Advance Analytical Course

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Lecture No. # 28

We were looking at the understanding of FTIR, that is, infrared spectroscopy. And, once we understand the working of IR, how do we then go on to understand the interpretation of the graph or the spectrum, which is generated by the IR machine. To be able to understand it in a more precise and accurate manner, we will try to take simpler examples in order to make you aware as to how to look for a group, functional group particularly in a spectrum generated by IR machine, and how do we try to interpret the positioning of that peak, and what does it relate to.

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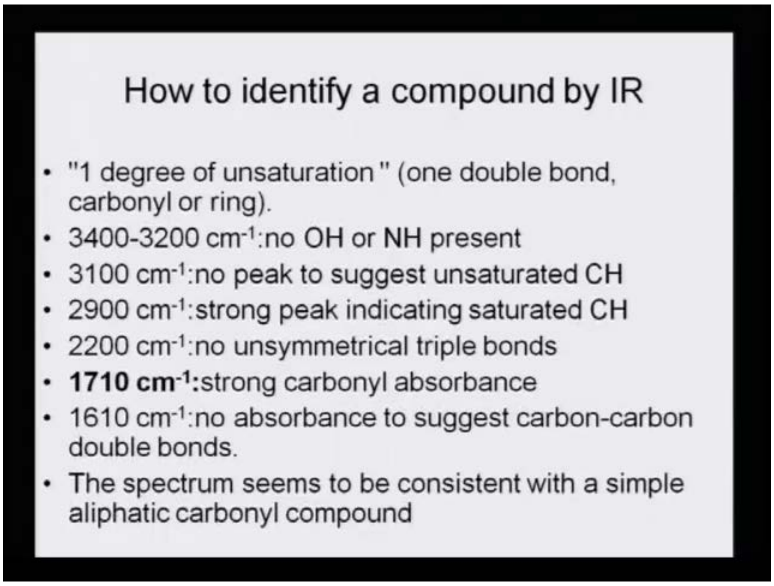

We will try and look at a very simple example, where there is only one unsaturation or 1 degree of unsaturation. It could be a double bond or a carbonyl ring or a benzonoide ring. Now, if there is a peak at 3400 to 3200 centimeter inverse, then we should expect an OH or an NH as told to you in previous lectures. But, since the spectrum does not show the presence of a peak in the region of 3400 to 3200 centimeter inverse, it means that OH and NH are absent. Similarly, if there is a peak at 3100 centimeter inverse, we should understand that the molecule definitely has unsaturated CH. However, if it does not have a peak in that region, that means unsaturated CH is absent. A strong peak appearing at 2900 centimeter inverse means that there is an indication that there is a saturated CH bond. If there is a peak appearing between 2400 to 2200 centimeter inverse or add 2200 centimeter inverse, it shows that there has to be an unsymmetrical triple bonds, either carbon-nitrogen triple bond or carbon-carbon triple bond. If there is a peak at 1700 region, particularly in this case, I have taken a peak at 1710 centimeter inverse and shows a very strong peak, it goes to understand that there is the presence of carbonyl group, that is, C double bond O. A peak appearing at 1610 centimeter inverse and is suggestive that there is a carbon-carbon double bond. So, this was one of the simplest spectra that I would like to introduce you to, because as and when we understand the simple ones, it will be able to be more understandable for you to see some complex IRs.

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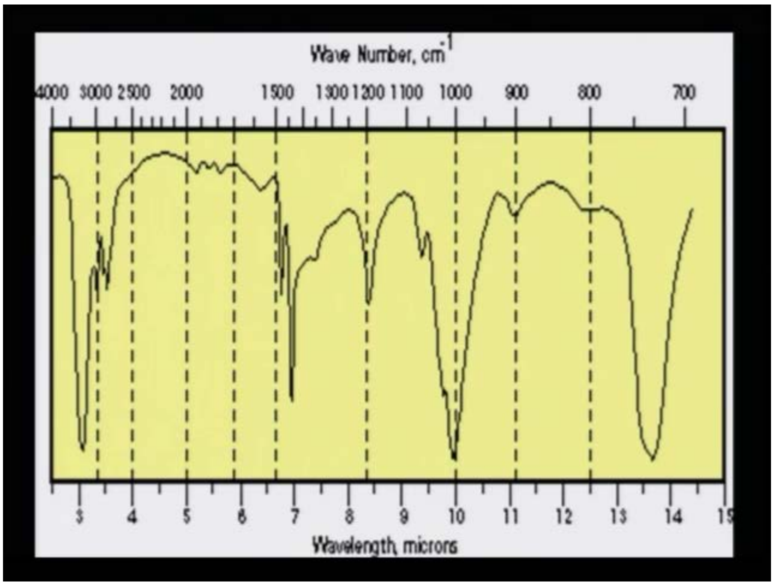

Now, looking at this particular IR, you will see that there are different types of functional groups and different types of peaks that are appearing. A peak that is appearing between 4000 and 3000, there must be the presence of OH or NH. Then, there is a peak appearing at the 3100; there is a peak appearing at 2900. So, it has both saturated and unsaturated CH bonding, and then, there is an intense peak of CO single bond between 1500 to 1300. So, these are indicatives of some of the functional groups that may be present. We do not try to look at the fingerprint region, because it is very complicated. Just the way our fingerprints are very different, similarly, the fingerprint regions of different molecules are bound to be different.

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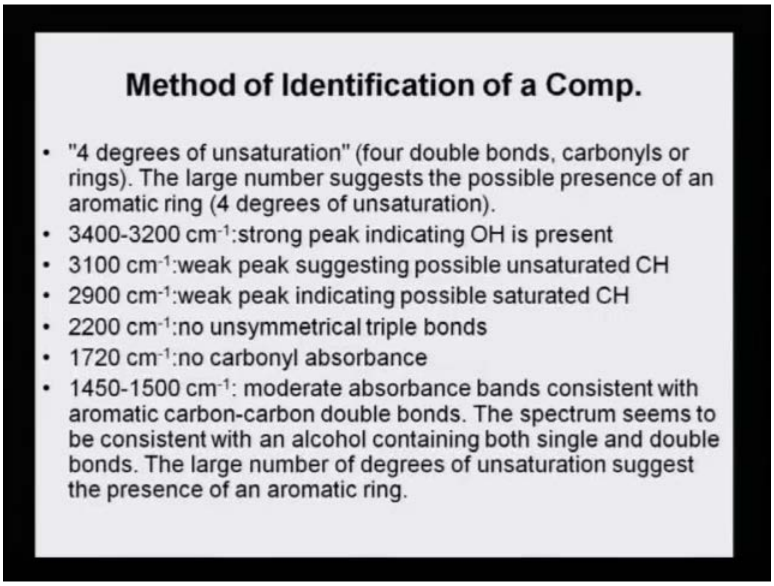

Now, taking an example of a little more complicated system, where there is 4 degrees of unsaturation, that means it may either have four double bonds or four carbonyl groups or four different types of aromatic rings. Large number suggests the possible presence of an aromatic ring and that itself may have 4 degrees of unsaturation. So, if we say that this molecule, I give you an indication that this molecule has 4 degrees of unsaturation. Unsaturation means any point where there is carbon-carbon double bond, carbon-oxygen double bond, carbon-nitrogen triple bond, or carbon-carbon triple bond. So, these are various possibilities or an aromatic system, where there is a benzonoide ring alternating with double bond, single bond; or, there could be an aliphatic system having four double bonds alternated by single bonds. So, these are the various possibilities that may be present when I say that there are 4 degrees of unsaturation.

Now, if you see a peak between 3400 to 3200 centimeter inverse, a strong peak indicating the presence of OH, and usually, the OH peak will be slightly broader as compared to the NH peak, that is, the amino peak; why, because the electro negativity of oxygen is more. So, there is a tendency of hydrogen bonding, and that is why the peak does not appear as sharp peak, but as a broad peak. Similarly, if there is a weak peak appearing at 3100 centimeter inverse as what we had discussed in the previous slide, it suggests that there is a possibility of unsaturated carbon-hydrogen bond. Similarly, if there is another weak peak at 2900 centimeter inverse, it indicates the possibility of saturated carbon-hydrogen bond. But, if there is no peak appearing at 2200 centimeter inverse, it means that there is no unsymmetrical triple bond; neither carbon-nitrogen triple bond nor carbon-carbon triple bond. A peak not appearing in the region of 1700, that means 1720, there is no peak. So, there is no carbonyl in that compound. So, definitely, out of that 4 degrees of unsaturation, carbonyl is ruled out.

1450 to 1500 centimeter inverse – if there are moderate absorbance bands consistent with aromatic carbon-carbon double bonds, the spectrum seems to be consistent with an alcohol containing both single and double bonds. The large number of degrees of unsaturation suggest the presence of an aromatic ring. So, if this is the situation, it is an indication that the four unsaturation are coming from an aromatic ring, because three are coming within the ring and one must be outside the ring, and then there is the presence of a strong OH bond. So, what does it go to prove, that it may be some kind of an aromatic alcohol, which has outside the ring, 1 degree of unsaturation.

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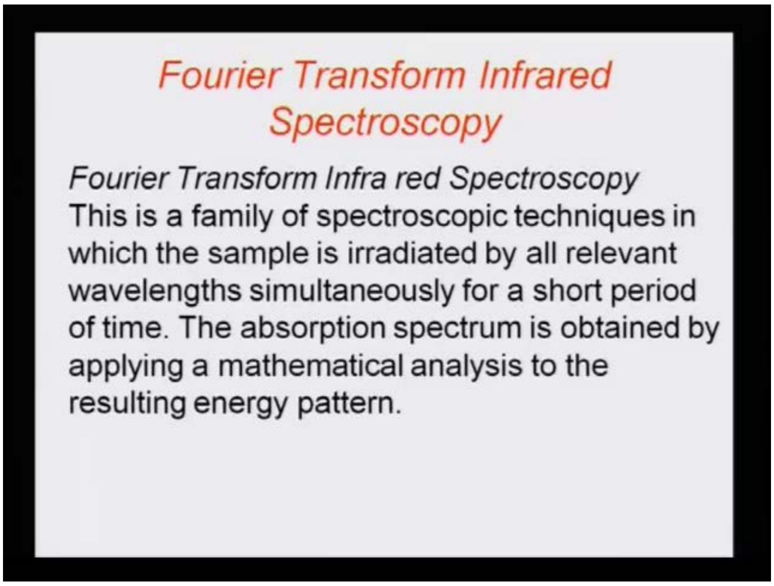

Now, coming to a most advanced infrared spectroscopic method, which is called as Fourier transform infrared spectroscopy, I would like to spend a little more time on this, because the machines that are used in today's analytical laboratories are all FTIRs. The IRs are now obsolete. So, what is so special about the Fourier transform infrared?

Obviously, it is advancement over the previous machines. The previous machine was simply a monochromatic beam entering the sample, and then, one-by-one, the wavelengths were being changed and the functional groups were being identified. As a result, the spectrum that was obtained was a very crude spectrum. But, when this new technique was adapted, many wavelengths were incident at the same time, and the spectrums that were generated by these different wavelengths were super imposed, so that a very sharp peak spectrum could be obtained from this spectroscopic method.

Fourier transform infrared spectroscopy – this is a family of spectroscopic techniques in which the sample is irradiated by all relevant wavelengths simultaneously in a short period of time. The absorbance spectrum is obtained by applying a mathematical analysis to the resulting energy pattern. So, what it is done, within the machine, there is a possibility to super impose these different spectra. And, the energies being similar, this is possible, because their energy patterns are very similar, and the outcome is totally super imposable. So, we go on super imposing and it takes very short time. That is also a beauty of this Fourier transform; that it not only enhances the speed of analysis, but it also increases the sharpness in the peaks of the spectrum, and that is why it is a more advance level of IR.

Coming to another technique of IR, which is in today's world of analysis, a big boon; why, because it can analyze many samples, which are not readily analyzed by FTIR, because of their sample preparation problem. Now, as I told you that in the sample preparation, there are three methods: either a gaseous sample can be introduced, where the cell path length should be very long between 5 to 10 centimeter; or, a liquid sample can directly be introduced; or, thirdly, it can be made into a solid sample by crushing it with sodium bromide and then making a pellet out of pressure. But, if these three methods are not acceptable or are not possible to the adapted for a sample preparation, how do we analyze that? For example, if there is a sample of polymer, how does one analyze a polymer directly on IR? It is impossible because it will not crush on to the KBr; it will not form a pellet; it can neither be dissolved nor made into a liquid sample, and leave alone the gaseous state. So, for such a sample, there was an introduction of yet another method, and this is a very well-accepted and very important method in the recent past that has been invented for IR spectroscopic method.

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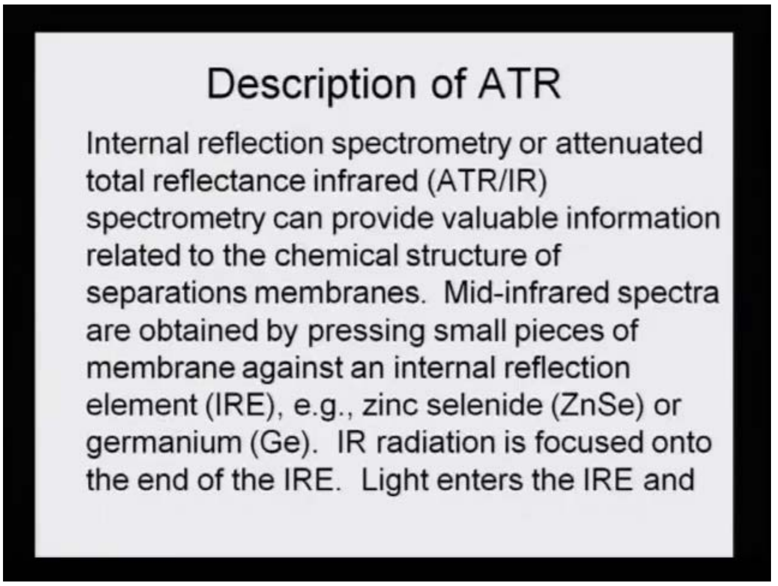

Internal reflection spectrometry or attenuated total reflectance infrared (ATR/IR) is the name of this new technique. Attenuated total reflectance spectrometry can provide valuable information related to the chemical structure of separation membranes. Mid-IR spectra are obtained by pressing small pieces of membrane against an internal reflection element, that is, IRE. There is a special material, which is called internal reflection element, and that material mostly comprises of zinc selenide (ZnSe) or germanium (Ge). So, it is the beauty that because of the use of either of these two compounds, zinc selenide or germanium, it is possible that IR radiation is focused on to the end of the internal reflection element, that is, IRE. And, the light enters the IRE and reflects down the length of the crystal. So, what it does? It first comes and gets incident on the IRE, that is, the internal reflection material element.

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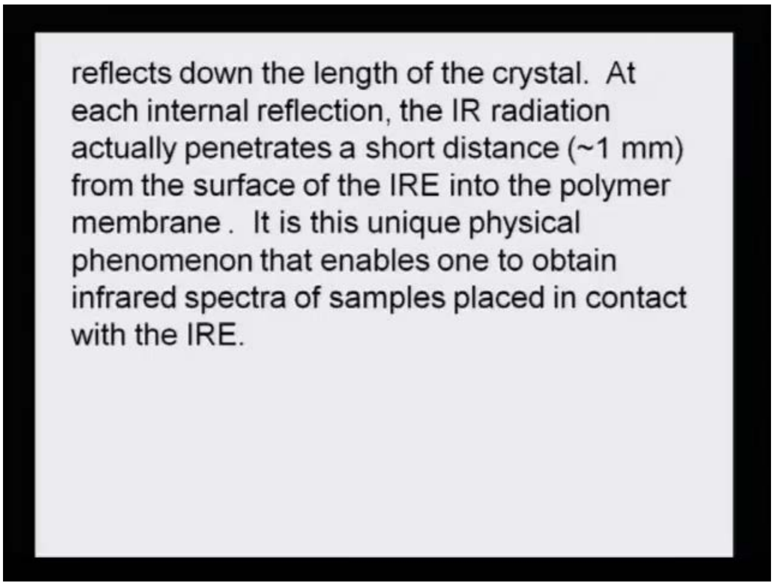

At each internal reflection, the IR radiation actually penetrates a short distance, that is, 1 mm into the surface from the surface of the IRE into the polymer membrane. It is this unique physical phenomenon that enables one to obtain infrared of samples placed in contact with the IRE. So, you see that the sample need not go through any sample preparation method; only thing is, a thin film of that material is made to sit on top of the IRE crystal. And, when the IR light is incident on this, it penetrates to the sample, reaches the internal reflection element, and then, you will see how the action takes place.

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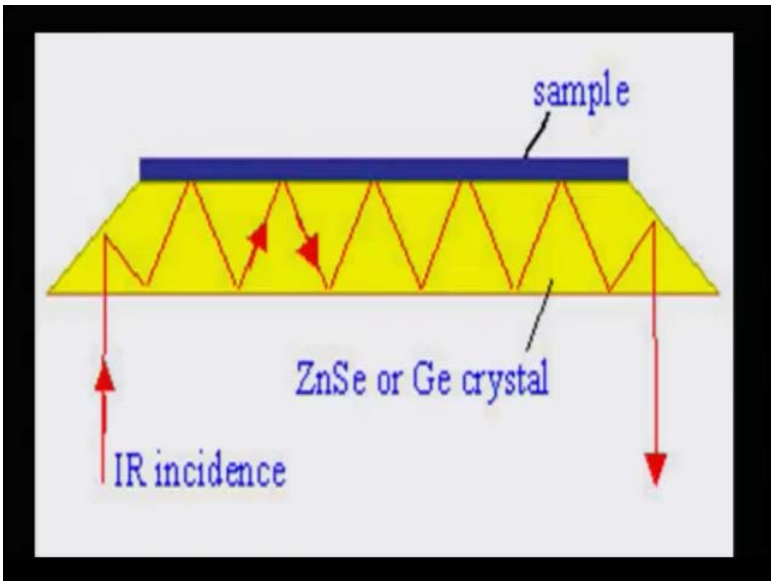

Now, you see that the infrared light is incident. And, here on the top, is the sample sitting. And, because of germanium crystal or zinc selenide crystal, there is an internal reflection that is taking place. And finally, it is transmitted out. So, this provides an internal kind of penetration up to 1 mm into the sample. And, that is possible, because these are highly transmitting materials, and that is the beauty of attenuated total reflectance method or ATR method.

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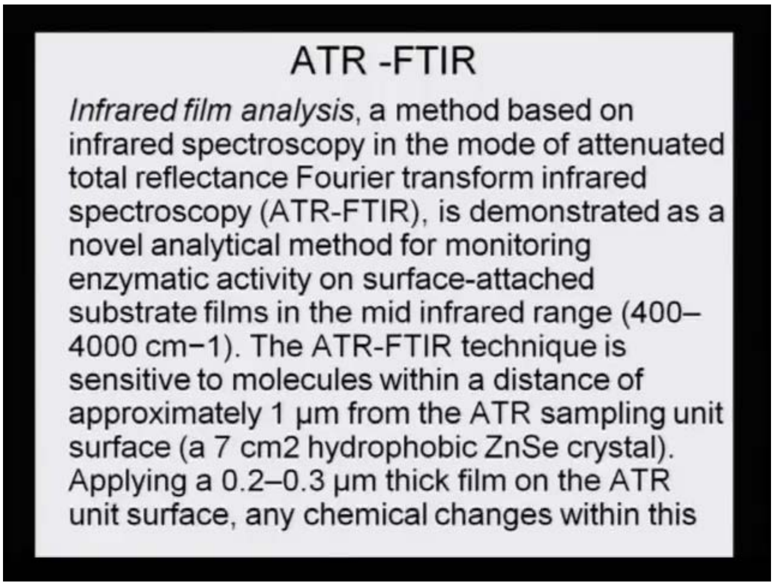

ATR-FTIR – infrared film analysis, a method based on infrared spectroscopy in the mode of attenuated total reflectance Fourier transform infrared spectroscopy. We call it ATR-FTIR, is demonstrated as a novel analytical method for monitoring enzymatic activity on surface-attached substrate films in the mid infrared region, that is, between 4000 to 400 centimeter inverse. The ATR-FTIR technique is sensitive to molecules within a distance of approximately 1 micrometer from the ATR sampling unit surface; a 7 centimeter square hydrophobic zinc selenide crystal is kept. Applying a 0.2 to 0.3 micrometer thick film on the ATR unit surface, any chemical changes within this film as well as at the interface can be continuously monitored. So, you see that the ATR has a zinc selenide or germanium film or crystal, which is hydrophobic, and it is just 7 centimeter square kind of area occupying surface. And, it has a thickness of 1 micrometer. And, on top of that, the sample is applied and the sample film thickness is 0.2 to 0.3 micrometer, and that is why it is able to penetrate into the sample.

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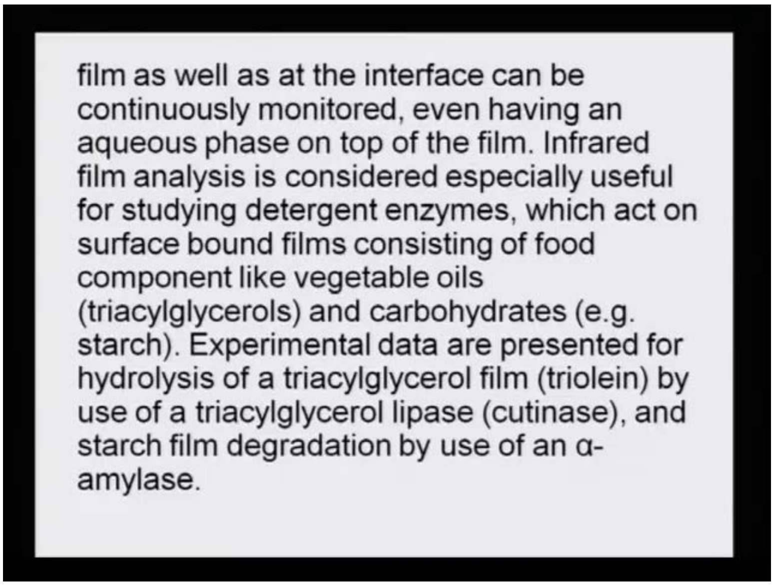

Infrared film analysis is considered especially useful for studying detergent enzymes, which act on surface bound films consisting of food components like vegetable oils, that is, the triacylglycerols, and the carbohydrates, that is, the starch. Experimental data are presented for hydrolysis of a triacylglycerol film, that is, the triolein by the use of a triacylglycerol lipase, that is, an enzyme, which is cutinase, and starch film degraded by the use of an alpha-amylase. So, when studies have to be made on enzymatic digestion of some of these biotic materials, it is a very good method, because in the process of hydrolysis, there is lot of water. When there is water in the sample, as I mentioned to you, the KBr pellet will dissolve. So, it has to be a very hydroscopic condition; that means there should be no moisture when IR samples are being run; why, because if the sample has OH and if the medium also has OH, it will be impossible to diagnose whether the OH peak is coming from the sample or whether it is coming from the sample preparation method. That is why even the zinc selenide and the germanium crystals are taken as very hydroscopic. So, there is no possibility of any OH peak coming from the sample preparation or sample keeping zone.

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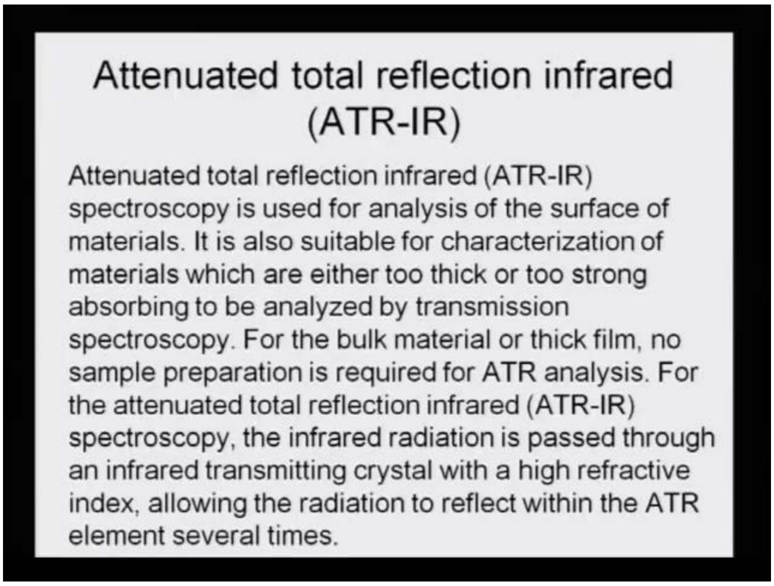

Attenuated total reflection infrared, that is, ATR-IR – attenuated total reflection IR spectroscopy is used for analysis of the surface of materials. It is also suitable for characterization of materials, which are either too thick or too strong absorbing to be analyzed by transmission spectroscopy. Now, it is to be understood that where is the necessity to use ATR-IR, and when to only use simple FTIR, because this becomes a big problem, what to use, when to use. For an analyst, it must be clear that attenuated total reflection infrared, that is, ATR-IR spectroscopy is only used for the analysis of surface material, because surface materials cannot be dissolved into liquid, cannot be made into solid samples. Therefore, they are just made to reside on these special internal reflection element crystals, and that is the reason why it is only used for surface material study.

It is also suitable for characterizations of material, which are either too thick or too strong absorbing to be analyzed by transmission spectroscopy. So, if they are too thick, the light will not pass through it; that is the infrared light. And, if they are too strong, even then, the radiations will not get absorbed and there will be no light transmitted out of this spectroscopic method. For the bulk material or thick film, no sample preparation is required for ATR analysis. As what I emphasized that the beauty of this ATR-IR is that there is no sample preparation; you can just take the polymeric film and place it on the internal reflection element, that is, the IRE, and then start the analysis. For the attenuated total reflection infrared spectroscopy, that is, ATR-IR, the infrared radiation is passed through an infrared transmitting crystal with a very high refractive index, allowing the radiation to reflect within the ATR element several times.

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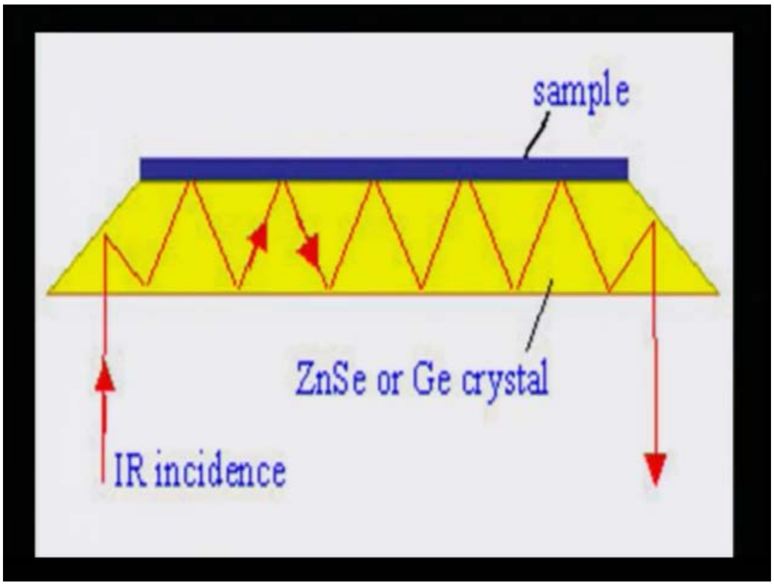

So, I just showed you an example or rather a typical graphical structure of the ATR. And, the IR incident light is then internally reflected several times, and each time, it penetrates about 1 millimeter into the sample; whereas, the sample thickness is very small or it could be even large, so that the information from the surface of the material can be attained very easily. So, this is the beauty of this particular machine, and therefore, by allowing the radiation to reflect several times within the element, that is, the germanium or zinc selenide, it can be attained very easily.

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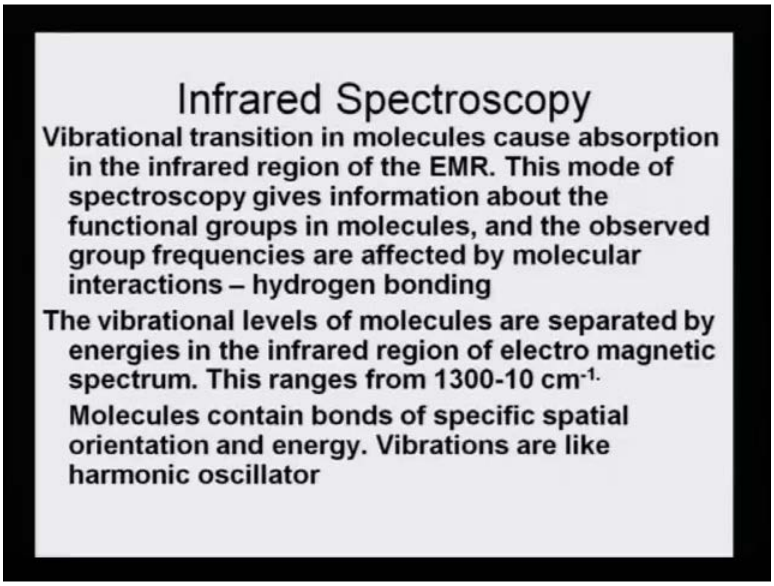

Now, coming to the use of this particular IR method (FTIR), how do we actually use IR for some analysis and how do we interpret the information? I have taken a very typical case; as what you would know, I would just have a recap of infrared spectroscopy before we start looking at the example of a particular analysis. Vibrational transition in molecules cause absorption in the infrared region of the EMR. So, the basis of analysis is that in the electromagnetic radiation or spectrum, there are regions of light, which falls in the region of IR, infrared region. They cause vibrational transition in the molecules. The bonds are simply vibrated, rotated, bended or oscillated, because of this energy being incident on the bond. This mode of spectroscopy gives information about the functional group in the molecule, and the observed group frequencies are affected by molecular interactions, that is, hydrogen bonding. I just briefly told you that how the OH peak is much broader as compared to the NH peak, which has lesser tendency for hydrogen bonding. Nitrogen is less electro negative; whereas, oxygen is more electro negative, and therefore, hydrogen bonding is more pronounced in the case of OH as compared to NH 2.

The vibrational levels of molecules are separated by energies in the infrared region of the electromagnetic spectrum. This ranges from 1300 to 1310 centimeter inverse. Molecules contain bonds of specific spatial orientation and energy. Vibrations are like simple harmonic oscillators. This ranges from 1300 to 10 centimeter inverse. The vibrational energies are of that level and so infrared region of the electromagnetic spectrum. It is that part of EMR that is causing the vibrational energy matching with the bonds. And, molecules which contain these bonds are in a specific spatial orientation and energy. So, once they get energized, they start moving or oscillating or bending in a simple harmonic oscillator type of situation. So, how does one make use of this particular phenomenon of molecules and their interaction with the infrared energy?

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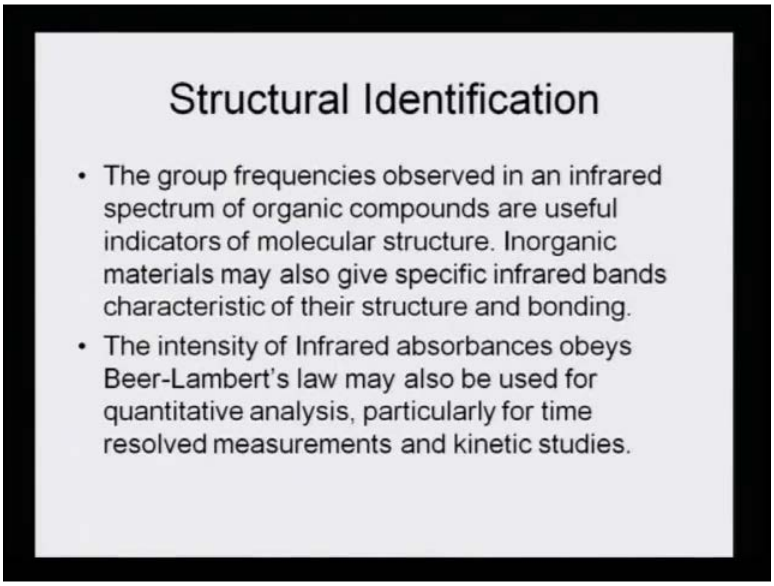

The structural identification – the group frequency observed in an infrared spectrum of organic compounds are useful indicators of molecular structure. Inorganic materials may also gives specific infrared bands characteristic of their structure and bonding. So, it goes to show that because of these matching of frequencies of the IR waves as well as of the molecular bond energy, because of this compatibility, it is possible to identify organic compounds from these vibrational energies. Even inorganic material may also certain specific infrared bands characteristic of their structure and bonding. So, wherever there are bonds, there would be bond energies. And, if these bond energies are matching with the incident light IR Energies, then there will be some kind of vibration or some kind of oscillation or some kind of bending caused by the incoming energy source.

The intensity of infrared absorbances obeys Beer-Lambert's law may also be kept for quantitative analysis, particularly for time resolved measurements and kinetic studies. Although so far, I was telling that it is a qualitative method; that means that IR only gives us the information whether a functional group is present or is absent. But, a more advanced level of IR machine can also help us in doing time resolve measurements and kinetic studies, whether a particular species is being is formed and what time it was formed; what are the transition states; and, how long did it take. So, all these kinetic studies also can be done by monitoring the reaction after every fixed time interval, and seeing what are the changes in the IR spectrum.

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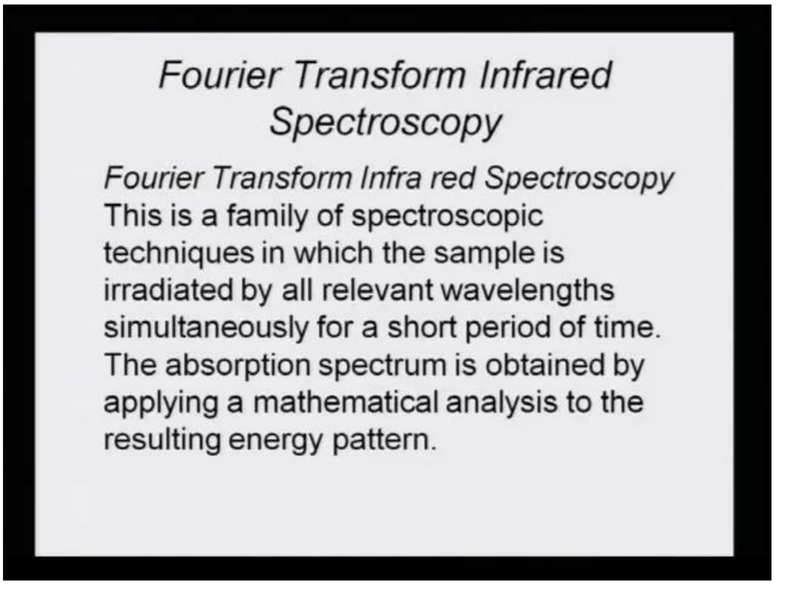

Fourier transform infrared spectroscopy I just discussed a while ago, is a spectroscopy and it is a family spectroscopic techniques in which the sample is irradiated by all relevant wavelengths. That means whatever are the wavelengths related to OH, NH, CO, are all made to be incident simultaneously in a short period of time, and that generates lots of spectra, which are super imposed. And, by mathematical analysis of the resulting energy pattern, one gets very sharp infrared spectrum.

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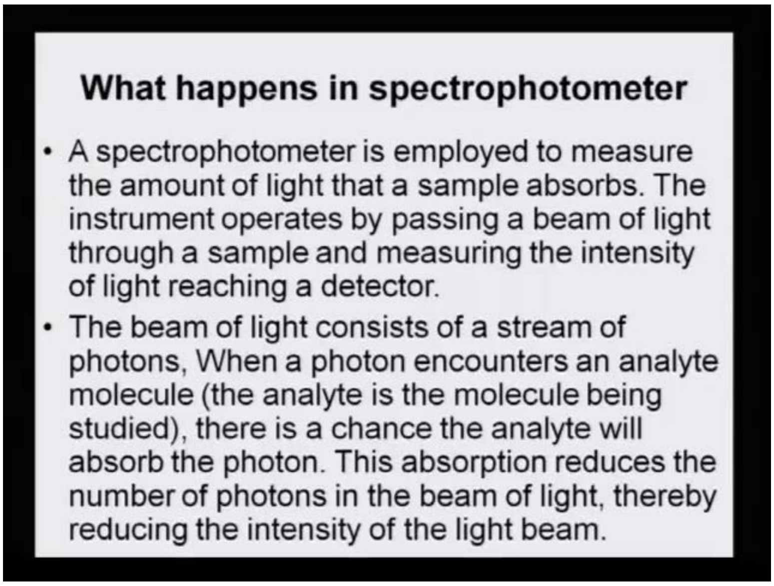

What happens in spectrophotometer? Now, how it does the machine work? How does it look like? A spectrophotometer is employed to measure the amount of light that a sample absorbs. The light instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector. As what I told in the case of UV or IR, we always have two lights passing: one through the sample and one going without the sample and the change in the intensities. That means one which does not hold the sample will remain unaffected; the incident light and the transmitted light will remain one and the same. But, in the case of the sample, if the sample is having the bonds and if the bonds have the same kind of energy capability, they will get excited. That means a part of incident light will be absorbed by the molecule to do these little vibrational activities, and the incident light will subsequently be smaller in its intensity. So, that is the overall overview of what happens in a spectrophotometer. But, the way it has been designed is that the instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching the detector.

The beam of light consists of stream of photons. When a photon encounters an analyte molecule, the analyte is the molecule being studied, there is a chance that the analyte will absorb the photon if they are of the compatible energies, if the energies are different. Suppose if we have a saturated compound and we are showing IR and it is not exiting the bonds, then what will happen, we will only have saturated CH; there is no functional group; there is no unsaturation; there is no acetylenic bond; there is no nitrile. Then, what will happen, the spectrum will have very few peaks. This absorption reduces the number of photons in the beam of light, thereby reducing the intensity of the light beam, which is coming out of the sample chamber. So, the transmitted light will always have a lower intensity as compared to the incident light if the part of light has been absorbed.

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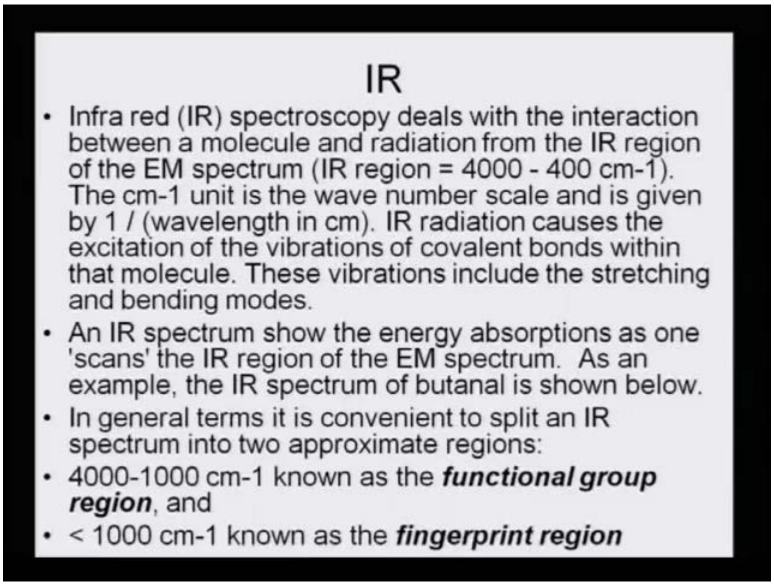

IR – infrared spectroscopy deals with the interaction between a molecule and radiation from the IR region of the electromagnetic spectrum, which is in the region of 4000 to 400 centimeter inverse. The centimeter inverse unit is the wave number scale and is given by 1 upon wavelength in centimeter. The IR radiation causes the excitation of the vibrations of covalent bonds within that molecule. So, this is like a recap. We are trying to remember what the salient features of IR are. IR machine only excites or is related to the interaction between a molecule and the bonds in a molecule and the radiation, which is coming from the IR region of the electromagnetic spectrum. And, it is always expressed in centimeter inverse, which is the unit for expressing IR. And, it is the wave number scale. So, it is not express in wavelength as what we expressed in UV. So, it has to be kept in mind that what is the unit, what the functionalities are, and what the information that we get is. And, when the molecule actually gets excited, the vibrations of the covalent bonds are what matter and what create a peak. These vibrations include the stretching and bending modes. So, because of this incident light, the bond, which was sitting or oscillating in a very slow manner gets excited and start bending and vibrating in a higher manner.

The IR spectrum shows the energy absorptions as one scans the IR region of the EM spectrum. In an example, the IR spectrum of butanal is shown below. In general terms, it is convenient to split an IR spectrum into two regions: I told you, the functional group region, which ranges from 4000 to 1000 centimeter inverse, and the region, which is below 1000 centimeter inverse, is known as the fingerprint region.

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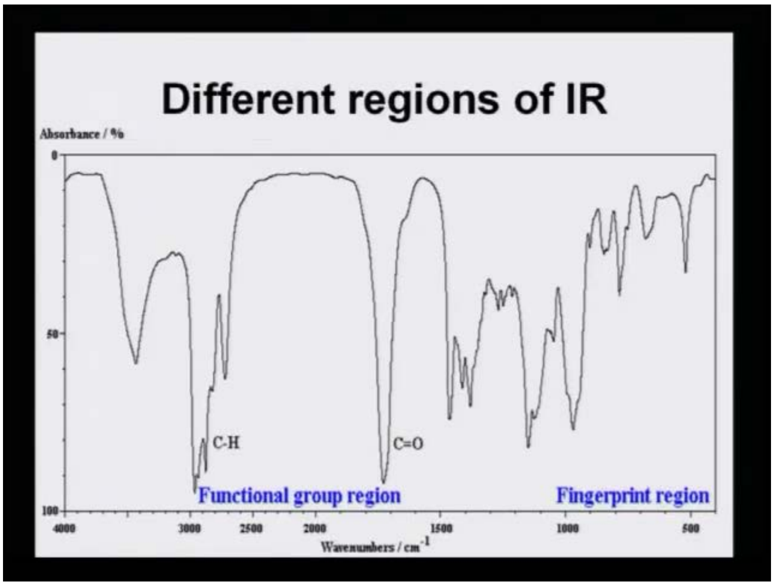

So, if we look at this particular IR, this is an IR of butanal; this is the region of the functional group region; and, this is the fingerprint region. And, you see, there is a very intense carbonyl stretching, which is showing at 1730, and that is from the aldehydic group of butanal. Butanal is butyraldehyde.

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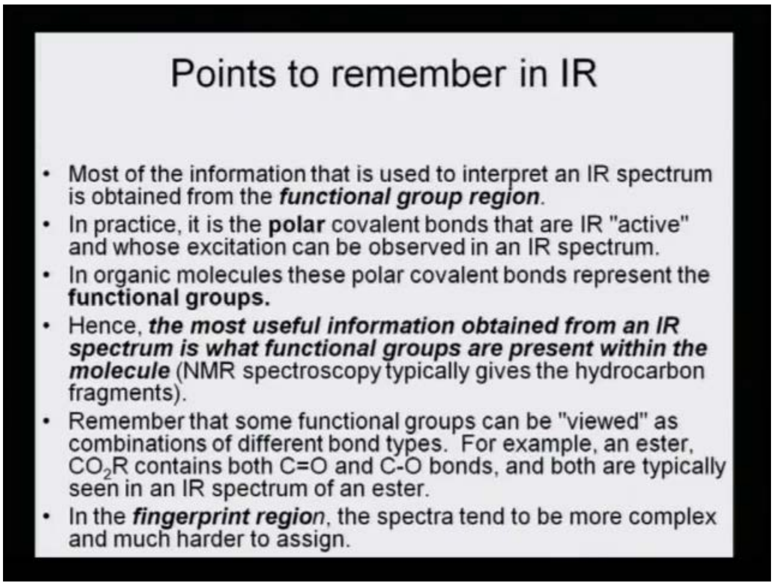

Points that need to be remembered when we are studying IR – most of the information that is used to interpret IR spectrum is obtained from the functional group region. That means if we want to get any information about functional group, we must look at IR whether a particular functional group, like OH, NH 2, carbonyl, acetylelic bond and nitrile bond, ethylenic bond are present or not. In practice, it is the polar covalent bonds that are IR active. This particular point is very important to remember that unless and until the molecule has a dipole moment or the bond has a kind of delta positive and a delta negative, it means that the molecule will not be IR active. Or, in other words, if we have to say that molecules, which are IR active must have polar covalent bond, what does the word polar covalent bond means? Covalent bond means equal sharing, but when the electro negativity of the adjoining atoms, which are participating in the covalent bond are not the same, then there is a creation of delta positive and delta negative. That means they are not completely polarized, but there is a partial… ; on one side, the bond will be more tilted than the other side. And, that is what we called as polar covalent bond. So, the mandatory factor for IR sensitivity of a bond is that it should have a polar covalent bond.

In organic molecules, these polar covalent bonds are represented as functional groups. So, it is this functional group, OH is a functional group; NH 2 is a functional group. Hence, the most useful information obtained from an IR spectrum is what functional groups are present within the molecule. So, if we have to conclude, then we say that IR gives the most important information; that it is giving us information about the molecule whether it has certain functional group or not, like NMR typically gives us the information about the number of hydrogen and the carbon skeleton. Remember that some functional groups can be viewed as combinations of different bond types. For example, an ester, CO 2 R contains C double bond O and C single bond O, and both are typically seen as IR of the ester. So, in the fingerprint region, the spectrum tends to be more complex and much harder to assign. So, we restrict our study only to the functional group region, because we need to get only the information of functional groups in a molecule.