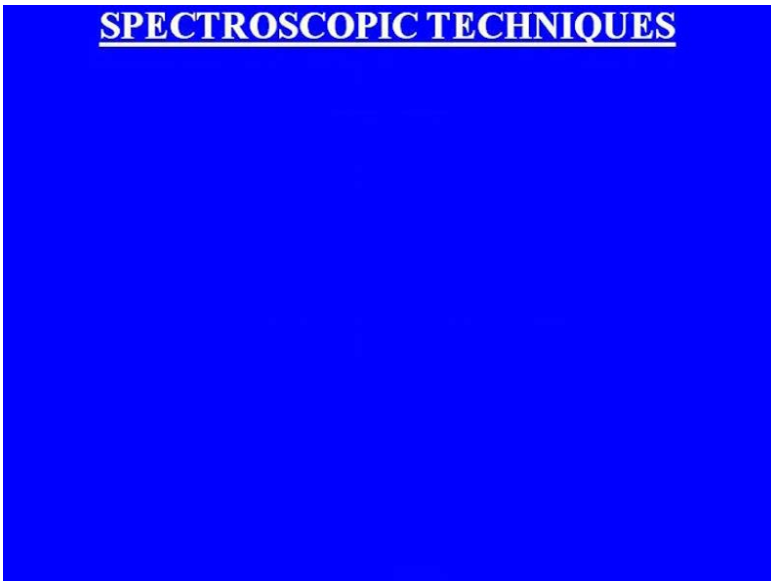
Analytical Technologies in Biotechnology
Prof. Dr. Ashwani K. Sharma
Department of Biotechnology
Indian Institute of Technology, Roorkee

Module - 6
Spectroscopic Techniques
Lecture - 1
Introduction and Basic Concepts

In previous lectures, we have covered 5 topics and these include microscopy, radio isotope technique, chromatographic technique, electrophoreses and centrifugation techniques. From this lecture, we are going to start a new topic and that is spectroscopic techniques.

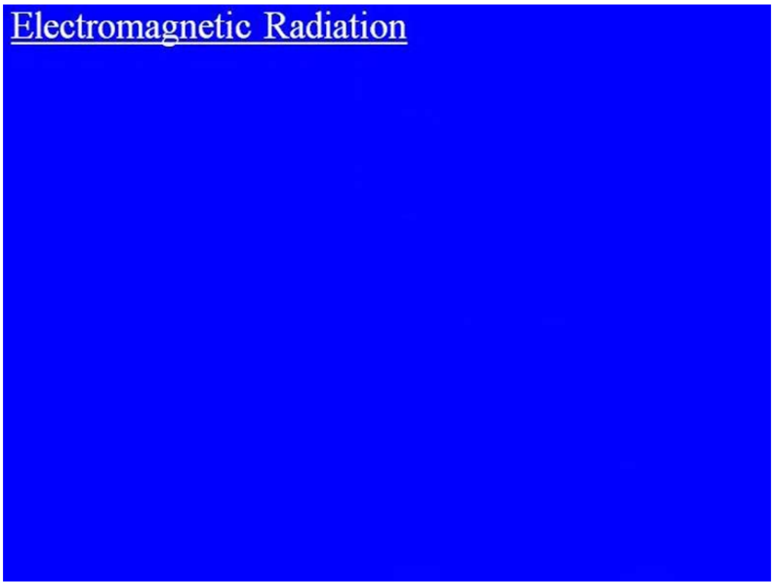
(Refer Slide Time: 00:48)



Now, spectroscopy can be defined as the study of interaction of electromagnetic radiation with metal and it is analysis. So, in spectroscopy the electromagnetic radiation say light, interacts with the metal in different ways, as we will discuss and this particular property of the metal to interact with the light, and then formation is derived is used for the analysis and characterization of small and large molecules. The interaction of electromagnetic radiation with metal or as we say spectroscopy is a quantum phenomenon or we can say observation of quantum mechanics.

And it depends on both the properties of the radiation and the structural composition of materials, which are involved in here interacting with the electromagnetic radiation. Now, different types of spectra obtained or the information, which is derived due to the interaction of the radiation with a metal, will lead to the understanding of these interactions and development of spectroscopic techniques, which will help in solving many biological problems, where one can characterize these molecules and know about them and likewise solve many biological problems.

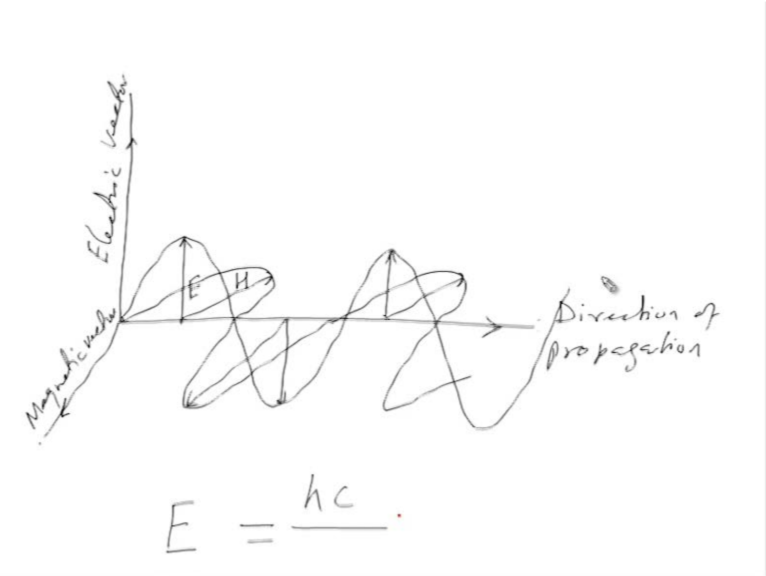
(Refer Slide Time: 02:32)



Little bit into electromagnetic radiation, in very simple terms, electromagnetic radiation can be defined as a form of energy, whose origin is the emission and absorption from charged particle, it is like coming out of the metal. So, in terms of classic physics, if we say electromagnetic radiation is mainly produced, when charged particles are accelerated by forces acting on them.

So, it comprises of both, the electrical as well as magnetic components, which are oscillating in directions perpendicular to each other, at a fixed intensity ratio and mutually, they are at right angles to the direction of propagation. The energy of wave or electromagnetic wave is given by Planck’s constant into the velocity of the light divided by the wave length that is or you can say h nu.

(Refer Slide Time: 03:48)

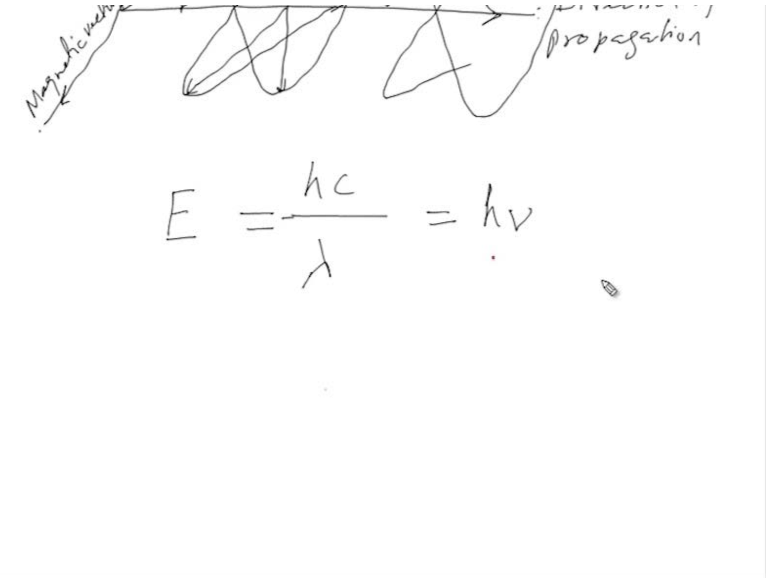


And let me show, you on this screen the typical electromagnetic radiation in a wave form, so like I said, it has 2 components, electromagnetic wave has 2 components, electrical and magnetic component. So, if I can draw very specific, so there will be 2 components, one is electrical electric, you can say electric vector and another is magnetic vector and if you consider a wave here. So, this is a magnetic vector at 90 degrees electric vector and this is the propagation of the light actually that is direction of propagation.

So, as we depict the wave nature of the light here, what we will have is if I just draw, supposing this is your one vector or one direction and if I say this direction as say electric vector here. Then there will be another direction, which will be perpendicular to this and which can be made in a different manner, likewise actually and this one here, this is you can say, you can give different term x or whatever and this is these are the 2 components of this wave, electric and magnetic components.

So, these are perpendicular to each other and then, to the direction of propagation here, so that is a typical electromagnetic wave, which has one component, which is perpendicular to 2 components, which are perpendicular to each other, one is electric component or electric vector another is magnetic component. And they are perpendicular to the direction of propagation, so that is a typical electromagnetic wave actually. Now, as we said, the energy of this wave, in terms of like energy of the, this wave could be expressed in term and in this equation here.

(Refer Slide Time: 06:25)



Where you have h is Planck's constant c is the velocity of light and lambda is the wavelength of the radiation. So, here it has particular like, this particular could be also like, it could be written in this way that is h nu, where nu is the frequency and frequency is expressed in terms of hertz actually that is one oscillation per second alright. So, this is typical representation of a electromagnetic wave here, let us come back, so what you have is your Planck's constant like, we have shown you h c upon lambda, c is the velocity of the light in this equation, which I have shown you and nu is the frequency of the radiation. If the frequency of the radiation is the is in reciprocal seconds and it is expressed in terms of hertz, which is one oscillation, per second is called one hertz.

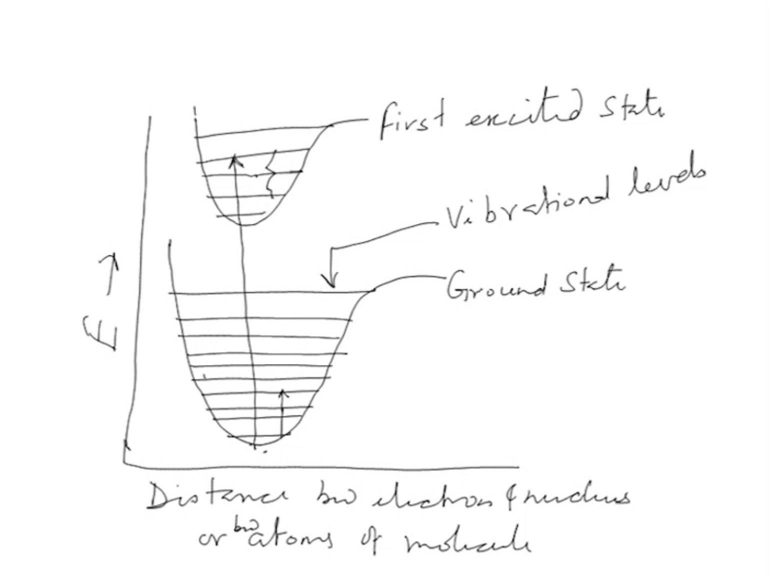
Now, electromagnetic wave or you can say light, can interact with metal, where it can be either absorbed or emitted or scattered. So, a molecule or atoms, they will either absorb an electromagnetic radiation or they might emit, as they go into a higher state and when come back to the ground state, they can emit a particular radiation or it is could be that electromagnetic radiations can be scattered also by the metal.

So, the atoms and molecules mostly exists only in discrete energy levels, what does that means, it means that electrons in atoms or a molecules may be distributed or they reside in ground state or which say lower level of energy. And they can be excited to a higher level or we call it excited state, where if exact amount of energy, which is equivalent to the difference in energy levels or excited electrons in higher state.

So, if it is a absorption, then absorption will occur only if, there is a equivalent amount that is the difference between the 2 energy states is absorbed and then, electron can be go to higher or excited state. Likewise, when an excited electron might come back to a lower level where again it will whichever level it comes, it will emit the equivalent amount of energy that is difference in energy between the levels.

So, light could be absorbed or as well as emitted by a molecule by undergoing transition and this particular phase, where a particular phenomenon, where the electron like undergoes transition from one transition state to another is known as, what you call transition here. And this transition is between discrete energy levels and these energy levels and other characteristics are predicted by quantum mechanics, let me show you on your screen these, what are these energy levels mean here alright. So, when we say energy levels like, I said these atoms have a discrete energy levels and they reside in these discrete energy levels and there is could be a ground state and higher excited states.

(Refer Slide Time: 10:38)



So, when we say these states, let us little bit understand them, if I, if we can just understand this particular one alright, now if we see these 2 here and this is ground state and this is the first excited state. Now, here in this one, so this is electronic state, you can say and this going from this particular one is electronic energy level, another one is in excited state that is the first excited state of that an electrode level. Now, within these electronic energy levels, there are subdivisions, which are known as vibrational levels actually.

So, these are called vibrational levels and these vibrational levels, will exist in the excited states also, here we have shown you the first excited state and they will be in here also. Now, when absorption or emission whatever occurs, say for example, absorption occurs, say from the first or from very ground level, to the higher, if I say it is going up to here. Then absorption has occurred the exact energy has been absorbed, which leads to the electron from this state to the say 4th vibrational state of first excited a state in electronic shell actually.

So, these are electronic shells and these are vibrational further division makes them into or divides them into vibrational levels and further vibrational levels could be divided into rotational levels, which are not shown in here. And this distance is this is distance between, electrons and nucleus or we can say atoms or we can say between atoms of molecule, of a molecule or between atoms in a particular molecule.

So, what you can see here is this is very clearly shown that, electronic the transition occurs from one electronic state to another, this transition could be between the vibrational levels of the 2 electronic states that is ground state. And first excited state and rotational levels are not shown, which could be further division of vibration levels actually. So, that is how likewise, if say this is transition from one level to another, but there could be like transition in within the electronic level between say vibrational levels, if I say here.

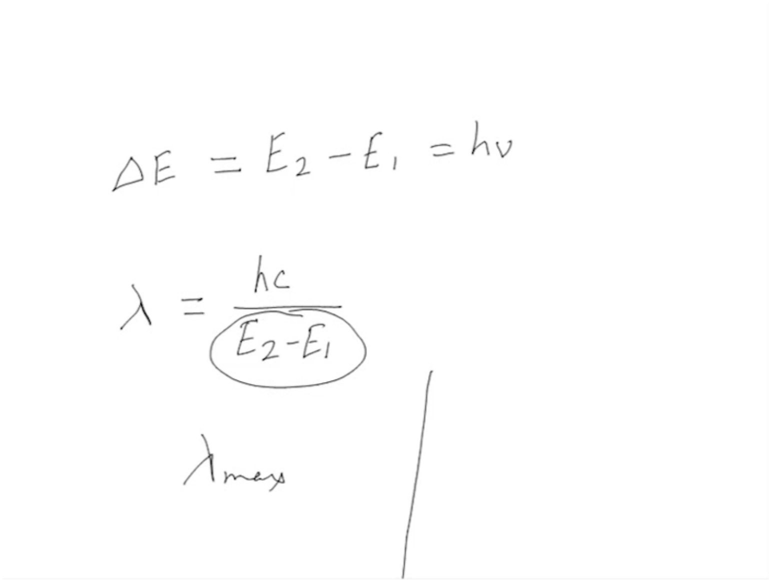
This is like changing vibrational level or in this particular excited state, it could be like this could be returning into different vibrational levels. So, this is how a transition takes place between different energy levels actually. So, I think, it should be very clear that how an electromagnetic radiation interacts with metal by a absorption or emission and electrons in atoms or molecules, which resides in the ground state or a lower level after absorption of the equivalent energy that is the energy difference in the 2 levels, can get a excited to a higher level.

So, and the reverse will happen emission actually, so a molecule that can be excited by absorption is known as chromophore actually, the excitation energy is usually converted to heat say by collision of excited molecule with another molecule or reemitted as fluorescents. In both the cases intensity of light transmitted by chromophore is less than, the intensity of the incident light. Now, will be discussing about a fluorescence spectroscopy, in later lectures, now the major energy level, as we have were discussing depends on spatial distribution of electrons.

And these are called electronic energy levels, so what I have shown you, these word electronic energy levels and I have shown you, the ground state and first excited state. Then there are vibrational levels within electronic levels or you can say in each electronic states, indicating various modes of vibration of this of the molecule, now these vibration this gives information about stretching and bending of various covalent bonds within the same electronic state. And then, there could be further smaller subdivisions, which were not shown in that diagram, these are called rational levels and they are not.

So, much used in biology in an energy level diagram, lowest electronic level is ground state and others are excited state and as we have shown the first excited, state in here. Now, absorption energy, absorption of energy can happen, only if the amount absorbed corresponds exactly to the difference between energy levels.

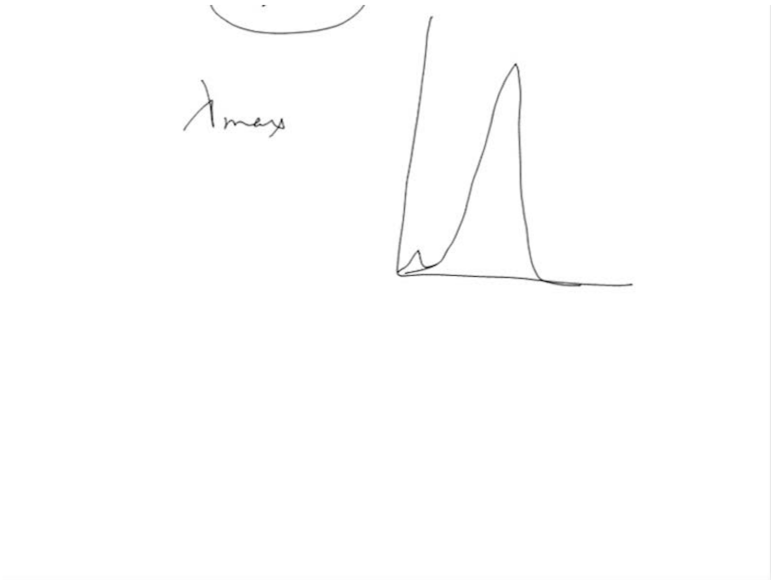
(Refer Slide Time: 17:23)



So, light of a particular wavelength, can only be absorbed only when there is a like, so if you consider this alright, so amount could be absorbed only, when like in terms of like say energy change, for electronic transition, we can say. But, you should have is that, change the difference in energy that is between the 2 states has to be equal to, so the energy absorbed should be equal to the difference between the 2 energy states that is the first ground state and then excited state.

And also between the vibrational levels, to which vibrational level that, particular electron is excited or in terms of like we can say, that if particular, if tells for wavelength of electromagnetic radiation then, we can say that it should be that, if it fulfills this particular one that is again this component here. Then, the wavelength the energy is will depend on the wavelength of electromagnetic radiation as well. And the wavelength, at which the maximum absorption takes place is called lambda max, lambda max means, the wavelength, at which a particular material or molecule absorbs the maximum in terms like.

(Refer Slide Time: 18:48)

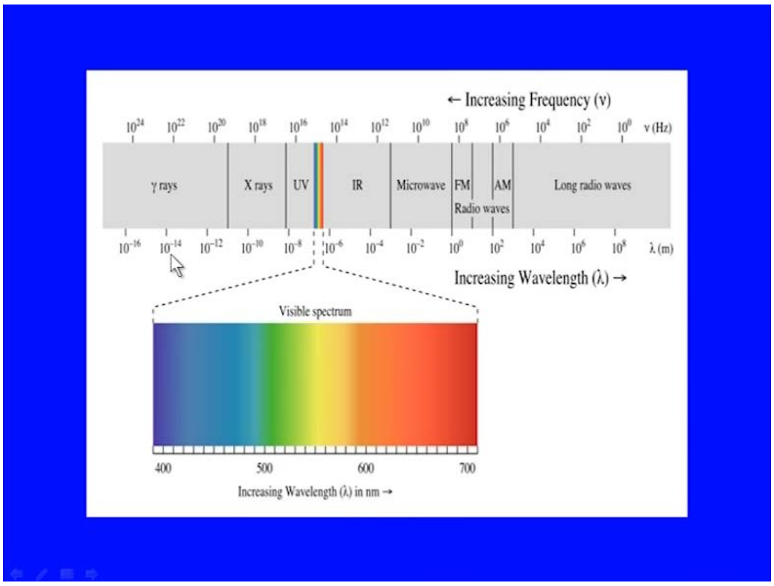


If I say in terms of absorption plot, if there is an absorption, which is large absorption, there could be minor absorption around here, but if there is a particular wave length gives maximum absorption, then this wavelength will be called lambda max. So, a plot of the absorption versus wavelength is called an absorption spectrum and this absorption spectrum comes from, the absorption of particular wavelengths actually, which is what, which is the information that we obtained from absorption spectrum.

Likewise, if it is a emission spectrum then, the spectrum or it will be called emission spectrum and there, we will be looking for the emitted wavelength, emitted radiations, rather than, absorbed radiations. So, these are 2, complimentary techniques, spectroscopic techniques, now E M radiation or electromagnetic radiations, they are classified by the frequency of the radiation or you can say in reverse, it is a wavelength, but on the basis of frequency, if we say.

And they will be categorized into different types of electromagnetic radiations, these could be radio waves microwaves, infrared radiation, visible light, ultraviolet radiation x-rays and gamma rays and the total radiations including all these frequencies form the electromagnetic spectrum. Now, different radiations of the electromagnetic spectrum has been utilized in the biological science, for variety of purposes, now if you can see on your screen.

(Refer Slide Time: 20:37)



This is the particular diagram to depict various kinds of electromagnetic radiations and as per their frequency and that is increasing frequency or increasing wavelength, as the frequency increases, wavelength decreases and vice versa. So, if you can see in here, there are gamma rays, at very high frequency then, x-rays and this frequency here is depicted in terms of hertz actually and wavelength here is depicted in terms of meters. So, if you see gamma rays with very high frequency then, x-rays then next in electromagnetic spectrum is u v that is ultraviolet rays.

Then after ultraviolet rays, these are visible spectrum that is what we can see or the colors, which we can perceive then, comes IR region infrared region in the spectrum, microwave then, radio waves and then long radio waves which are present in here. Now, if we consider like I said there are different radiations of the electromagnetic spectrum have been utilized in the biological science, for a verity of purpose. If I compare in here this one, if you can, if little bit, we can go into that then, u v and visible region.

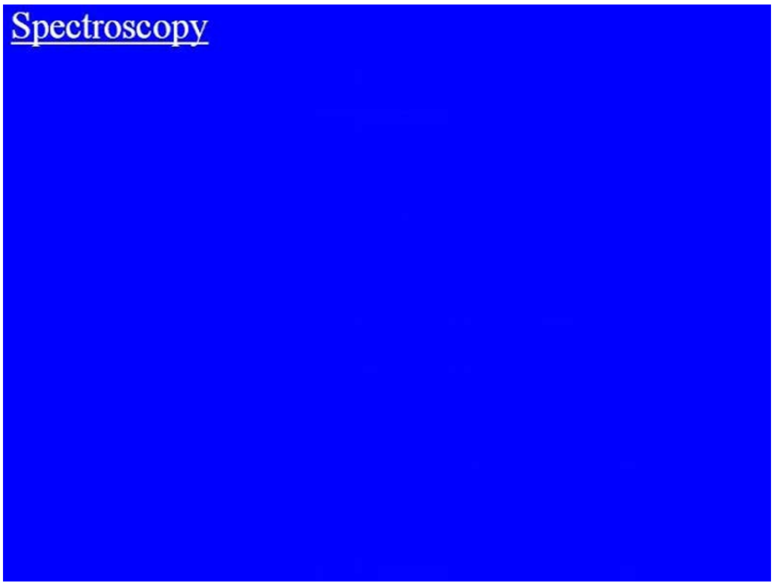
These are utilized and little bit of IR region, these are utilized, for as a standard, what you call this particular region from u v, till little bit of IR is for a standard absorption spectroscopy. And here, in absorption spectroscopy mainly inner shell at the lower electrons and outer shell electrons are considered here, for standard absorption spectroscopy. Then second is in IR region, you have like, we have told you about, vibrational and rotational thing.

Now in the IR region, there is a vibrational part is taken and this is utilized, for infrared in vibrational region, it is utilized for infrared and raman spectroscopy. So, here in higher regions, there will be nuclear magnetic resonance spectroscopy, which will be utilized. So, these are like different like, another important thing is where, we are utilizing in u v visible, where absorption spectroscopy is being used, this is in electronic level actually, where inner shell and outer shell electrons are taken.

Infrared spectroscopy is performed, as per vibrational shell here or vibrational level, these are transition, I am talking about here, it is a electronic transition, these are vibrational transition and as you go for say, nuclear magnetic resonance, it is a nuclear transition, we can say. So, these are different parts of the spectrum or electromagnetic, different types of electromagnetic radiations and the types of transitions, we were talking about and to how to, what kind of techniques have been developed on that bases.

So, in summary we can say that, u v based and even particular x-rays are taken as standard absorption spectroscopy with electronic transition, from x-rays till u v based little bit of infrared. Then, infrared is mostly vibrational transition and it is utilized for raman spectroscopy and infrared spectroscopy and then finally, you have nuclear magnetic resonance, which is in say microwave region here, which is based on nuclear, this resonance actually. So, these are different types of transitions, which we were which have been utilized for obtaining information regarding the metal that is interaction of the metal, with the electromagnetic radiation.

(Refer Slide Time: 25:10)



At so as we have seen that is spectroscopy is the study of interaction of energy with the metal and it is analysis and this has originated, this is spectroscopy originated with the study of visible light dispersed by the prism according to it is wavelength. So, like as we know that, visible light is a combination of different wavelengths and when they pass through the prism, they are dispersed actually, according to their wavelengths, as we have talked about this in microscopy also.

So, in daily life, also the colors of things can be taken, as an example of spectroscopy, because objects absorb light of a particular frequency and emit a particular frequency. And failing in the visible, which are falling in the visible range and giving it a particular characteristic color actually, so that, we can see it. So, this is like in daily life we are observing different colors, because of like absorption and emission of a particular frequency wavelengths.

Now, the principle concept of the spectroscopy is resonance and the corresponding resonant frequency that is, it is resonating between the 2 different energy states. So, there is a major or principle concept is resonance and resonant frequencies, in quantum mechanical terms resonance can be defined as the energy equivalence between 2 stationery states of a system, which is provided by the external energy source.

Now, spectroscopy is a very wide field here and where multiple subdivisions exist and it is and it is classified in a verity of ways in like, nature of radiation, types of interaction and types of materials being studied. Traditionally, spectroscopy has been restricted to where, particular absorption or emission takes place that is a particular molecule or atoms absorbed, a particular wavelength or electromagnetic radiation of a particular energy.

And gets excited that information or where, emission takes place, because of emission of a particular radiation and those information are taken and that is the our characteristic or you can say traditional spectroscopy. And many techniques, which might not have fallen in spectroscopy earlier, have been regarded or considered in terms of the spectroscopy, like for example, scattering actually, where or interaction in terms of not absorption or emission.

But, say in terms scattering of light that could be one form, where that could also be called as a particular kind of spectroscopy, as we will discuss, as we go along here. So, it could be divided like a spectroscopy has become a very wide field and lot of multiple subdivisions. And it could be divided into various ways for example, what are the nature of radiations are being used, what are the types of interactions, which are happening and what are the types of materials, which are being studied.

Let us take each one of them separately here, in terms of nature of radiative energy or radiations, which will include radiations belongs to different parts of the electromagnetic spectrum. Particles it could be like electromagnetic spectrum, which as we have seen from right from x-rays till microwaves and then, ultraviolet and visible rays infrared part of the spectrum all these are utilized, where interactions with metal takes place.

Also other than, electromagnetic spectrum, there could be particles like electrons or neutrons, which could be taken like there is electrons when they, pass through metal, they could be scattered or there could be other interactions likewise, neutrons can interact in various ways. And at times could be also say waves like, say pressure waves or others, used as energy source for a spectroscopic study.

So, it is not a typical only electromagnetic spectrum or electromagnetic radiations, which are interacting, it could be in the form of particles like, electrons and neutrons as well, where scattering or other interactions might occur will also be covered under spectroscopy. Then, there are types of interactions actually, now there could be different types of interactions that will form the bases of spectroscopic technique, actually and as we were, talking the traditionally absorption and emission were, the main constituent or main types of interactions, which were considered or covered under the spectroscopy.

But, there could be lot of other interactions like, scattering, which could be elastic scattering and reflection or we call it as diffraction, in many comes like a x-rays and electron scattering and crystallography. Like x-ray crystallography, where electrons like, x-rays are diffracted by the molecule, which are arranged in a crystal in a particular regular array. So, that could be elastic scattering could be one form of interaction, which is also comes under spectroscopy then, they could be inelastic scattering, where it could be like, it might involve change of energy between the radiation.

And the metal and that will shift the wavelength of this cathode radiation and it will include like, raman and compton scattering. So, there could be both, elastic scattering like in x-ray crystallography or x-rays in electronics scattering in crystallography or it could be inelastic scattering, where change in or shifting of the wavelength of the scattered radiation could occur and that includes raman and compton scattering.

There could also be coherent or resonance spectroscopy in coherent or resonance spectroscopy like, radiative energy, couples the 2 quantum states of the material in coherent interaction. And that is sustained by the radiating field one example is N M R spectroscopy is widely used method in this typical example of resonance method. So, there could be different types of interactions right from absorption emission, which are traditionally considered and then, could be scattering that is elastic scattering or inelastic scattering and then, there could be a resonance spectroscopy or interactions as well.

Apart from types of radiation or types of interaction also one other thing should be considered in here, when we are talking about a spectroscopy that is types of materials, which are involved in here. So, this spectroscopic techniques are designed to interact with specific kinds of materials, which could be like, it could be atoms only, in terms of atomic absorption and atomic emission spectroscopy, like we will be talking about atomic absorption and emission spectroscopy, where absorption and emissions often referred to as atomic spectral lines.

And these atomic spectral lines are due to the an electronic transition of an outer shell electron to an excited state and atoms of different elements have distinct spectra or we can say, spectral lines. On the bases of these spectral lines, there it could be spectral lines, in terms of absorption of particular lines or it could be in terms of emission lines emission spectra, where a particular wave length have been emitted actually.

Both ways these have these are characteristics of a particular atom and these are very helpful in recognizing or identifying the particular types of elements or atoms, in these sort of spectroscopy. So, one is interaction could be with like materials like atoms, another could be interaction with the molecules, molecular spectra can be obtained, due to electron spins states, like where could be, one is electron spin states could be there. Another could be molecular rotations actually, which could also utilized for deriving information then, molecular vibrations, which occur all the time and electronic states.

So, spin states could also be utilized and electronic state, which we have discussed earlier could be utilized. Then, there are vibrations are which are also happening and these are relative motions of the atomic nuclei and are studied by both infrared and Raman spectroscopy. Electronic excitations as we have discussed are studied using visible, ultraviolet and fluorescence spectroscopy, whereas for example, there could be, so we have discussed about atoms molecules.

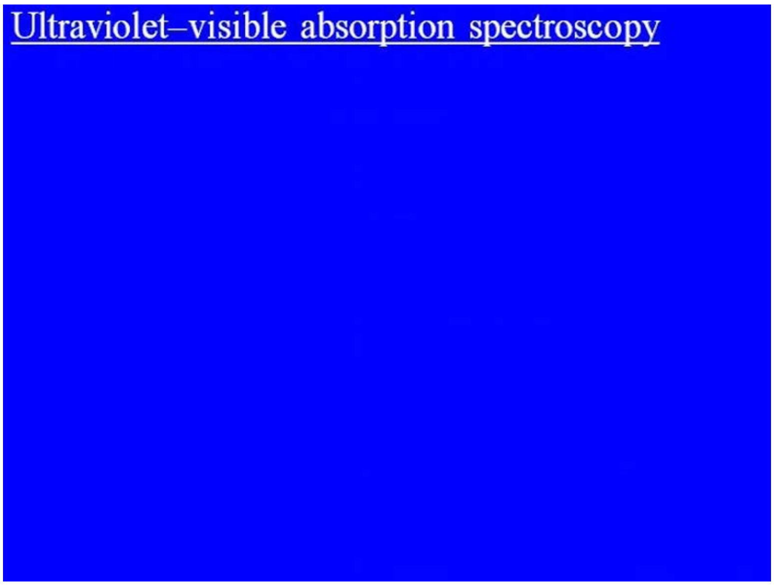
There could be crystals as well actually like, I said these are regular arrangement or regular array of atoms and the regular arrangement of atoms in a particular array in crystals. And these regular lattice structure of crystals will scatter x-rays electrons on neutrons and these are utilized for crystallographic studies, where the structure, 3 dimensional structure of molecules, could be obtained by generating the electron density maps.

So, this is like particular way that is scattering method, could be utilized for crystals for deriving information that is a structural and chemical compositions, then nuclei or metal could be interacting like, nuclei also have a distinct energy states and distinct nuclear spin states. And these can have their energy separated by a magnetic field and which, forms the bases for N M R spectroscopy. So, in n m r spectroscopy, it is the nuclear resonance which is utilized or magnetic nuclear magnetic resonance, which is utilized for deriving information.

So, these are different ways like, you can say a different types of spectroscopic techniques, which are on the bases, on which these spectroscopic technique could be put in and different ways like of interaction or types of radiation or different types of matters are discussed in here. So, alright this was the basics, certain basic concepts in terms of electromagnetic radiation, in terms of a types of interactions, which are happening and how this information could be derived.

What are the different spectroscopic techniques, which have been developed like on the bases or electronic transition or on the bases of say vibrational transitions or say nuclear transitions. The techniques like absorption techniques or say infrared technique, spectroscopy or say nuclear magnetic resonance spectroscopy have been developed. Also other techniques, which will be included in here like, x-ray diffraction or x-ray scattering and mass spectrometry, which is not typical spectroscopy technique, as we have explained. In traditional it is not typical spectroscopy technique, but very useful very powerful technique and is important, so we have included it in here.

(Refer Slide Time: 38:40)

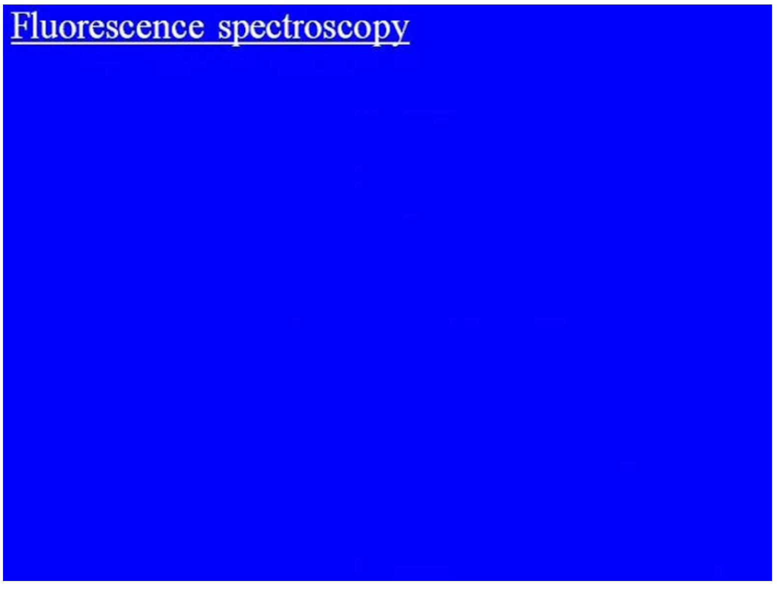


So, the techniques, which are little bit into the techniques, which we are going to discuss in this section here, one is ultraviolet visible absorption spectroscopy, which we are going to discuss here. In here, when electromagnetic radiation passes, through a transparent material, a portion of radiation may be absorbed and as a result of energy absorption atoms or molecules will pass from a state of low energy that is ground state to state of higher energy that is excited state.

And ultraviolet visible spectroscopy is based on the transitions of electrons from one atomic orbital or molecular orbital to another and due to the absorption of the electromagnetic radiations. So, we are going to discuss in detail about, this technique, in coming lecture, then another based on vibration transition is infrared spectroscopy, which deals with the infrared region of the electromagnetic spectrum, having higher wavelength and lower frequency then visible light.

It consist of 3 regions that is near 0.8 to 2.5 micrometer midpoint 2.5 to 25 micrometer and far infrared that is 25 to 1000 micrometer and based on the facts that are molecules absorb specific frequencies in this range that are characteristic of the structure and absorb frequency resonance resonates within the frequency of the vibration of the group. So, IR active molecule require the change in the dipole moment the IR spectrum is recorded by passing a beam of infrared light, through the sample and when, the frequency of the infrared beam is equal to the vibrational frequency of the bond IR absorption occurs.

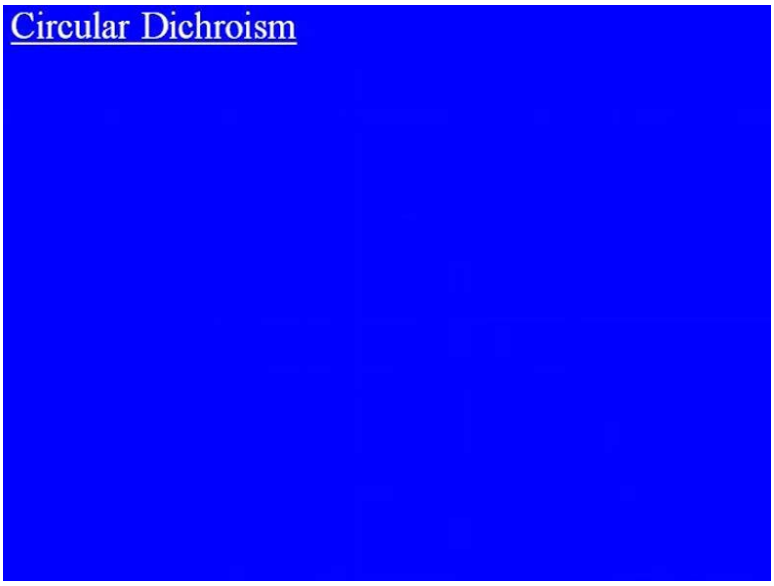
(Refer Slide Time: 40:33)



Then, next technique, we are going to discuss about, it fluorescence spectroscopy, fluorescence spectroscopy is a type of spectroscopy, where fluorescence from a sample is analyzed. The phenomenon involves the absorption of light usually in ultraviolet region, that will excite the electrons in molecules of the certain compound and will cause them to emit light at a longer wavelength, when they come back to the ground state, usually in visible region.

So, that we are going to discuss in coming lectures, then also we going to discuss, we have included certain other techniques, like which are typically, spectroscopy techniques of course.

(Refer Slide Time: 41:17)



Like circular dichroism, which are very useful in where is biophysics applications, circular dichroism here the electromagnetic radiation consist of an electric and magnetic field that oscillates perpendicular to each other and to the direction of the propagation. So, while linearly polarize light occurs, when the electric field vector oscillates, only in one plane and circularly polarized light occurs, when the direction of the electric field vector rotate about, it is propagation direction, while the vector retain the constant magnitude.

At single point in space the circularly polarized, vector will trace out circular over the one period of the wave frequency, hence the name has been given. So, the circular dichroism refers to the differential absorption of the left and right circularly polarized light. The phenomenon of circular dichroism is exhibited by kairali molecules and hence biological molecules are excepted to exhibit the same, due their dextrorotatory levorotatory components.

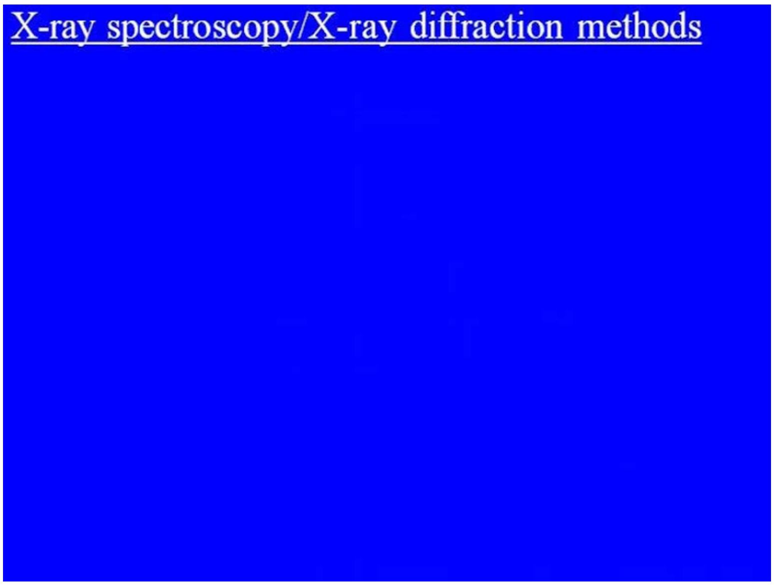
More over a distinct secondary structure in part the characteristics c d spectrum and like alpha helices and double helices in d n a have characteristics c d signatures, the far u v c d spectrum of proteins can reveal important characteristics of their secondary structure.

And c d spectrum can be rear used to estimates the fraction of molecule that is the in alpha helix confirmation. So, the beta sheet confirmation the and also some other, like say random coil confirmation could be obtained, these fractional assignments place important constraints on the possible secondary confirmation that protein can be in.

Then, we are going to discuss about nuclear magnetic resonance spectroscopy, which explode the magnetic properties of certain atomic nuclei to determine physical and chemical properties of the atoms or molecules, in which they are present. The method is based on the nuclear magnetic resonance and provides a detailed information about, the chemical environment reaction state and structure of the molecule. Basically, when sample contains n m r active nuclei for example, 1 h or 30 c or 19 f or 31 p, these have fractionally spin of half.

And when, they have placed in magnetic field, they exist in 2 spin states pulse half, which is aligned magnetic field and minus half, which opposes the magnetic field and difference in energy between the 2 spin states will depend on the external field strength. When these are radiated sample is radiated with the radio frequency energy, which corresponds to the except spin state separation of the specific sort of nuclei that will cause excitation of nuclei, from plus half state, spin state 2 higher state, and resonant frequency absorption and the energy absorption of the energy and intensity of the signal proportional to the magnetic field. And there could be lot of different kinds of n m r technique, which could be one dimensional technique, for smaller compounds to 3 or 4 dimensions techniques for large molecules, like protein or nucleic acids.

(Refer Slide Time: 44:57)

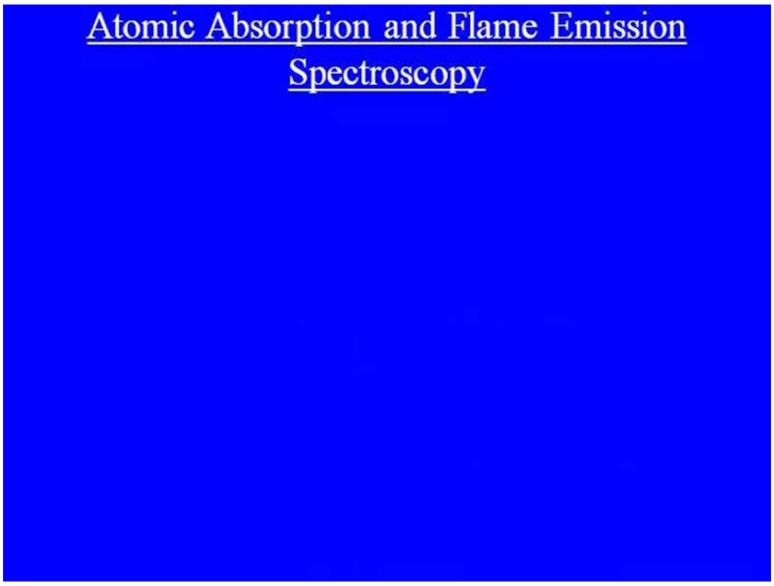


Another technique, we are going to talk about is x-ray spectroscopy and will be more talking about x-ray scattering or x-ray diffraction methods, now x-ray spectroscopy refers to the spectroscopic technique, for characterization of different material using, x-ray excitation. Now, when an electron from inner shell of atom is lost, due to some sort of excitation, then vacancies field with an electron from the outer shell, and the difference in energy emitted as x-ray of appropriate wavelength, characteristic for the element.

So, the analysis of spectrum of x-rays provides a qualitative information about, chemical compositions and comparison of spectrum with spectra of standards of known compositions provides quantitative results. Now, x-rays can be absorbed biometry also, x-ray absorption spectra then, there could be x-ray fluorescence analysis also, using x-ray fluorescence.

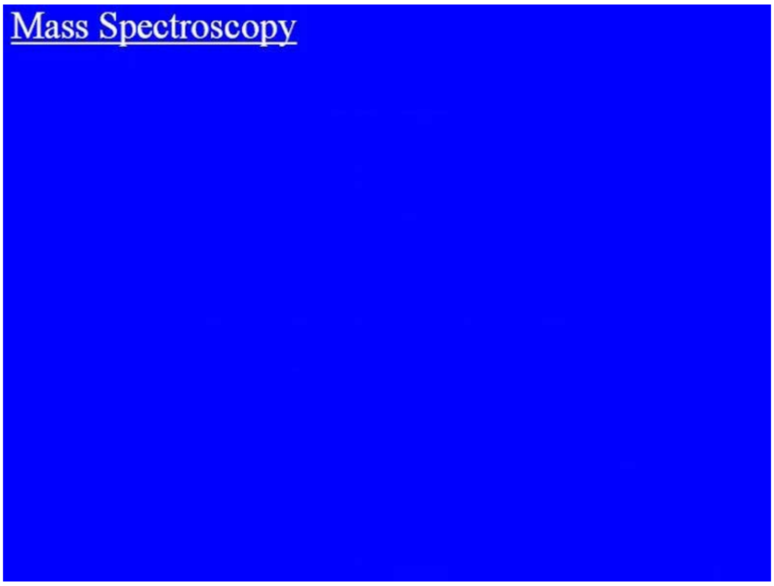
Apart from this, there are x-rays scattering diffraction technique, which reveal information about, the crystallites structure or structure of the molecule and these techniques are based on scattering or we can say diffraction of x-ray beam by crystals at different angles and intensities. And from these angles and intensities of the diffracted beam 3 dimensional distribution of density of electron within crystals is obtained and therefore, the mean positions of atoms in the crystal and the structural information can be derived.

(Refer Slide Time: 46:36)



So, dealing about, this in later chapters then, also we are going to discuss about atomic absorption and flame emission spectroscopy atomic absorption and flame emission spectroscopy methods are very powerful tools, for both qualitative and quantitative analysis. In atomic absorption technique absorption of radiant energy by free atoms in gas state occurs and that is the bases for obtaining the information, where as in emission of flame emission technique, there is intensity of the light of a flame or a plasma, at particular wavelength is emitted and this gives information. Wavelength of atomic spectral lines helps in identification of elements by the intensity is proportional to the number of atom present.

(Refer Slide Time: 47:25)



Finally, we are going to discuss about, mass spectroscopy or mass spectrometry to be a to say mass spectrometry is an analytical tool used for measuring, the mass to charge ratio of the sample that are charged. The instrument that do for same are called, which does, it which calls mass spectrometers and which has ionizer analyzers and detectors here the sample as to be introduced into the ionization source of the instrument.

Once in the side the ionization source the sample molecules are ionized and because ions are easier to manipulate the neutral molecules, these ions are extract into the analyzed region of the mass spectrometer. Where are there separated according to the mass to charge ratio and the separated ions are detected and thus signals send to the data system, where the these ratio and the z are ratio are stored, together with relative abidance for presentation in the format of mass to charge ratio spectrum.

The analyzer and detector of the mass spectrometer and often the ionization source to are maintain under high vacuum to give the ions are reasonable chance from 1 ion to another of the instrument to the other, without endurance from air molecules. Entire operation of the mass spectrometer and often the sample introduction process also is enter complete data system controlled on modern mass spectrometers.

And this technique is utilized for various things like are determining masses of particles for determining the alignmental compositions, for illustrated in the chemical structure and etcetera, and this also we are going to discuss in coming chapters or coming lectures.

So, in this lecture to summarized, we discussed about, basic concepts in terms of electrometric radiation and spectroscopy, which is the interaction of the metal with electromagnetic radiation.

We have discussed about different types of spectroscopy and the basis for, those spectroscopic techniques like, electronic transitions or vibrational or say nuclear resonance and the lot of differences spectroscopic techniques have been developed. The area of the spectroscopy also be widen to include more spectroscopic techniques, like on the basis of scattering and other phenomenons. So, in the will be starting with specific techniques, in the next lecture and in the next lecture, we are going to discuss about, the first technique that is u v visible spectroscopy.

Thank you.