Advance Analytical Course

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Lecture No. # 27

Near infrared spectroscopy – near infrared spectroscopy, which we will refer as NIRS is a spectroscopic method utilizing the near infrared region of the electromagnetic spectrum from about 800 nanometers to 2500 nanometers.

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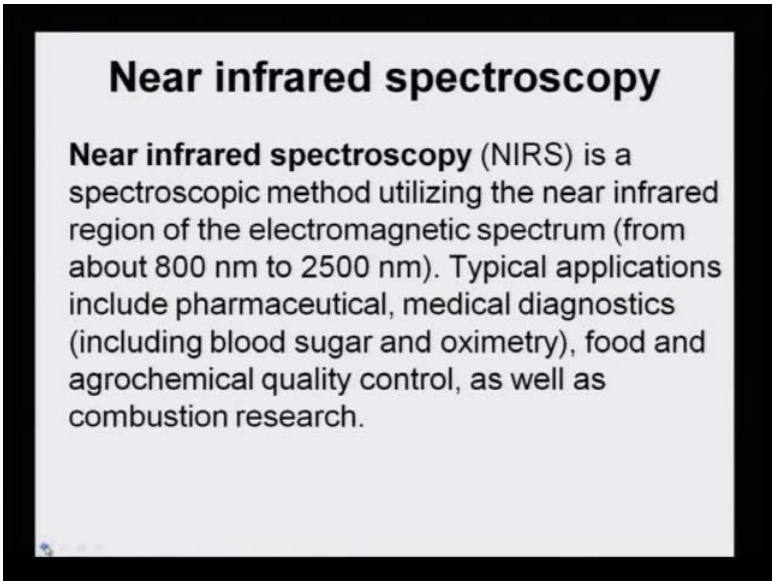

Typical applications include pharmaceutical, medical diagnostics including blood sugar and oximetry, food and agrochemical quality control, as well as combustion research. So, you see, it has a versatile area in which it can be used. And, the information imparted by the near infrared spectroscopy can be so important that it can serve in the areas of pharmaceutical, medical diagnostics including blood sugar, in the area of food and food chemistry, in the area of agrochemical quality control, and in the area of combustion research.

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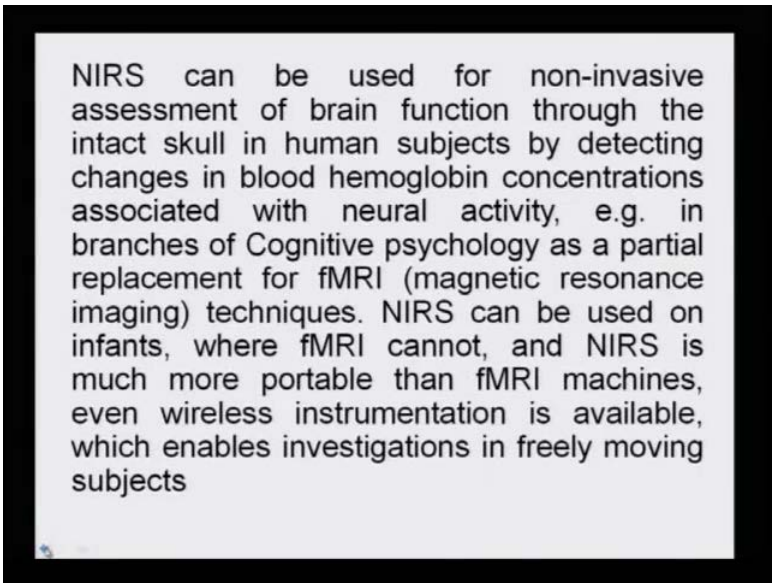

NIR can be used for non-invasive assessment of brain function through the intact skull in human subjects by detecting changes in blood hemoglobin concentrations associated with neural activity, that is, in branches of Cognitive psychology as a partial replacement of fMRI (magnetic resonance imaging) techniques. NIRS can be used on infants, where fMRI cannot, and NIRS is much more portable than the fMRI machines, even wireless instrumentation is available, which enables investigations in freely moving subjects. So, you see that it is even superior in certain cases, where the magnetic resonance imaging, that is, MRI cannot be done or is not very fissile to be done.

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Near infrared spectroscopy has a very short history. Over the last few years only, it has come into existence and so much of use. Near-infrared (NIR) spectroscopy has rapidly developed into an important and extremely useful method of analysis. In fact, for certain research areas and applications ranging from material science via chemistry to life sciences, it has become an indispensable tool, because this fast and cost-effective type of spectroscopy provides qualitative and quantitative information not available by other techniques. So, you see that it is ranging from material science to chemistry to life sciences, and it is one of the most effective and fast methods of spectroscopic technique. And, many a times, no other tool or technique can actually match with it; it is so indispensible.

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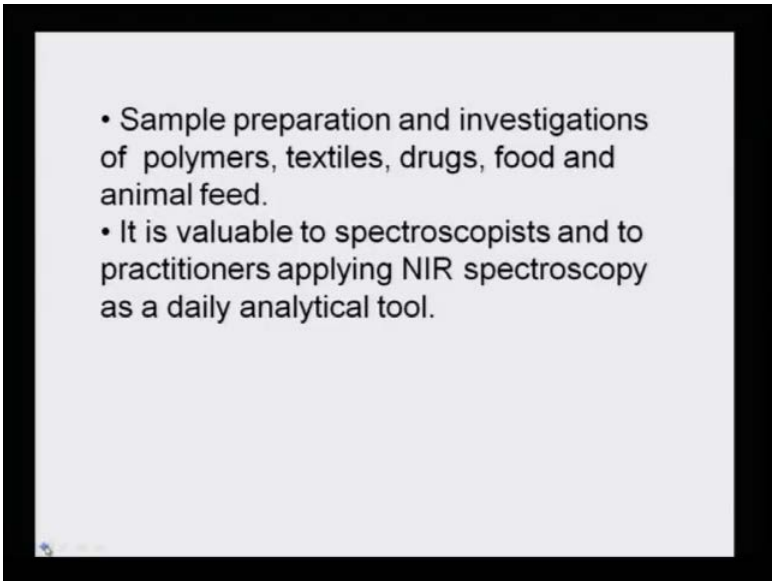

Sample preparation, investigation of polymer, textile, drug, food and animal feed is also very fissile. It is valuable to spectroscopists and to practitioners applying NIR spectroscopy as a daily analytical tool. So, it has become so popular that every day we can use it. And, sample preparation for any type of sample, be it polymer sample, textile sample, drug sample, food sample, biological material, animal feed, name it – all can be analyzed with great effectivity and with great fastness.

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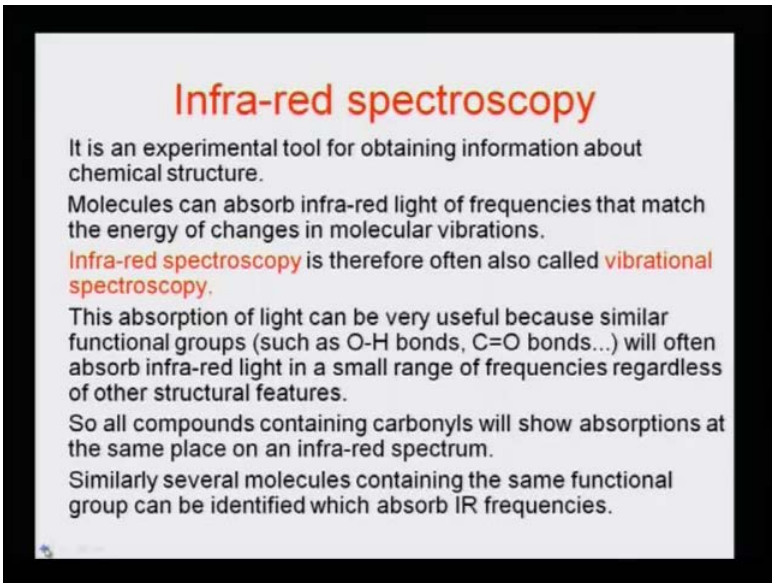

Coming to the main infrared spectroscopy, which is from the range of 4000 to 400 centimeter inverse – it is an experimental tool for obtaining information of chemical structure. As what I rightly pointed out, that it gives an idea about the functional groups presence; whether a particular functional group is present or not. And, that would come from the fact that these functional groups have some arrangement, which causes more vibration than a normal C-C or C-H bond.

Molecules can absorb infrared light of frequencies that match the energy of changes in molecular vibrations. Again, let me emphasize one point very clearly that infrared energy will not be absorbed if the molecule does not have an arrangement matching with that. If the matches are right, then absorption will take place; but, if the matches are diverse, the absorption of infrared light will not take place. As a result, it will not culminate in any kind of data. Infrared spectroscopy is therefore often called as vibrational spectroscopy.

This absorption of light can be very useful, because similar functional groups, such as O-H bonds C-O bonds will often absorb infrared light in a small range of frequencies regardless of other structural features. Let the molecule have any kind of big structure, huge molecular structures, but if it has these functionalities like OH group, NH group, carbonyl group, it is bound to absorb infrared light and excite, and give vibrational and rotational information, because of these absorption. So, all compounds containing carbonyls will show absorption at the same place on an infrared spectrum. Similarly,

several molecules containing the same functional group can be identified, which absorbs IR frequency. So, if several molecules have the same functional group, will show peaks at a particular region. And, that has come from the fact that it has absorbed the IR frequency and culminated in a spectrum.

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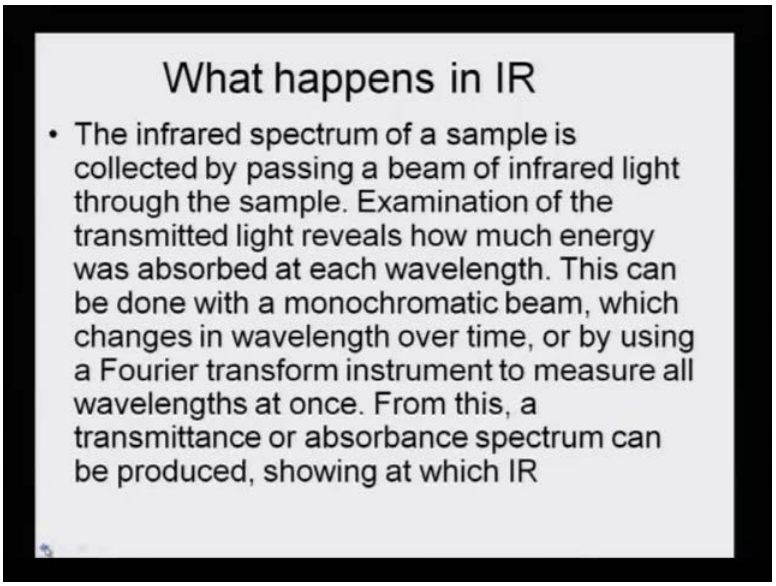

What happens in IR? To be able to understand it in a better manner that what kind of excitations are we looking at; what is the energy level of these infrared radiations; and, how do they excite the molecular bonds in the vibrational forms, in the rotational forms, the bond can bend; the bond can vibrate, can oscillate, can move like a pendulum, and that all happens, because the infrared light is being shown on that particular bond, or bonds of a molecule. The infrared spectrum of a sample is collected by passing a beam of infrared light through the sample. Examination of the transmitted light reveals how much energy was absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by passing a Fourier transform instrument to measure all wavelengths at once. For this, a transmittance or absorbance spectrum can be produced, showing at which IR wavelength, what excitation has taken place. So, you see, it is a very simple device; there is a source of IR and monochromatic beam; one-by-one, it is shown on the molecule. The wavelength, which matches with the bond structures, desirability will excite; otherwise, it will just go unnoticed, and that is what the crux of giving information about the molecular structure or the functional group is.

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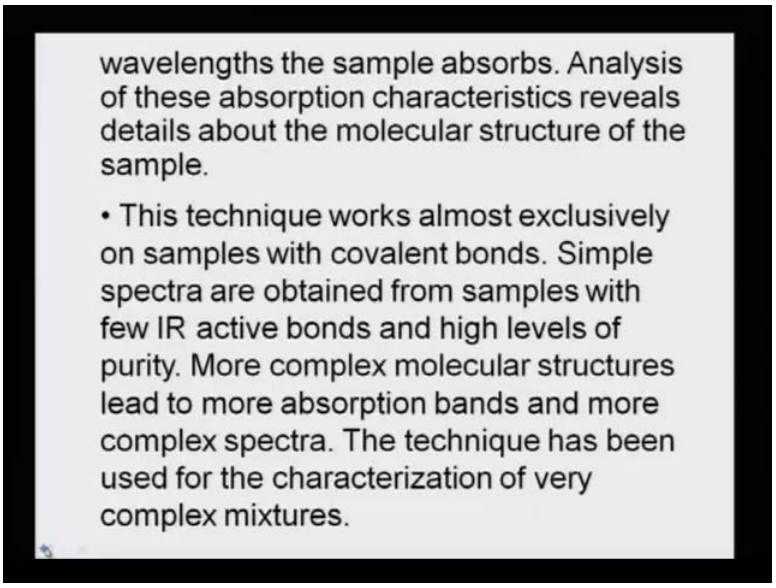

Wavelength of the sample that absorbs will be known. Analysis of these absorption characteristics reveals details about the molecular structure of the sample. This technique works almost exclusively on samples with covalent bonds. Similarly, simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra. The technique has been used for the characterization of even very complex mixtures. It does not matter what the entire molecular structure of a compound may be, but if it has certain functional groups, they will be revealed by the absorption of IR frequencies, and that will culminate in an IR spectrum. That is what I have told you.

Now, as what I explained in the previous slide, that either a monochromatic beam can be used slowly one-by-one at a time, or a Fourier transform, that means, all the wavelengths are flashed through the molecule and all the spectrum are over linked over each other. And, there are thousands of such spectrums that are recorded in a second, and when they are superimposed, and then, the spectrum that comes out is much clearer and much intense; the peaks are well-defined in the case of Fourier transform machines.

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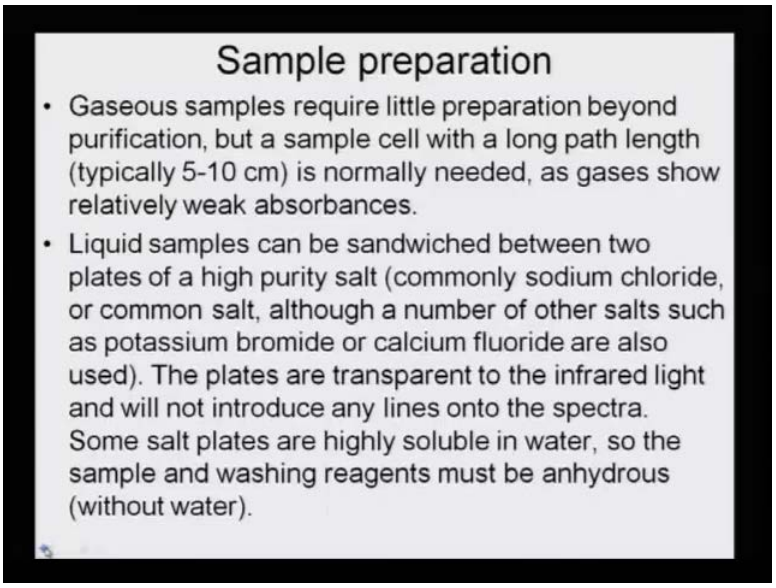

Sample preparation – now, obviously, as I have been talking, time and again from the very beginning, when we were doing chromatographic techniques, and now, we have reached spectroscopic techniques, I have been telling that sample preparation is the most crucial step. If the sample in not prepared correctly, it will never give an analytical data, which is correct. For gaseous samples, it requires little preparation beyond purification, but a sample cell with a long path length, typically 5 to 10 centimeter, is normally needed, as gases show relatively weak absorbance. It is a little tough to analyze gaseous samples in an IR machine. So, some adaptability has to be done; (( )) preparation of the gases as such. The gas should be pure and it should be allowed. Only the path length or the cell length should be higher. Normally, it is 1 centimeter for a solid sample or a liquid sample, but in this case, the sample path length is kept as 5 to 10 centimeters.

Liquid samples can be sandwiched between two plates of high purity salt, commonly, sodium chloride or common salt, although a number of other salts, such as potassium bromide, calcium fluoride are also used. The plates are transparent to the infrared light and will not introduce any lines onto the spectra. Some salt plates are highly soluble in water. So, the sample and washing reagents must be anhydrous without water. Now, what is done, there are two circular plates of sodium chloride; and, between these two plates, the liquid sample is sandwiched. It forms a thin film, and this thin film is then analyzed on the IR machine. But, instead of using sodium chloride pellets, one can use potassium bromide or calcium fluoride pellets also. But, it has to be kept in mind that

these pellets must be kept under dry condition, because they react with water or moisture. So, there should not be any water or moisture while cleaning these pellets for the next analysis.

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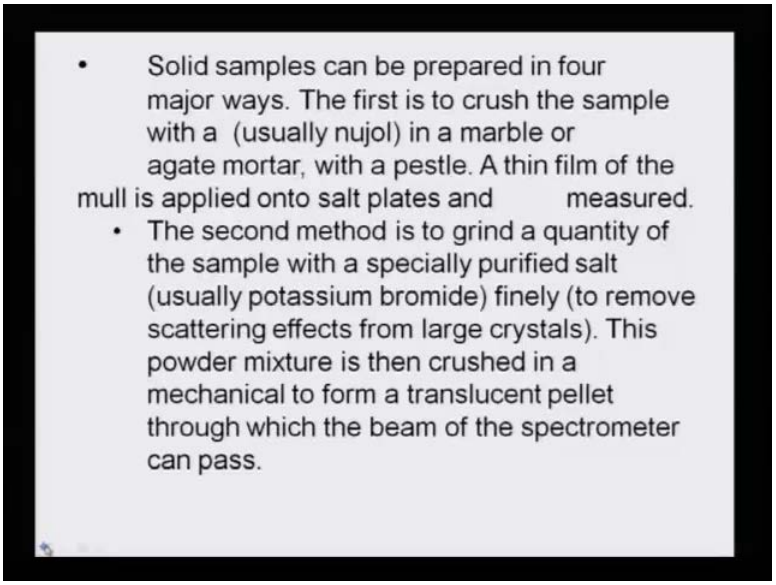

Solid samples can be prepared in four major ways. The first is to crush the sample with a usual nujol or a marble or agate mortar, with a pestle. A thin film of mull is applied onto salt plates and measured. The second method is to grind a quantity of sample with a specially purified salt, particularly, potassium bromide finely crushed to remove scattering effects of the large crystals. This powder mixture is then crushed in a mechanical or to form a translucent pellet through which the beam of spectrometer can pass. So, one method is that it is just crushed into the nujol gel, and it is just agitated with it and it is put in the cell. Otherwise, the second method of making the sample is by making potassium bromide pellet with the sample, and so, they are first crushed together, and then, put in a mechanical press. And then, a very translucent thin pellet should be formed, which is the sample and, because potassium bromide does not absorb by any infrared radiation. That is why it can be easily used with the sample.

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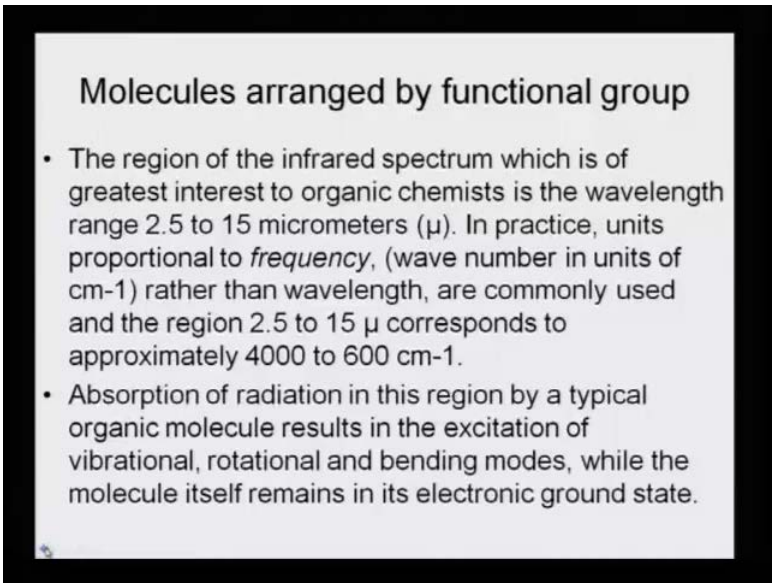

Molecules arranged by functional group – the region of infrared spectrum, which is of greatest interest to organic chemists is the wavelength ranged between 2.5 to 15 micrometers. In practice, units proportional to frequency, that is, wave number in units of centimeter inverse, rather than wavelength, are commonly used and the region 2.5 to 15 micron corresponds to approximately 4000 to 600 centimeter inverse. And, in some machines, it could range between 4000 to 400 centimeter inverse also. As we saw in some cases, we refer the unit, particularly in UV visible spectroscopy, we make use of wavelength; whereas, in the case of IR, we refer in terms of frequency. So, that is the only difference, but we have to refer to the light with some unit; whether it is a wavelength, where it is lambda max or expressed in nanometers; or, whether it is frequency, which is expressed in centimeter inverse, there has to be a unit associated. Any number without a unit has no meaning. So, when we are talking about infrared and its absorption frequencies, we talk in terms of either 2.5 to 15 micron, or we say it is in micrometers; or, we refer it as 4000 to 600 or 400 centimeter inverse.

Absorption of radiation in this region by a typical organic molecule results in the excitation of vibrational, rotational and bending modes, while the molecule itself remains in its electronic ground state. So, there is again a big difference. In the case of UV light, what was happening, the molecule as a whole was getting excited; the electrons were living their ground state and going to the excited state; whereas, in IR, what happens is that there is no molecular excitation of this large type; there are only small vibrations of

the bonds. So, the energy just sensitizes the bond to either vibrate or to rotate or to bend, and that is all it does. The work of an IR light and that itself, gives us some information about the presence or absence of a functional group in a molecule.

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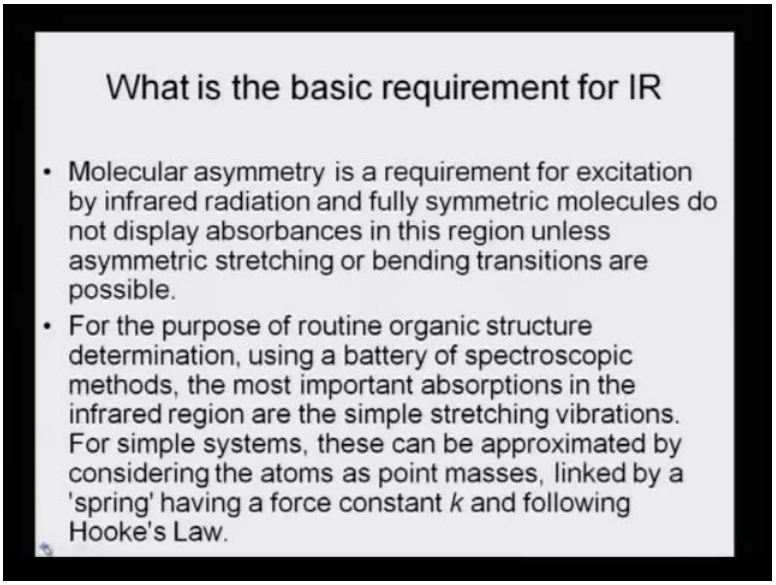

What is the basic requirement for IR? How do we say that the molecule is IR active or inactive? There has to be some basis and what is that basis? Molecular asymmetry is a requirement for excitation by infrared radiation and fully symmetric molecules do not display absorbances in this region unless asymmetric stretching or bending transitions are possible. So, for a molecule to be IR active, the mandatory facts are that there should be a fundamental molecular asymmetry; asymmetry means the molecule should not be equal on both the sides; or, there should be some part, which can create asymmetry just by stretching or bending.

For the purpose of routine organic structure determination, using a battery of spectroscopic methods, the most important absorptions in the infrared region are the simple stretching vibrations. For simple systems, these can be approximated by considering the atoms as point masses, linked by a spring having a force constant k and following the Hooke's law. So, if you imagine that an atom is a point and the bond is just like a spring, then what happens to the movement of the spring, is expressed by the Hooke's law. One has to extend the imagination of looking at the atom as ball and spring

kind of arrangement one after the other. And, what happens to these bonds, which are acting like springs?

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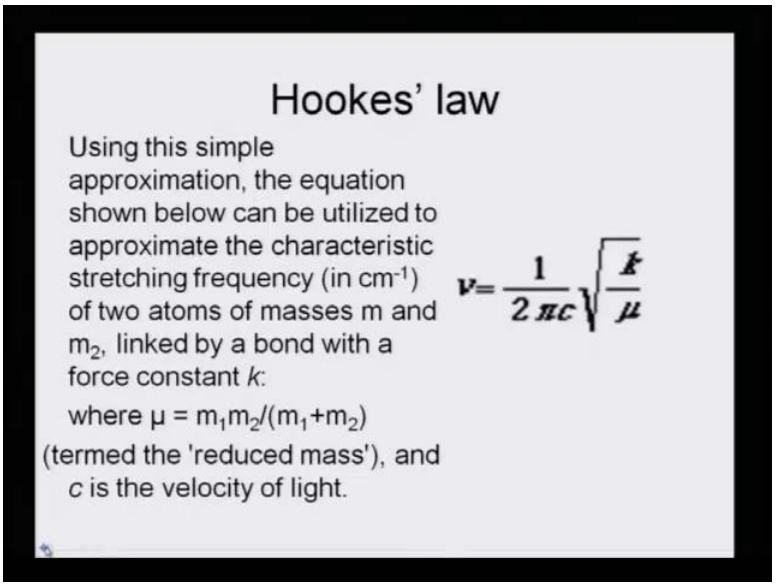

Hooke's law – using this simple approximation, the equation shown below can be utilized to approximate the characteristic stretching frequency expressed in centimeter inverse of two atoms of masses m and m 2, linked by a bond with a force constant k; where, nu is equal to m 1 m 2 upon m 1 plus m 2, and termed the reduced mass, and the c is the velocity of light. So, this is how the expression of energy is expressed in an IR, and it solely depends on Hooke's law; whereas, in the case of UV, it was Beer's-Lambert's law.

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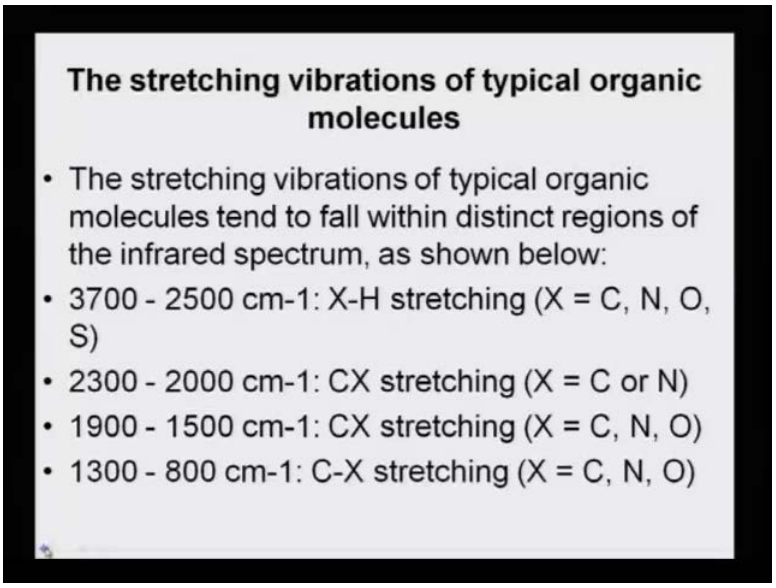

The stretching vibrations of typical organic molecules – the stretching vibrations of typical organic molecules tend to fall within distinct regions of the infrared spectrum, as shown below. It will show a peak in the region of 3700 to 2500 centimeter inverse if there is a hetero atom attached to hydrogen. Such hetero atoms could be carbon, nitrogen, oxygen. There will be a peak appearing between 2300 to 2000 centimeter inverse, where there is CX stretching. So, these are all stretching bond vibrations that we are discussing at the moment in an organic molecule. And, these stretchings could be – the X could be C-C, C double bond C or C double bond N. There could be a peak appearing between 1900 to 1500 centimeter inverse with the CX stretching, where X could be C, N, O. And, there would be a peak appearing between 1300 to 800 centimeter inverse in a CX stretching, where X could be again C, N, O. So, these are the probable regions of looking for a peak. And, the different types of hetero atoms attached to carbon could be either nitrogen oxygen or carbon itself.

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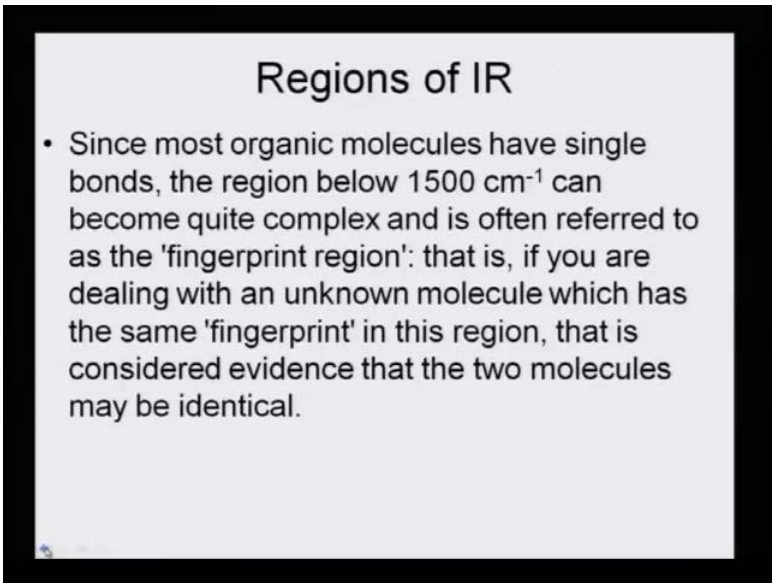

Regions of IR – since most organic molecules have single bonds, the region below 1500 centimeter inverse can become quite complex and is often referred to as the fingerprint region, that is, if you are dealing with an unknown molecule, which has the same fingerprint in this region, that is considered evidence that the two molecules may be identical.

Now, fingerprint region – as the name suggests, that means that finger… We all have very specific fingerprints. And, if I have a certain fingerprint, it does not mean that you will also have the same fingerprint, and that mixes two different identities. Similarly, if the finger prints in a molecular spectrum are matching, it shows that the molecules are same, but this is a very complicated area of the IR spectrum, and it must be only dealt with a specialist. It is not for commoners to use this area. However, as a spectrum detailing, we only use the other part, the functional group part, which gives information about the presence or absence of O-H group, carbonyl group, amino group and so on.

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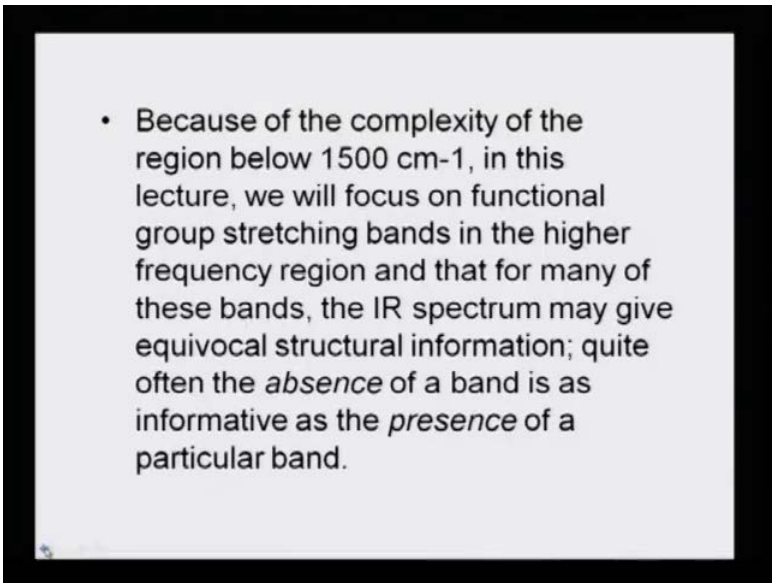

Because of the complexity of this region below 1500 centimeter inverse, in this lecture, we will focus on functional group stretching bands only in the higher frequency region and that for many of these bands, the IR spectrum may give equivocal structural information; quite often, the absence of a band is an information as the presence of a particular band. As what I said, the fingerprint regions are very specific. So, we need not worry about that part, rather we should focus more on understanding the functional group region, which is at a higher region. And, this region gives us an idea whether a particular functional group is present or it is absent; both the information are important. In order to understand the complete structure of a compound, it is important to be able to evaluate how many types of different functional groups are present or absent.

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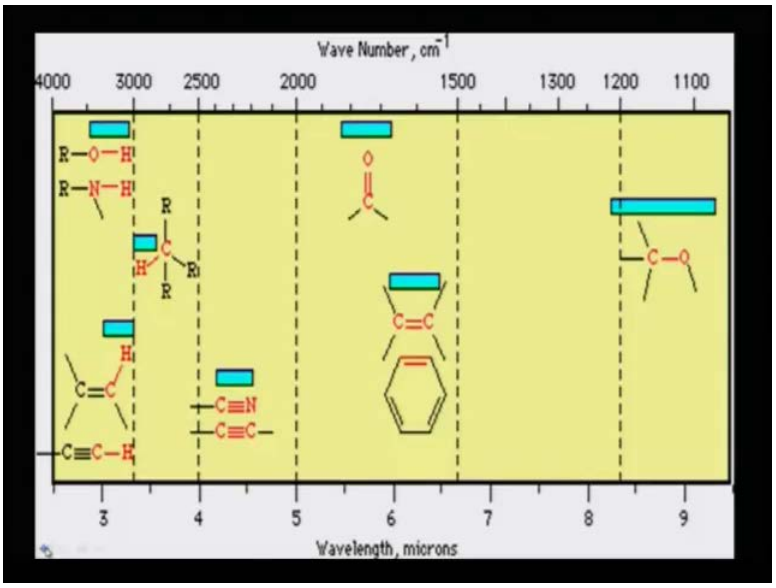

So, if we look at this, it will give us an idea. This particular slide I have prepared only because I should give you the idea how functional groups of different types O-H, N-H, C double bond C, C triple bond C; all these come between the regions of 4000 to 3000. Then, in the case of alkyl region, we have the region between 3000 to 2500. But, for acetylenic and synide group, the peak appears between 2500 to 2000. Similarly, for carbonyl groups and C C double bond groups, it will show peaks in the region between 2000 to 1500 and so on. So, this up to 1500 centimeter inverse is the region, which is of the functional group region, and that gives much more information to us as what is required when we are studying the molecular structure.

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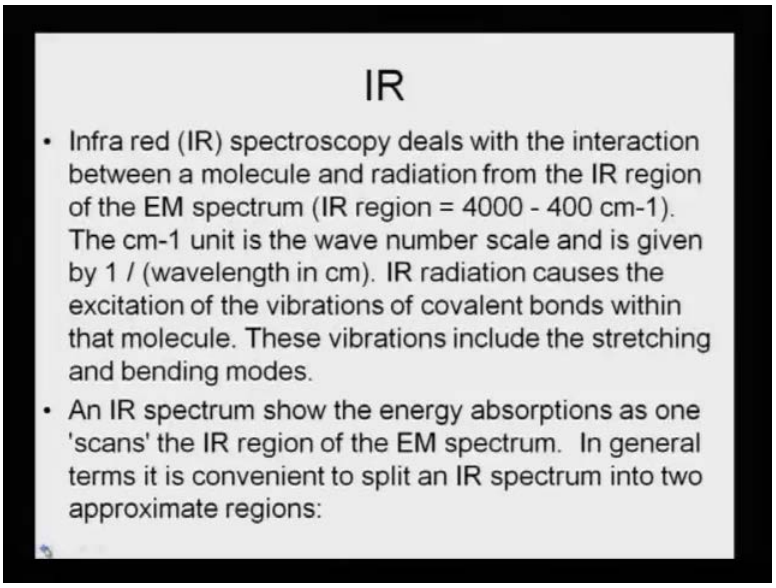

IR – infrared spectroscopy deals with the interaction between a molecule and radiation from the IR region of the electromagnetic spectrum. The IR region as what I told a little while ago is 4000 to 400 centimeters inverse. The centimeter inverse unit is the wave number scale. That also, I have mentioned a little while ago. IR radiation causes the excitation of the vibrations of covalent bonds within that molecule. So, in a molecule, when the IR light is shown, the bonds are simply excited; only those bonds are excited, which match the energy of the excitation and the incident; only those will get affected. These vibrations include the stretching and the bending modes.

An IR spectrum shows the energy absorptions as one scans the IR region of the electromagnetic spectrum. In general terms, it is convenient to split an IR spectrum into approximately two regions as what I mentioned a little while ago.

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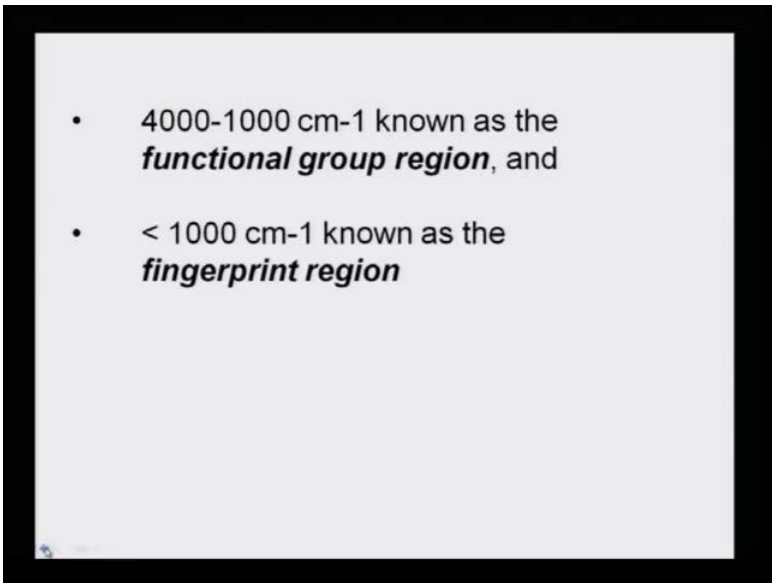

Regions between 4000 to 1000 centimeter inverse is known as the functional group region. And, the region, which is lower than 1000 centimeter inverse is known as the fingerprint region, which is not of common use for an analyst. It is for a specialist to study the fingerprint region.

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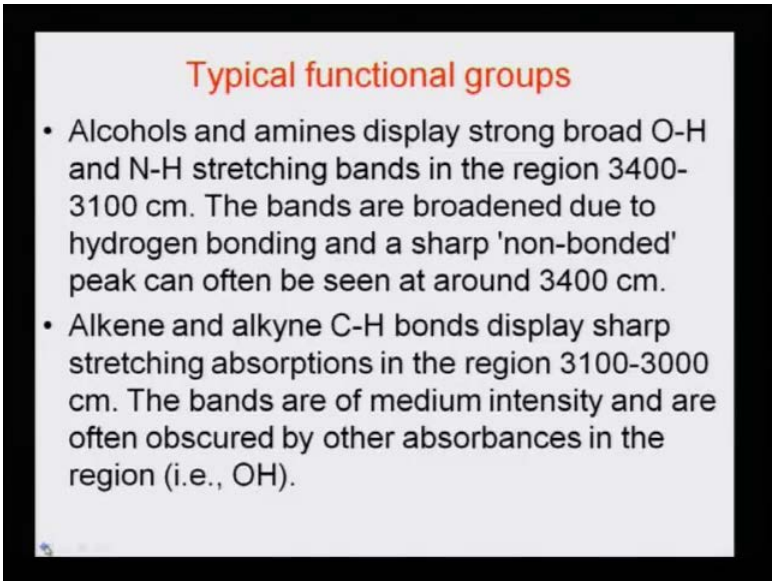

Typical functional groups – alcohols and amines display strong broad O-H and N-H stretching bands in the region between 3400 to 3100 centimeter inverse. The bands are broadened due to hydrogen bonding and a sharp non-bonded peak can often be seen at

around 3400 centimeter inverse. So, if there is a peak appearing at 3400 centimeter inverse or nearby and a sharp peak, it should be taken into account that O-H or N-H is definitely present in the molecule.

Alkene and alkyne C-H bonds display sharp stretching absorptions in the region between 3100 to 3000 centimeter inverse. The bands are of medium intensity and are often obscured by other absorbances in this region; that is, sometimes it is over shadowed by the OH, because OH is usually not a sharp P, it is a broad P. So, that completely covers this region also. But, one can expect the acetylene and the alkene C-H bonds to be present in this region. If the molecule shows some kind of broadening of OH, one can expect that these may be also present in the molecule.

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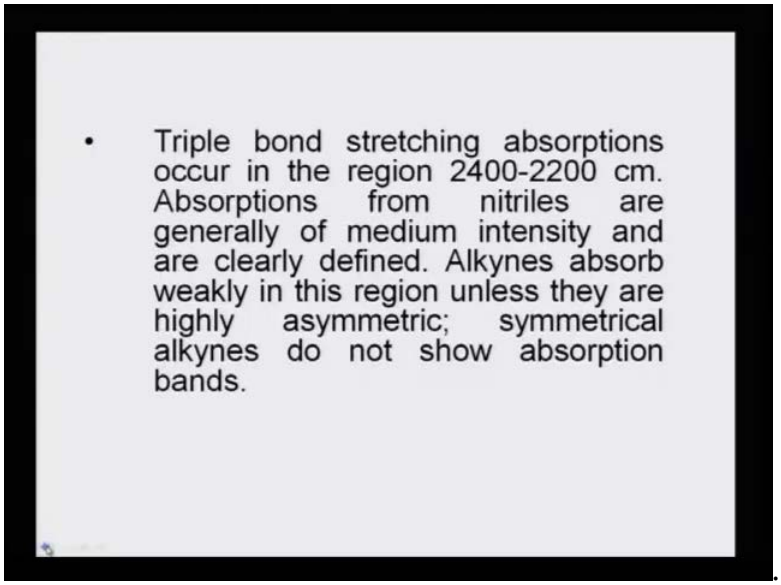

Triple bond stretching absorptions occur in the region between 2400 to 2200 centimeter inverse. Absorptions from nitriles are generally the medium intensity types and are clearly defined. They are very nice sharp peaks, but not very long ones. Alkynes absorb weakly in this region unless they are highly asymmetrical; and, symmetrical alkynes do not show absorption at all in this band. So, one has to keep in mind that the edge plays a very role big role. On one side, you can have another carbon chain, but at the end, it should be a terminal alkyne to be noticed. And, the peak that appears between 2400 to 2200 centimeter inverse is typically for alkynes or for nitriles.

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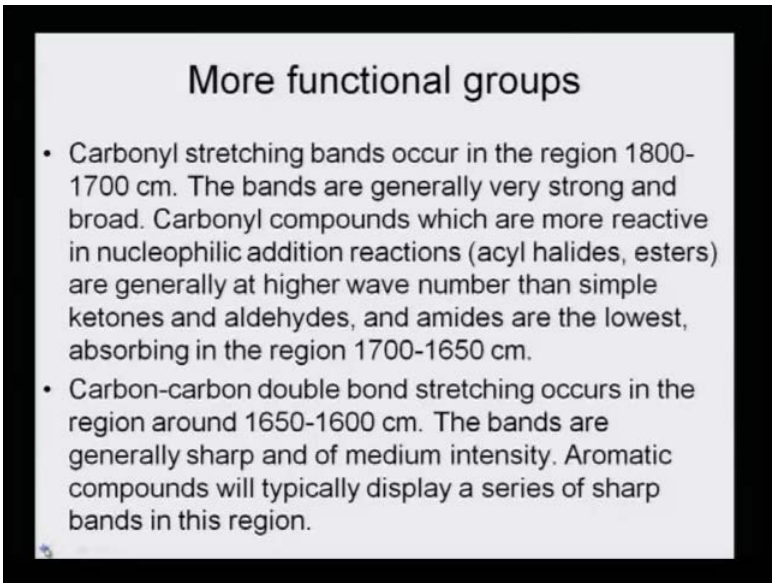

More functional groups like carbonyl stretching bands occur in the region of 1800 to 1700 centimeter inverse. So, if there is a peak appearing in this region, one can assume that it is coming from the carbonyl stretching. The bands are generally very strong and broad. Carbonyl compounds, which are more reactive in nucleophilic addition reactions (acyl halides, esters) are generally at higher wave number than simple ketones and aldehydes, and amides are the lowest absorbing in the region of 1700 to 1650 centimeter inverse. Now, you see, that as we go along, different types of carbonyl compounds like acyl halides, esters have a higher wave number than the simple ketones and the aldehyde; and, the amides have the lowest, and they come at the other end, which is between 1700 to 1650. So, in a way, one can imagine that carbonyl ranges from 1800 to 1650.

Carbon-carbon double bond stretching occurs in the region between 1650 to 1600. So, you see small demarcations, but yet they have well-defined areas where you can find carbon-carbon double bond and carbon double bond oxygen in very specific regions. So, they give information about the molecule whether the molecule has carbonyl group or whether the molecule has ethelinic group. The bands are generally sharp and of medium intensity. Aromatic compounds will typically display a series of sharp bands in this region. So, if the compound is aromatic, that means if it has a benzonyde structure, even then we can expect the functional group to be as C double bond C.

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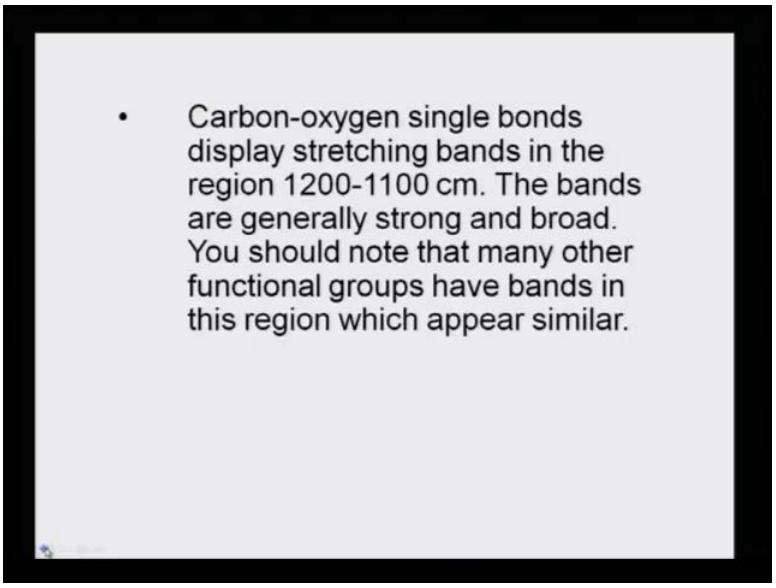

Carbon-oxygen single bonds display stretching bands in the region 1200 to 1100 centimeter inverse. The bands are generally strong and broad. You should note that many other functional groups have bands in this region which appear similar. So, there it can be a little deceptive, nevertheless one can expect that if there is a sharp peak in the region of 1200 to 1100 centimeter inverse, it could come from carbon-oxygen single bond also.

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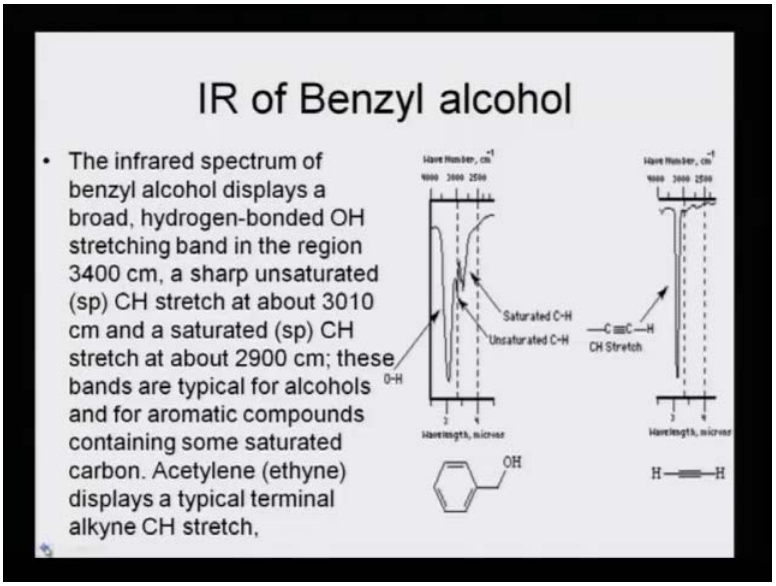

Now, if we look at the IR spectrum of benzyl alcohol – a very simple type of compound I have taken for an example, the infrared spectrum of benzyl alcohol displays a broad,

hydrogen-bonded OH stretching band in the region at 3400 centimeter inverse, a sharp unsaturated CH stretch at about 3010 centimeter inverse and a saturated CH stretch at about 2900 centimeter inverse. These bands are typically for alcohols and for aromatic compounds containing some saturated carbon. Acetylene displays a typical terminal alkyne stretching, and therefore, it has… The next spectrum that is shown here shows a very sharp spectrum between 4000 to 3000 very single; and then, it will also show in the regions of 2400 to 2100 centimeter. And, that will also characterize the presence of acetylene. So, these are like small molecules, but they have been shown precisely, only that region has been magnified for your convenience, so that you are able to understand how these peaks are appearing, how do they look in this spectrum. The peaks are looking as though it is inversely laid down, and that is how the IR spectrum looks like.

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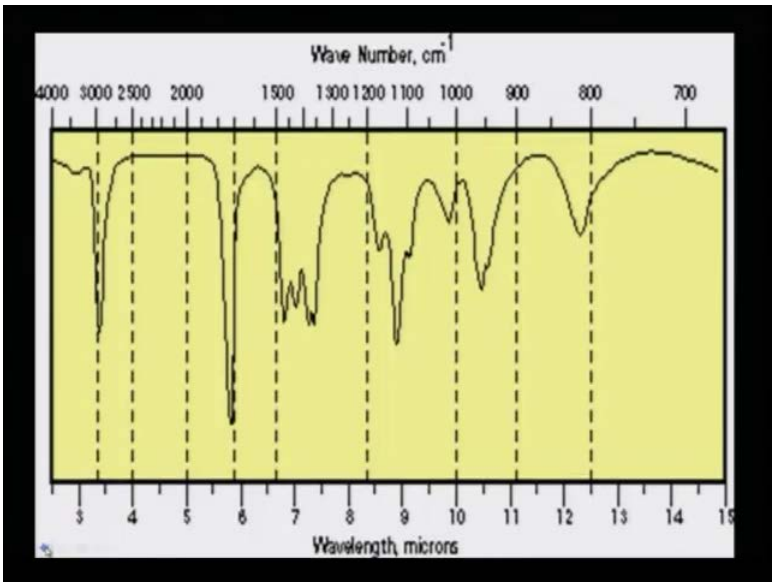

If we look at another compound, you will see that there is a sharp peak at 3000; there is another sharp peak at 1700. So, what do we infer from here? That it has C-H stretching or it can have NH, but it is not at 3400 centimeter inverse. So, it cannot have OH and NH, but it has C-H stretching. It has a carbonyl, and so on and so forth. And, it also has a carbon-oxygen single bond in the molecule, because there is a sharp peak between 1200 and 1100. So, from the given information that I have given you so far, at least you will be able to appreciate whether a functional group is present or not. When you look at the IR spectrum, you will not be saying that oh! all the peaks are opposite; no, this is how the IR spectrum looks like. The peaks are moving downwards and they are peaks, which

have arisen from some kind of vibrational, rotational or bending action caused by the IR light on these particular bonds.

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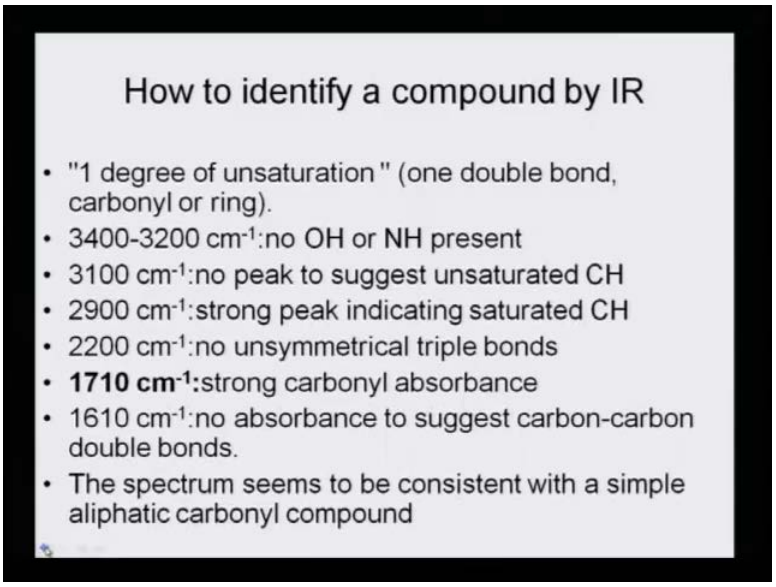

How to identify a compound by IR? How much degree of unsaturation is present? If there is only one double bond or carbonyl in a ring, how do we identify? If there is no peak at 3400 to 3200 centimeter inverse, that means that there is no OH or NH present. Second, if there is no peak at 3100 centimeter inverse, it suggests that there is no unsaturated CH. Similarly, if there is a peak, which shows at 2900 centimeter inverse, it indicates that there is a strong peak of saturated CH. Similarly, if there is no peak at 2200 centimeter inverse, that means there is no unsymmetrical triple bond; either the C triple bond N or the C triple bond C. But, there is an intense peak at 1710, which shows that there is a strong carbonyl absorbance. And then, there is a peak at 1610 centimeter inverse; if there is no such peak, then it also suggests that either it is not showing or it is showing; if it is showing, then there is a presence of carbon-carbon double bond. The spectrum seems to be consistent with a simple aliphatic carbonyl compound. So, if there is a simple aliphatic carbonyl compound, there is no carbon-carbon double bond, and it simply has a carbonyl stretching, which is shown by the highlighted number 1710 centimeter inverse.

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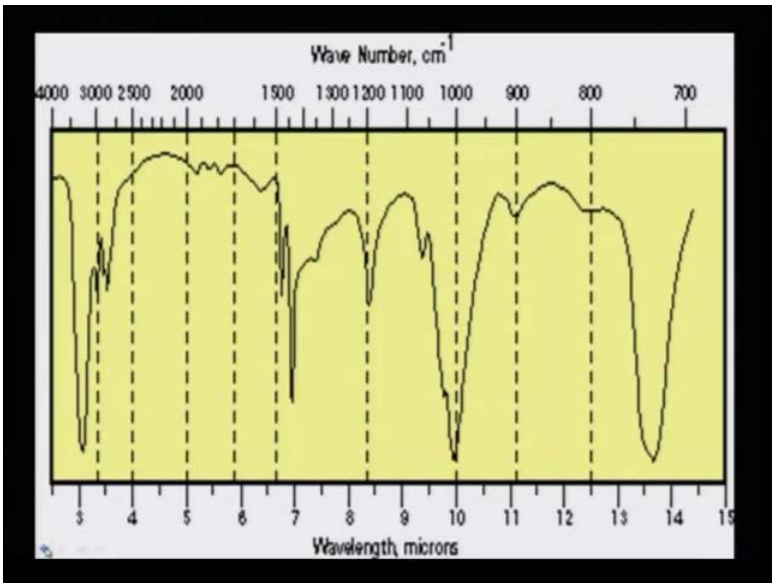

And, this is what the spectrum looks like. So, you see, it is a very simplified spectrum. Do not look at the fingerprint region, only look at the functional group region, and you will be able to see that there are not too many peaks in certain areas; there only certain peaks in these areas; and, they need to be understood very clearly. Only the C-H stretching of the saturated type is present, which comes as a sharp peak; there is no carbonyl present in this particular case, but there is a peak coming between 1500 to 1300, and so on and so forth. So, one can see that this way we can go on looking at different types of compounds.

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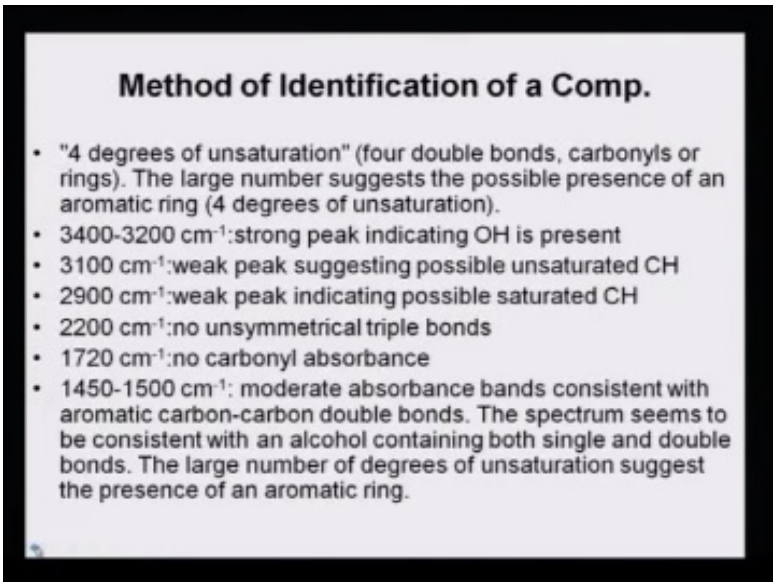

And, identifying them by means of this little information that I have given to you, the information how to identify if a peak appears in a spectrum; then, those regions are very well-defined, and IR functional groups can be easily identified.