well
UV regions are involved in the electronic transitions in the molecules. The spectroscopic methods using UV or visible light therefore com



Coming to infrared: In infrared the energy associated with molecular vibration fall in the infrared region of electromagnetic spectrum. Infrared Spectroscopy is therefore also known as vibrational Spectroscopy because it is working with the vibrational level of the atom that operates there. It is very useful technique for functional group identification in organic compounds. In addition, Infrared and Raman spectroscopy also operate in that level, and that is why infrared and Raman is competitive. They are affecting a single molecule in two different ways. So combining infrared Spectroscopy and Raman spectroscopy would always give us more information and in the latter part of this module will discuss about this in details. Visible: Our eyes detect visible light. Fireflies, light bulbs and stars all emit visible light, whatever you see is visible. These are involved in electronic transition in the molecule. So these are all in the visible range from 400 to 780 nm wavelength.

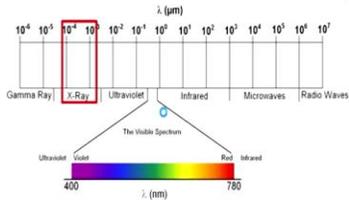
Ultraviolet radiations emitted by the sun and are the reason skin tans and burns. HOT object in space emit UV radiation as well. UV regions are involved in the electronic transition in the molecule. The spectroscopic method using UV or visible light, therefore, they are very well used in UV visible spectroscopy.

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X-ray: A dentist uses X-rays to image your teeth, and airport security uses them to see through your bag. Hot gases in the Universe also emit X-rays

X-rays are high energy electromagnetic radiation and causes transitions in the internal electrons of the molecules

Gamma ray: Doctors use gamma-ray imaging to see inside your body The biggest gamma-ray generator of all is the Universe



X-Ray: We go for our dental problem. Dentist use X-ray to image of our teeth. And in airport security, they use them to see through your bag. Hot gases in the universe also emit X-rays. X rays are high energy electromagnetic radiation and causes transition in the internal electrons of molecule as we have discussed when we are going to X-ray crystallography.

Gamma ray: Doctors use Gamma ray imaging to see inside your body. The biggest gamma generator is the universe.

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Molecular Mechanism of Interaction of EMR with Matter:

In order to interact with the electromagnetic radiation, the molecules must have some electric or magnetic effect that could be influenced by the respective components of the radiation

*Proton & Neutron
→ q/e*

In NMR spectroscopy, the nuclear spins have **magnetic dipoles** aligned with or against a huge magnetic field. Interaction with radiofrequency of appropriate energy results in the change in these dipoles

Rotations of a molecule having a net **electric dipole moment**, such as water will cause changes in the directions of the dipole and therefore in the electrical properties

We have seen spectra and that was starting of our conceptualization of Spectroscopy. We assume that looking at those lines we could differentiate Hydrogen Helium and mercury, but now if you see something more interesting with this, give rise to complete field of study of different

elements at atomic states, molecular interaction and is called Spectroscopy and we now know that the phenomenon is due to the interaction of matter with light.

But now we have something additional to look at. If you look at those lines, and now if you put wavelength and colour you will see that the bands are even more categorized. So you get more characterization of those spectra lines. So, that would help you towards developing core concept of the spectra these lines which we have called spectra. The representations of the lines are called spectrograph.

The complete system including the light source (a means to collect the light that interacts with the tested items) for measurement is called spectrophotometer. So these are the basic terminologies, the spectra the spectrograph, the diffraction grating Monochromator and spectrophotometer. So, looking at the molecular mechanism of interaction of electromagnetic radiation with matter, in order to interact with the electromagnetic radiation, the molecules must have some electric or magnetic effect that could be influenced by the respective components of the radiation.

In NMR spectroscopy, as we now know in very detail, the nuclear spins have magnetic dipoles aligned with or against a magnetic field. If you remember the last module, you could easily remember that we have the nuclei, the nuclei have protons and neutrons, why Proton have charge and spin, the neutron have only spin and because of the presence of them, they are working as a tiny magnet, once there is a external field then they would react to them, they would respond to them giving us characterization of that element.

Rotation of a molecule having and net electric dipole moment such as water will cause changes in the direction of the dipole and therefore in the electrical property.

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Molecular Mechanism of Interaction of EMR with Matter:

Vibrations of molecules can result in changes in electric dipoles that could interact with the electrical component of the electromagnetic radiation

Electronic transitions take place from one orbital to another. Owing to the differences in the geometry, size, and the spatial organization of the different orbitals, an electronic transition causes change in the dipole moment of the molecule

Vibration of molecule can result in changes in electric dipole that could interact with the electrical component of the electromagnetic radiation. Electronic transition takes place from one orbital to another. Owing to the differences in the geometry, size, and the spatial organization of the different orbital, an electronic transition causes change in the dipole moment of the molecule helping us to characterize a particular atom, material, polymer, substance and whatnot.

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Interaction of EMR with Matter:

Absorption-Light is absorbed

Emission-Light is emitted or released

Transmission-light is allowed to pass through

Reflection-light is reflected or bounced away

Diffraction-shows wave nature

Refraction-shows particle nature

Interference-light is disturbed

Scattering-light is dispersed

Polarization-light vibration is restricted to one direction

So what are the types of electromagnetic radiation interaction with matter? Absorption of light is absorbed. Emission, light is emitted or released, transmission light is allowed to pass through, reflection light is reflected on bounced, diffraction shows wave nature, refraction shows particle

nature, interference of light is disturbed, and scattering light is dispersed, and polarization of light vibration is restricted to one direction so these are the interactions.

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Spectrum:

Spectrum –graph or plot of intensity of absorbed/emitted radiation by sample versus frequency or wavelength

Types of Spectra:

Continuous Spectra–Spectra obtained when white light passed through a prism

Absorption Spectra–Spectra obtained by absorption of electromagnetic radiation to the atoms, ions or molecules of sample (UV/Visible, etc.)

Emission Spectra–Spectra obtained by emission of electromagnetic radiation to the atoms, ions or molecules of sample

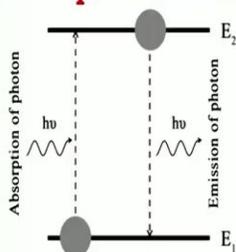
The diagram illustrates three types of spectra. The 'Continuous Spectrum' is shown as a smooth, continuous gradient of colors from red to violet. The 'Emission Spectrum' is shown as a dark background with several discrete, bright colored lines. The 'Absorption Spectrum' is shown as a continuous rainbow gradient with several dark, vertical lines superimposed on it.

Coming to Spectrum: Spectrum is the graph or plot of intensity of absorbed or emitted radiation by sample versus frequency or wavelength. Types of spectra: three types of spectra we generally see. Continuous spectra, spectra obtained when white light passes through the prism. Absorption spectra; spectra obtained by absorption of electromagnetic radiation of the atoms, ions and molecules of sample (UV-visible anything).

Emission spectra: Spectra obtained by emission of electromagnetic radiation to the atoms, ions or molecules of the sample. These are the common type of Spectroscopy we have observed in our experiments.

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Absorption and Emission:



Absorption of radiation is the first step in any spectroscopic experiment
Absorption spectra are routinely recorded for the electronic, rotational, and vibrational spectroscopy
It is therefore important to see how an absorption spectrum looks like

A transition between states takes place if the energy provided by the electromagnetic radiation equals the energy gap between the two states *i.e.* $\Delta E = h\nu = hc/\lambda$

This implies that the molecule precisely absorbs the radiation of wavelength, λ and ideally a sharp absorption line should appear at this wavelength

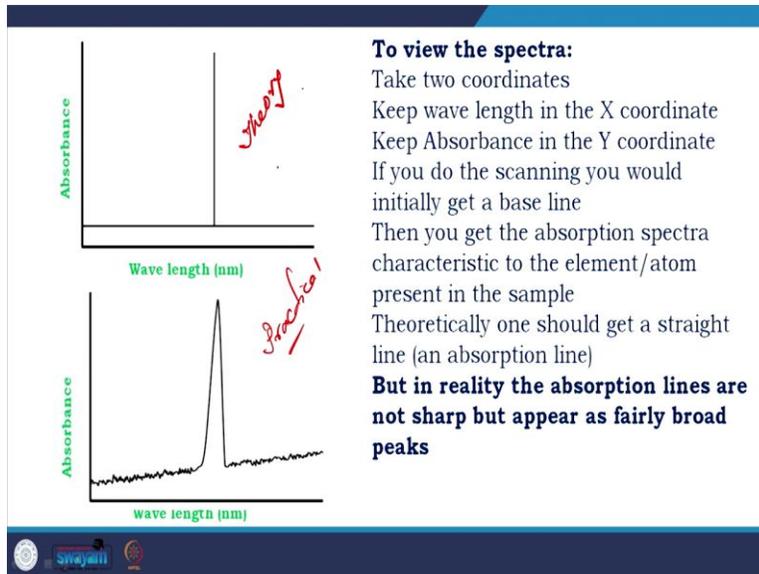
Coming to Absorption and Emission: When a photon comes and interacts, it changes the electronic state of the atom or molecule and then emits. So, absorption of radiation is the first step in any spectroscopic experiment. Absorption spectra are routinely recorded for the electronic, rotational, and vibrational Spectroscopy. It is therefore important to see how an absorption spectrum looks like. A transition between states takes place if the energy provided by the electromagnetic radiation equals the energy gap between the two States.

$$\Delta E = h\nu = hc/\lambda$$

The ΔE the change of energy

This implies that the molecule precisely absorbs radiation of wavelength λ and ideally a sharp absorption line should appear at this wavelength.

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So to view the spectra you have to take two coordinates. See here we have taken 2 co-ordinates. I put wavelength in the X co-ordinate, and I put absorbance in the Y co-ordinate. Then get a baseline very solid without noise baseline. Then you get the absorption spectra, and in some portion of that, you get a line of absorption for the characteristic of that atom or molecule. So in theory, we should get straight line (an absorption line).

But in reality the absorption line could not be that sharp, it appears as fairly broad peaks there and I have made the experimental, and you see that there is a band rather than line. So you would not get a theory like straight line rather you get a band because there are different experimental consequences which we are going to discuss.

(Refer Slide Time: 39:28)

Reason behind absorption line becoming broad peaks:

Instrumental factors:

The slits that allow the incident light to impinge on the sample and the emerging light to the detector have finite widths

Consider that the transition occurs at wavelength, λ_t . When the wavelength is changed to $\lambda_t + \Delta\lambda$ or $\lambda_t - \Delta\lambda$, the finite slit width allows the radiation of wavelength, λ_t to pass through the slits

As a result a finite absorbance is observed at these wavelengths

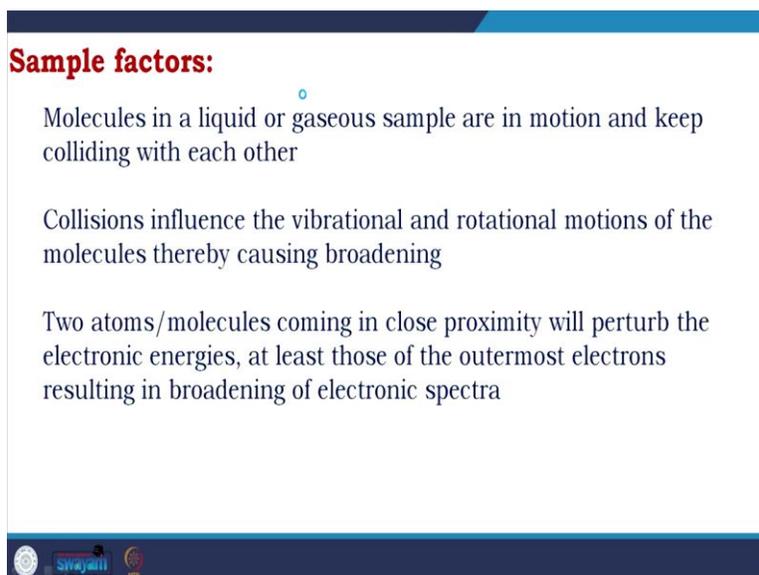
The absorption peaks are therefore symmetrical to the line at $\lambda = \lambda_t$

Why the absorption line Giving broad pick?

- 1) Instrumental factor. The slits that allowed the incident light to impinge on the sample and the emerging light to the detector have finite width.

Consider that the transition occurs at wavelength, λ_t . When the wavelength is changed to $\lambda_t + \Delta\lambda$ or $\lambda_t - \Delta\lambda$, the finite slit width allows the radiation of wavelength λ_t to pass through the slits. As a result a finite absorbance is observed at those wavelengths. The absorption peaks are therefore symmetrical to the line at $\lambda = \lambda_t$, that is why the broadening happened.

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Sample factors:

- Molecules in a liquid or gaseous sample are in motion and keep colliding with each other
- Collisions influence the vibrational and rotational motions of the molecules thereby causing broadening
- Two atoms/molecules coming in close proximity will perturb the electronic energies, at least those of the outermost electrons resulting in broadening of electronic spectra

2. Sample factors: Molecules in a liquid or gaseous sample are in motion and keep colliding with each other. Collisions influence the vibrational and rotational motion of the molecules thereby causing broadening. Two atoms or molecules coming in close proximity will perturb the electronic energy, at least those of the outermost electrons resulting in broadening of the electronic spectra.

Motion of molecule undergoing transition also causes shift in absorption frequency and that is significant and that is known as Doppler broadening. So instrument factor, simple factor.

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Intrinsic broadening:

Intrinsic or natural broadening arises from the Heisenberg's uncertainty principle which states that the shorter the lifetime of a state, the more uncertain is its energy

Molecular transitions have finite lifetimes, therefore their energy is not exact. If Δt is the lifetime of a molecule in an excited state, the uncertainty in the energy of the states is given by:

$$\Delta E \times \Delta t \geq h/4\pi$$

$$\Delta E \times \Delta t \geq h/2$$

where, $h = h/2\pi$

Now intrinsic broadening: Meaning Intrinsic or natural broadening arises from Heisenberg Uncertainty Principle. Which states that the shorter the lifetime of a state the more uncertainty is its energy? Molecular transitions have finite life time as we know, therefore their energy is not exact. If Δt is the lifetime of the molecule in an excited state. The uncertainty in the energy of the state is given by

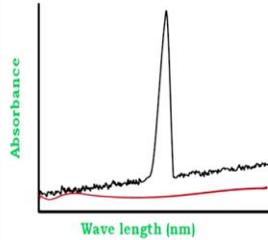
$$\Delta E * \Delta t \geq h / 4\pi$$

$$\Delta E * \Delta t \geq h / 2$$

Where $h = h / 2 \pi$

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Reason behind absorption line becoming broad peaks:



Another two more features which are worth noticing are the fluctuations in the baseline and the baseline itself, which is not horizontal

The small fluctuations in the baseline are referred to as noise

Noise is the manifestation of the random weak signals generated by the instrument electronics

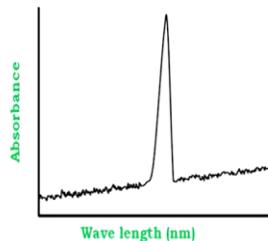
To identify the sample peaks clear of the noise, the intensity of the sample peaks has to be at least 3-4 times higher than the noise

A better signal-to-noise ratio is obtained by recorded more than one spectra and averaging; the noise being random gets cancelled out

There are other reasons behind this lines becoming broad peaks which are worth noticing are the fluctuations in the baseline, and baseline itself (which is not horizontal). The small fluctuations in the baseline are referred to as noise. Noise is the manifestation of the random weak signals generated by the instrument electronics. To identify the sample peaks clear of the noise. The intensity of the sample peaks has to be at least three to four times higher than the noise. Better signal to noise ratio is obtained by recording more than one spectras and averaging them, the noise being random gets cancelled out.

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Reason behind absorption line becoming broad peaks:



Instrumental factors are responsible for the non-horizontal baseline observed here

The light sources used in the instruments emit radiations of different intensities at different wavelengths and usually the detector sensitivity is also wavelength-dependent

A reasonable horizontal baseline for the samples can easily be obtained by subtracting the spectrum obtained from the solvent the sample is dissolved in

Instrumental factors are responsible for the non horizontal baseline observed there. The light sources used in the instrument emit radiation of different intensity the different wavelength and usually the detector sensitivity is also wavelength dependent. A reasonable horizontal baseline for the samples can easily be obtained by subtracting the spectrum obtained from the solvent the sample is dissolved in.

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Spectroscopy: Quantitative Measurement

Spectrophotometer - an instrument that measures the amount of light absorbed, or the intensity of color at a given wavelength.

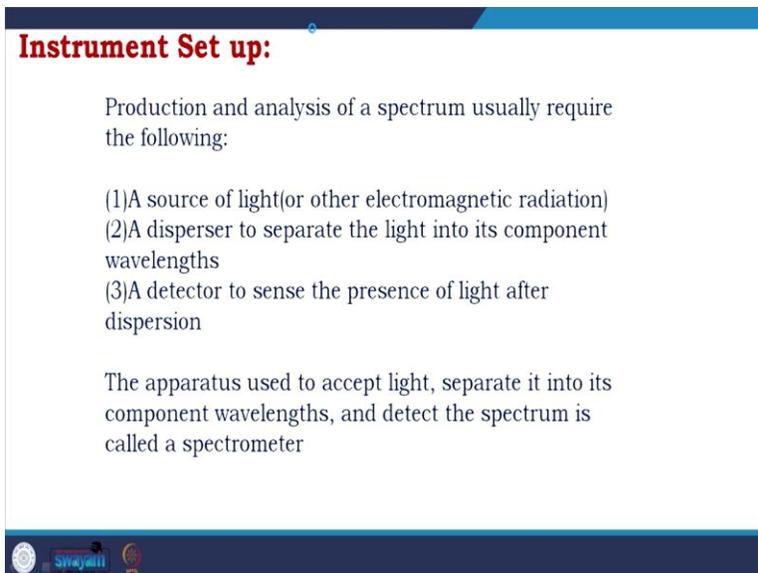
The **intensity of color** can be given a numerical value by comparing the amount of light prior to passing it through the sample and after passing through the sample.

These **quantitative measurements** of light absorbed are the **Transmittance** and the **Absorbance**.

Coming to quantitative measurement: So as I told spectrophotometer and instrument that measures the amount of light absorbed or the intensity of colour at a given wavelength. The intensity of colour can be given in numerical value by comparing the amount of light prior to

passing it to the sample and after passing through the sample. This quantitative measurements of light absorbed are the transmittance and the absorbance which we talked about.

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Instrument Set up:

Production and analysis of a spectrum usually require the following:

- (1) A source of light (or other electromagnetic radiation)
- (2) A disperser to separate the light into its component wavelengths
- (3) A detector to sense the presence of light after dispersion

The apparatus used to accept light, separate it into its component wavelengths, and detect the spectrum is called a spectrometer

The slide features a dark blue header with the title 'Instrument Set up:' in red. Below the title, the text is centered. At the bottom of the slide, there is a dark blue footer containing three small logos: a circular emblem on the left, the word 'Swayam' in the middle, and a circular emblem on the right.

So, the instrument set up: The production and analysis of spectrum usually require the following. A source of light or other electromagnetic radiation, a disperser or grating to separate the light into its component wavelength, a detector to sense the presence of light after dispersion. The Apparatus used to accept light, separated into its component wave and detect the spectrum is called spectrometer.

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Lambert-Beers's Law:

- Light of a particular wavelength enters the 'sample'
- Light *scatters* from particles in solution (no momentum change) reducing light transmission
- Light is *absorbed* by molecules/particles (momentum change) and remitted at different wavelengths, reducing light transmission



Lambert Beer's law: The light of a particular wavelength enters the sample, I_0 is the initial intensity and it change which to I_1 , l is the path length and c is the concentration. ϵ is the constant we will talking about it. Light scatters from particles in solution (no momentum change) reducing light transmission. Light is absorbed by molecules/particle (momentum change) and re-emitted at different wavelengths reducing light transmission.

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Lambert's Law:

When a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the **length** of the absorbing medium increases.

Beer's Law:

When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the **concentration** the absorbing medium increases.

Lamberts law: When a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

Beer's law: When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

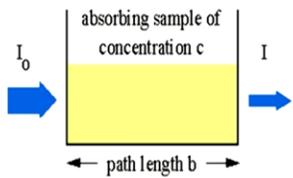
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Transmittance is given by the equation:

$$T = I/I_0$$

where I is the intensity of the light after it has gone through the sample & I_0 is the initial light intensity.

Absorbance is related to the %T:

$$A = -\log T = -\log(I/I_0)$$


The diagram shows a yellow rectangular cell representing an absorbing sample. A blue arrow labeled I_0 points into the cell from the left, and another blue arrow labeled I points out of the cell to the right. The cell is labeled "absorbing sample of concentration c" and "path length b".

Transmission T is given by the equation

$$T = I / I_0$$

Where I_0 is the initial intensity and I is the intensity of the light after it has come to the sample

Absorbance is related to the percentage of T

$$A = -\log T = -\log (I / I_0)$$

absorbing sample of concentration c and changing to I this is the path length.

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Absorbance Measurements:

Beer-Lambert Law - the linear relationship between absorbance and concentration of an absorbing species

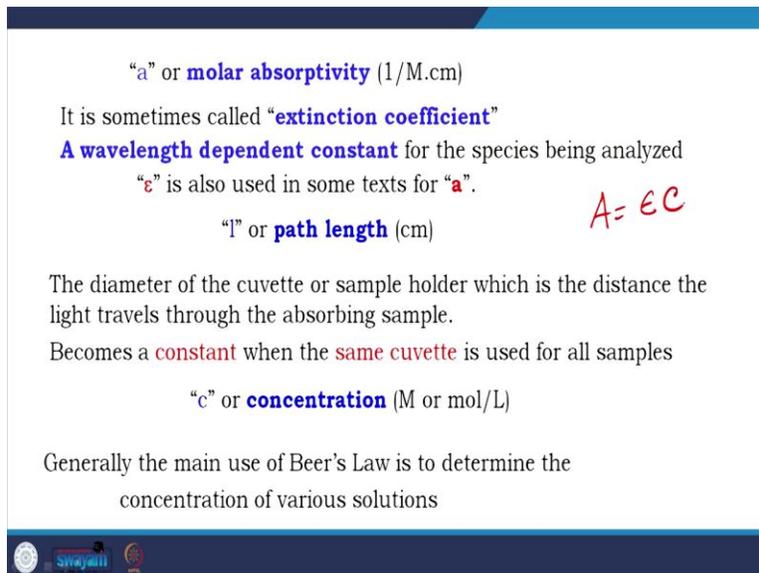
$$A = alc$$

A is the absorbance
"a" is molar absorptivity in $L/[(\text{mole})(\text{cm})]$
"l" is the path length in cm
"c" is the concentration of the analyte (sample) in mol/L

So, as Beer-Lambert law, the linear relationship between absorbance and concentration of an absorbing species, absorbance equal to A or $\alpha l c$ or a or $\text{Alpha } l$ into c, a is the absorbance

the capital A is molar absorptivity as I told it would also called Alpha Epsilon in litre per mole centimeter, l is the path length in centimeter, c is the concentration of the analyte in mole per litre.

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“a” or **molar absorptivity** (l/M.cm)
It is sometimes called “**extinction coefficient**”
A wavelength dependent constant for the species being analyzed
“ ϵ ” is also used in some texts for “a”. $A = \epsilon c$

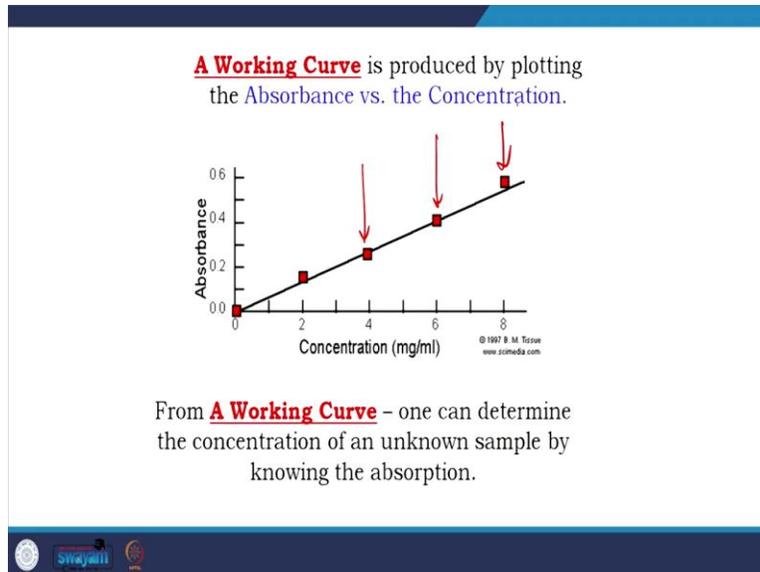
“l” or **path length** (cm)
The diameter of the cuvette or sample holder which is the distance the light travels through the absorbing sample.
Becomes a **constant** when the **same cuvette** is used for all samples

“c” or **concentration** (M or mol/L)
Generally the main use of Beer's Law is to determine the concentration of various solutions

a as I told its molar absorptivity it is sometime call extinction coefficient a wavelength dependent constant for the species being analysed with Epsilon, l is the path length in centimetre the diameter of the curvette or sample holder, which is the distance the light travels through the absorbing sample becomes a constant when the same curvette is used for samples, c is the concentration. Generally the main use of Beers Law as to determine the concentration of various solution.

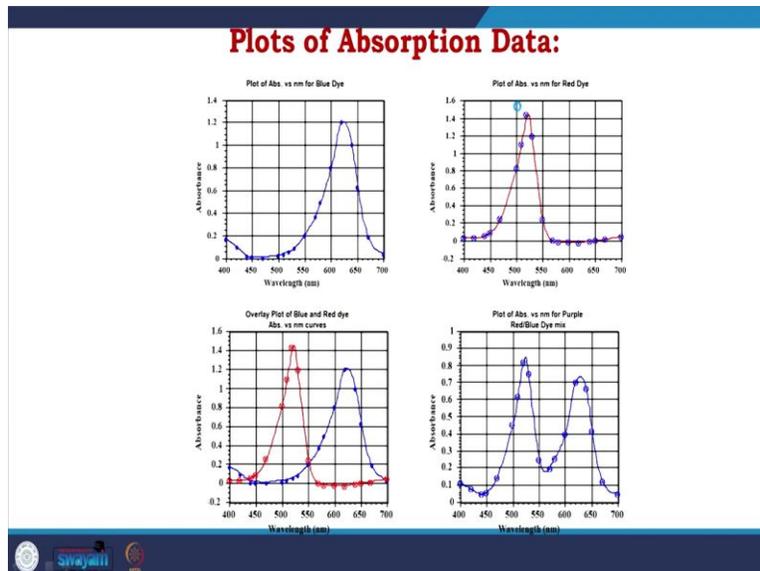
How as we know A equal to epsilon cl, so this could be fixed by fixing the cuvette. This is characteristic. So by measuring observance we could find the concentration.

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So, working curve is produced by plotting absorbance versus the concentration. So here you observe absorbance versus concentration it is a straight curve and from there you could get to know the required concentrations. From a working curve one can determine the concentration of an unknown sample by knowing the absorbance. This is what it greatly used.

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Suppose this is a plot of an absorption data. You have plot of the blue dye, you have a plot of the red dye. Now you overlay the plot now you could have the plot of absorption versus the nanometre of red and blue dye mix. So in that way you could have characterized them you could have known the concentration and that is why it is very common used because you get any spectrophotometer and you could have calculate the absorbance.

Any unknown samples you are working on you make the solution and you could get the concentration of the unknown sample. So we have discussed about the general principle of Spectroscopy and how Spectroscopy would be used.

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Type of Spectroscopy	Wavelength Range	Wave number range	Type of quantum transition
Gamma Ray	0.005-1.4 Ang	-	Nuclear
X-ray absorption, emission, fluorescence and diffraction	0.1-100 Ang	-	Inner electron ²
Vacuum ultra violet absorption	10-180nm	1×10^6 to 5×10^4	Bonding electron
UV-Visible absorption, emission, fluorescence	180-780 nm	5×10^4 to 1.3×10^4	Bonding electron
Infrared absorption and Raman Scattering	0.78-300 mm	1.3×10^4 to 3.3×10^1	Rotation/vibration of molecules
Microwave absorption	0.75-3.75 mm	13-27	Rotation of molecules
Electron spin resonance	3.0 cm	0.33	Spin of electron in a magnetic field
Nuclear spin resonance	0.6-10 m	1.7×10^2 to 1.0×10^3	Spin of nuclei in a magnetic field

We talked about the electromagnetic spectra in the electromagnetic spectrum. There is Gamma ray; Gamma ray is used for Quantum transition at the nuclear level. X-ray absorption emission, fluorescence and diffraction they are affecting the inner electron, vacuum, ultraviolet absorption using bonding electron, UV-visible absorption, emission fluorescent Spectroscopy. Again, they are affecting bonding electron, infrared absorption and Raman scattering.

Also they work on 0.78 to 300mm they are working on the rotation and vibration of molecules micro absorption spectroscopy working on 0.75 to 3.75mm rotation of molecules, electron spin resonance Spectroscopy around 3 centimetre wavelength range. Spin of the electron in magnetic field. This is kind of similar as we think about in the case of a NMR. So nuclear spin resonance is the spin off in a nuclei magnetic field 0.6 to 10 metre and so as I told these are the different parts.

Gamma ray working on the nuclear level and gradually the wavelength range is enhancing energies decreasing so it form the high energy to low energy and reversed in wavelength. In the

next few classes we would talk about different Spectroscopy like UV-visible absorption spectroscopy, circular dichroism spectroscopy, fluorescence, infrared, Raman also I would add a new technique. I want to discuss about Raman microscopy and Raman crystallography. Thank you for listening if you have any question, please keep writing us, thank you.