Medical Biomaterials
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Lecture - 15
Analytical Tools

In the previous class I mentioned that we can measure the hydrophobic hydrophilic nature of surfaces by using an instrument called goniometer which measures the contact angle of a surface when a water droplet is placed on it. So, I thought today I will show you a demonstration of how to use that particular facility that is called goniometer.

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This is goniometer a very simple set up we can place a drop of water ultrapure water on a surface. It could be a polymer surface it could be a metal surface. And there is a light that is passing and there is a camera which captures the droplet and angle that droplet makes and it comes on to a computer screen and the computer software helps you to calculate the angle that the water droplet makes on the surface.

So, lower the angle of contact then we call it hydrophilic. Higher the angle we call the material as hydrophobic. So, when as I mentioned that hydrophilic materials attract less of bacteria hydrophobic materials attract more of bacteria and so on. So, generally we will like to make the surface very hydrophilic. So, let us test it out I am taking a sample this is a polyester sample. Polyesters are widely used in medical especially in vascular

graphs and even in some of the diaphragms. So, it is a biodegradable because it has got a ester bond. So, what we do is we place this sample then I turn this knob. So, that the water comes down and settles down as a single drop. Even from the computer screen you can follow the drop coming down and settling down on the surface.

So, now I will slowly turn can you see the drop there the drop comes down. So, you can see the water droplet had made itself settling down with respect to the surface. Now it measures the software automatically measures both the angles this is called the receding this is called the advancing angle sometimes the receding and advancing angles could be different and we will take the average of that. Generally, it is 40 50 degrees' angle. We can call it hydrophilic, 70 80 hydrophobic if it is above 100 degrees, we will call it super hydrophobic there are like carbons which are very super hydrophobic. So, it is quite simple this data can be captured. And then we can do many at different points we can take an average and so on.

Let me try out another sample, for you to again view it this is another polymer PVA. Again I make water droplet as you can follow, as I keep turning the knob clockwise the drop comes down slowly. So, you do not have to let the drop fall and splash itself. So, again we can see drop makes an angle of around 72 73 degrees with respect to the horizontal. This is how we calculate the contact angle, and as I said lower is the contact angle higher is the surface energy surface is called hydrophilic higher is the contact angle lower is the surface energy and it is called hydrophobic.

So, attachment of bacteria depends very much on this hydrophilic, hydrophobic the plasma interaction that is blood components with the surface depends on the hydrophobic, hydrophilic nature of the surface. So, this plays a very important role, and as soon as we design a polymer and as soon as we make the surface modified we have to measure it is contact angle.

We will continue on analytical tools for determining the properties of various biomaterials, which we prepare we have been talking about microscopes different types of microscopes.

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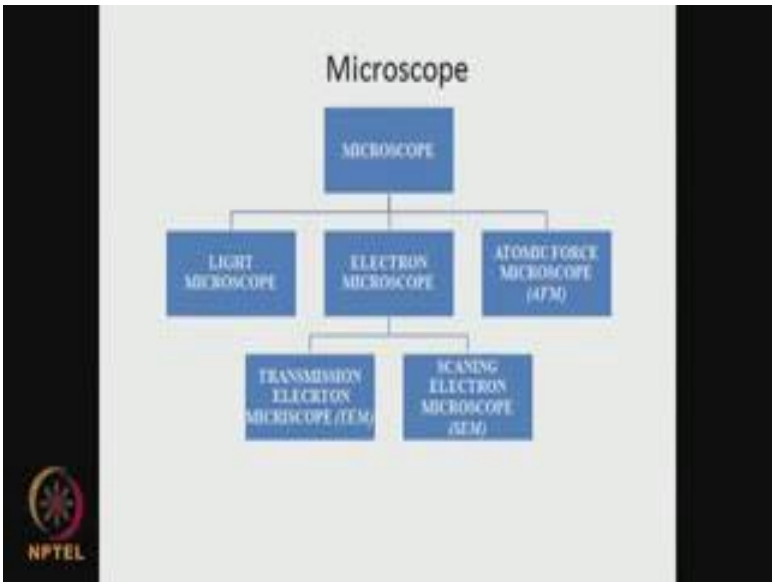

The light microscope the electron microscope and atomic force microscope there are 2 types of electron microscope, one is called the transmission electron other is called the scanning electron transmission as the name implies the electron beam passes through the sample, whereas, in scanning electron microscope it is reflected back. Then atomic force microscope is not a really a microscope in the sense of light and electron. Here a can notilever moves and the movement of the can notilever the up and down movement of the can notilever, because of the indentation or the roughness of the surface is captured.

So, basically in atomic force microscope the roughness of the surface is captured. And that is represented in a pictorial form. So, all these microscopes are very important we use quite a lot in biology; the light microscope is used to look at bio films, if you want to go deeper and look at the bacterial attachment, and we can go into scanning electron microscope if you want to look at nanoparticle. If you want to look at drug delivery system use transmission electron microscope or atomic force microscope, because these are having higher resolution, we can go even up to one nanometer scanning electron I would say about 20 30 nanometers may be00 nanometers.

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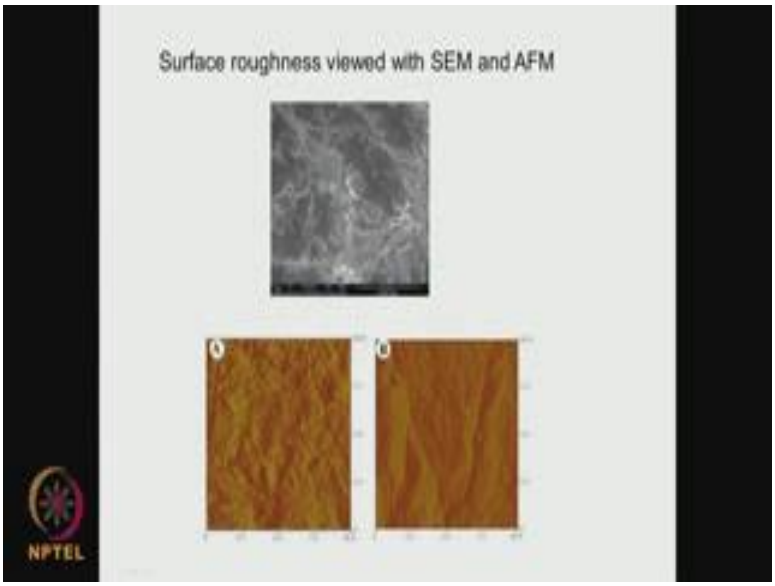

So, for example, if you look at this picture this tells you the surface roughness. This is a polymeric material. It gets rough when it gets incubated for a very long time in body fluids. So, this is a scanning electron picture of this roughness and the same thing is shown through AFM. So, we can get better resolution with the atomic force microscope. So, it is very important to know whether the surface gets roughness; what is the scale of roughness and when the surface gets affected and so on. So, these are quite powerful tools.

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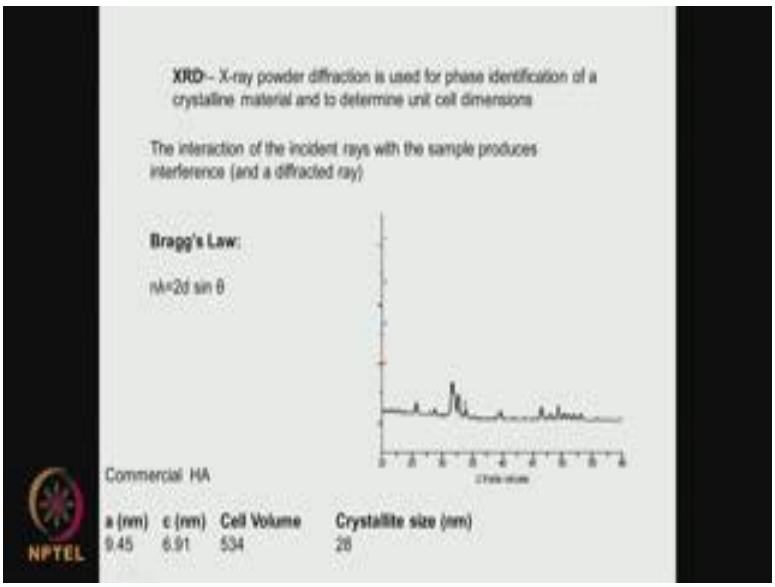

Now, let us go to the next analytical tool, like X-ray powder diffraction. X-ray powder diffraction technique. This is used for face identification of the crystalline material. So, when the material is in crystal form. For example, if you look at metals if you look at some polymers which are in crystal form inorganic material in crystal form and then this is very useful, we can look at whether material is crystalline or whether it loses crystallinity, because it stays inside the body. Because crystallinity has lot of effect on other properties like the melting may be the degradation and so on. For example, amorphous material degrades faster when compared to crystalline material.

So, crystallinity has a very important bearing number 1, number 2 when you are adding some other metals into existing crystalline system the crystallinity changes. So, we can monitor that sort of factors. We can also get the dimensions of the crystal that is called unit cell dimension. So, all these could have obtained from X-ray powder diffraction which is quite an important bulk property. So, the interaction of the incident rays with the sample produces the interference which is observed, that is the diffracted ray is observed and the various crystalline phases are seen in the diffracted ray. If you remember in your school day physics you would have studied something called Braggs law right Braggs law. So, we have an incident ray which has got wavelength of lambda, n could be one here d is dimension of crystal theta is the diffracted. So, we use this formula from there we can calculate d.

So, for example, this is a typical X-ray diffraction picture. This is commercial hydroxyl apatite. So, we can see some sharp peaks here right which is characteristics of hydroxyl apatite. So, we can go to literature and then find out whether these values are matching. So, we can say I have a hydroxyl apatite, and the x axis generally you plot 2 theta that is here right 2 theta you plot here and then from this, using this formula from the theta value we can calculate the dimensions of the crystal. So, crystal size is 28 nanometers crystal volume is 500 and 34 nanometer cube and the crystal cell has a 9.45 and c is 6.91 nanometers.

So, any crystal it is a 3 dimensional you will have a b c. As 3 dimensions' alpha beta gamma as it is angles. So, from these 2 theta value we go to literature and see whether it matches exactly with hydroxyl apatite. So, we can tell whether we have really prepared hydroxyl apatite. And if I am going to do modifications these 2 theta values can get shifted or the crystallinity will be lost. So, we make lot of conclusions based on that. So,

X-ray is very useful we call it powdered diffraction because we take powder X-ray is nowadays, used quite a lot for protein structure determination.

So, it is called a diffractometer X-ray diffractometer. If you want to know the protein structures the locations of various the atoms. So, it is a very powerful tool X-ray diffractometer is used to determine the protein structure. There is a protein data bank pdb and if you go into that you see the 3 dimensional structure of large number of proteins. So, crystallizing proteins and then looking at their 3 dimensional structures again we use X-ray diffractometer.

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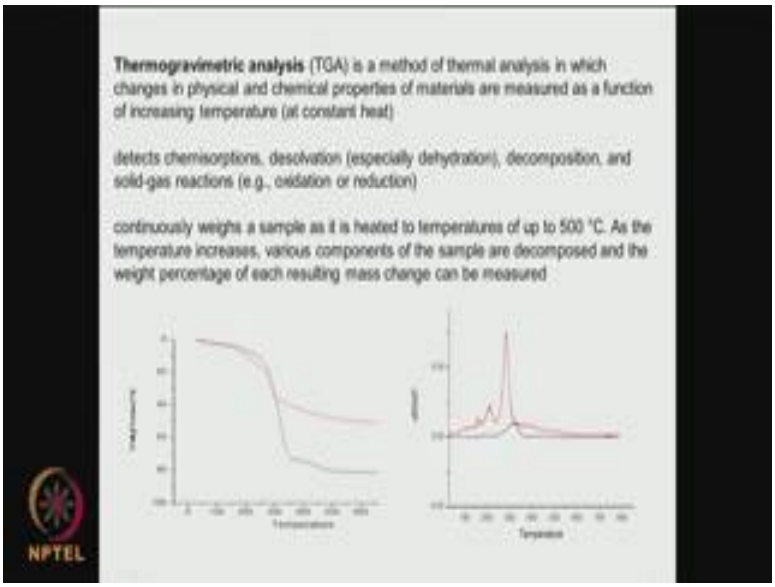

Now, let us look at another technique it is called TGA turbo gravimetric analyzer or turbo gravimetric analysis. So, it is a method of thermal analyses in which change in physical chemical properties of materials are measured as a function of temperature. So, this look at this picture; so we increase the temperature and we monitor the weight loss, f from there we make lot of sense at which temperature the initiation of the weight loss starts and how much is the weight loss and what is the final ash left behind; that means, it is not completely removed.

So, some ash is left behind. So, TGA we call it is a very useful technique especially we use quite a lot in polymeric system. Because the place where the temperature at which the weight loss started becoming maximum. It is a useful value to have sometimes some polymers may have 2 temperatures at which weight loss may happen. Initially there

could be loss of some hydroxyl groups which are easily removable and there could be another temperature higher temperature where it could be more difficult for it to lose it is weight.

So, we can detect chemisorptions dissolvation decomposition solid gas reaction. So, basically what does it do it continuously weighs a sample as it is heated? So, we can go up to 500 600 even 800 as the temperature increases various components of the sample are decomposed. So, the material loses it is weight. So, it comes down. So, for example, in this picture if you see one material loses lot of weight another material loses only little weight. So, it makes lot of sense and also when takes a differential d weight by d temperature and if you plot that you can see here. So, this red one for example, this particular material is losing especially at this temperature, whereas this material is losing at this particular temperature.

So, that way it may be difficult to look from this graph, but if we take a differential d w by d t and plot temperature, it gives you a very nice picture of where the materials are at what temperature each of these materials are losing their weight. So, it is a very powerful technique to understand what types of materials are there. For example, you take a blended polymer one polymer, may lose weight at one particular temperature another may lose weight at another temperature. So, depending upon the composition of the 2 polymers you may get gravimetric a thermogram like this you know it may fall and then after sometime another fall can happen.

So, this is a very useful technique to understand the phase change of materials especially in the area of polymer. And then we can also know if there is a coating which is easily evaporated, so as soon as the temperature is increased whatever coating will get removed. So, there will be a weight loss we can see that. So, we can identify what is the stability of the material especially if you are going to do sterilization of the biomaterial if you are going to do steam sterilization. So, steam sterilization we say about 100 degrees or 110 degrees. So, then coating or other material should not start going out of the polymer.

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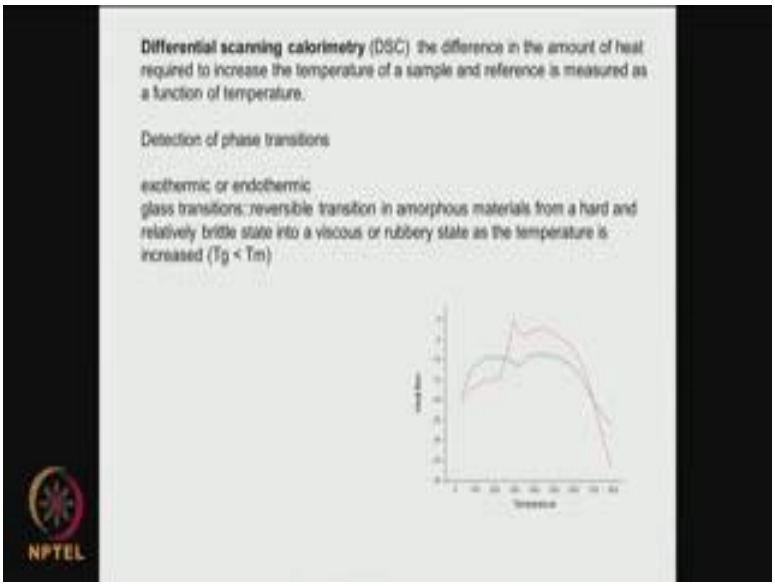

So, those things can be easily determined or identified when we run a TGA. TGA is quite useful in that then similar to TGA, we have something called differential scanning calorimeter or calorimetry. So, what it does is it measures the difference in the amount of heat required to increase the temperature of the sample. So, here you plot heat flow versus temperature where as in TGA we plot weight loss versus temperature. So, DSC heat flow versus temperature. So, if I am converting a material from say solid to liquid I need to put in heat. So, the heat flow will be positive. So, if I am converting from liquid to solid. So, it will be reverse. So, heat flow will be negative. So, we can detect the phase transition.

So, there is a measurement with respect to the reference. So, you are measuring the heat input or heat coming out that is heat flow in or heat flow out with respect to your reference. So, we can detect phase transition. So, we can look at exothermic or endothermic type. So, whether the heat is given out by the material or heat is taken up by the material. So, we can measure something called glass transition that is especially polymers exhibit this type of behavior glass transition, this is a reversible transition in amorphous material from hard relatively brittle stage into a viscous or rubbery stage as the temperature is increased Tm is the melting temperature Tg is called the glass transition temperature, Tg is always less than Tm, T m is the melting temperature that could be very high 300 400 degrees whereas, glass transition can happen at 340 50 degrees.

So, at this temperature there is a phase change from a hard and relatively brittle state to a viscous or rubbery state. So, that is called glass transition. So, some materials can have even 30 degree is a glass transition. So, when we are talking about body temperature we have to be very careful because it will be changing it is phase. That is called glass transition and that will be much smaller than melting temperature, that is Tm melting temperature as I said for example, can happen at 300 or 400. So, you have to put in lot of heat for melting as this graph shows. Whereas, glass transition could be around 30 or it could be 20 or it could be around 100 up to that range. So, material is becoming from hard to more of a viscous or a rubbery state.

So, 2 different thermal techniques one is called the TGA thermal gravimetric. So, weight loss as a function of temperature another is the heat input or output as a function of temperature. So, when we do this on polymers or polymer blends, we understand quite a lot of details how stable material is with respect to changing heat do they change their phases as well what is the glass transition temperature of the material. All those details we can try to identify using these type of techniques then we have lot of spectroscopies used in biology.

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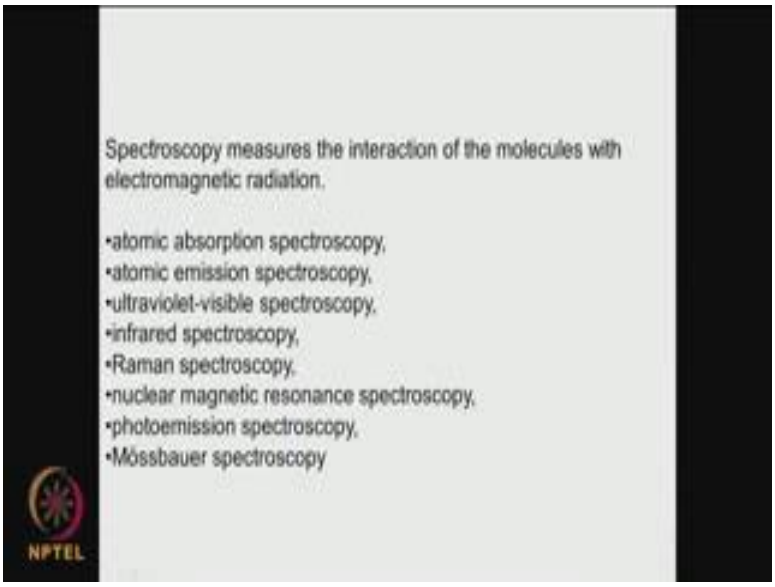

So, spectroscopy measures the interaction of the molecule with the electromagnetic radiation the spectroscopies could be atomic absorption spectroscopy atomic emission spectroscopy ultra violet visible, that is UV visible spectroscopy infra-red spectroscopy

Raman spectroscopy nuclear magnetic resonance or NMR then photoemission spectroscopy Mossbauer spectroscopy.

So, if you look in the area of biology biomaterials, we use quite a lot of this UV visible and IR. IR is used to look at the functional groups, UV visible is used quite a lot in biomolecules spectroscopies like NMR mostly used by synthetic organic chemist to identify characterize the product which they are producing. Atomic absorption we can use it to determine elements the various elements metallic elements present in a material we can use for example, yesterday I talked about EDX energy dispersive X-ray which also can be used to determine elements, but mostly EDX operates looks at only surface where as a can be a bulk and we can find out what are the various elements present in the material.

So, we will talk more about these 2 actually, we will not spend much time on remaining infrared IR or FTIR. Fourier transform infrared is a very powerful and useful tool which we will be looking at quite a lot in biomaterial area.

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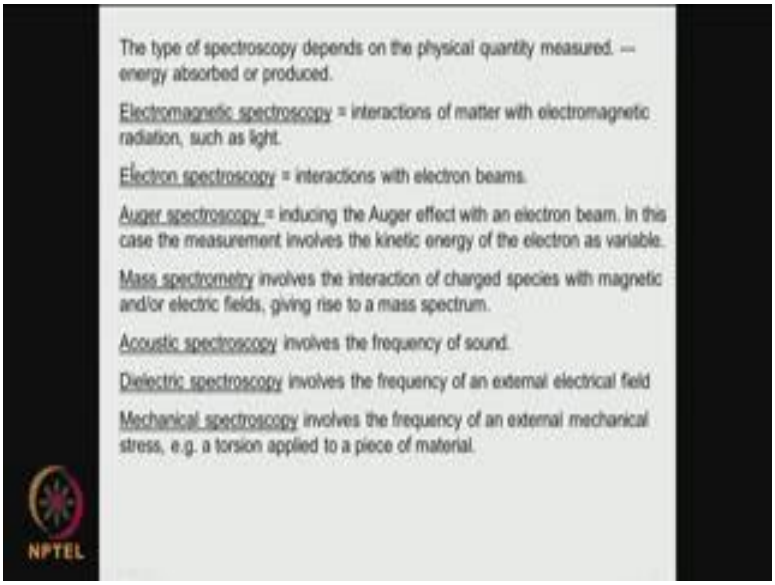

So, if you look at electromagnetic spectroscopy, there is an interaction of matter with the electromagnetic radiation. Electron spectroscopy interaction with electron beams, auger spectroscopy it is inducing auger effect with an electron beam; so the measurement or looking at kinetic energy of the electrons. Mass spectrometry involves interaction of charged particles with magnetic or electric fields. We are going to look at this more in

detail, because if you are make a material like an inorganic or you are making organic polymer I need to know what is the mass. So, mass spectrometry is very important acoustic spectrometry mostly solid material involves the frequency of sound we can look at cracks especially in aeronautics engineering. They use quite a lot of this dielectric spectroscopy involves frequency of external electric field mechanical spectroscopy involves frequency of external mechanical stress.

So, later on I am going to talk more on mass spectrometry. Electron spectroscopy is more like scanning electron and so on actually. So, some of them we will not bother some of them we will talk little bit in more detail, because they are all involved in an area of biomaterial. And so, as I said again we will spend a more time on infrared spectroscopy because we can look at the functional groups that are present on a surface and if I do some modifications what is happening to those functional groups and as a function of time.

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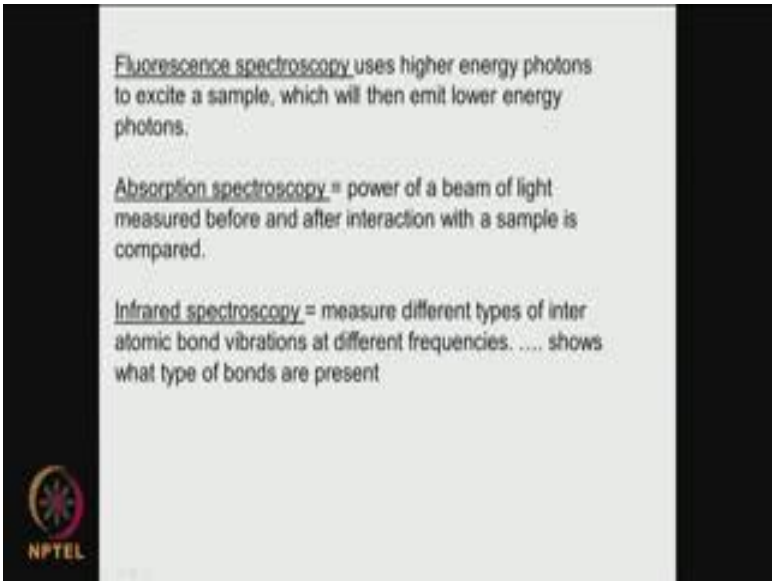

If there is oxidation of your material, we can find out those things using an infrared spectroscopy. Then we have the fluorescent spectroscopy which uses higher energy photons to excite a sample.

So, when you energize a sample it will emit lower energy photon. So, that lower energy photon is characteristics of the sample. Then we have the absorption spectroscopy. So, there is a power of beam of light measured before and after interaction with the sample.

So, we compare how much of the material is absorbed then of course infrared spectroscopy.

So, it measures different types of inter atomic vibrations. So, you could have vibrations of the bonds between 2 atoms or there could be a bending and there could be torsion. So, the different frequencies based on the frequencies we can tell what type of bonds that are present. So, all these spectroscopies are used as I said in biomaterial they are used in chemical analysis. They are used in organic chemistry they are used quite a lot in inorganic chemistry as well.

So, we will talk little bit in more detail on some of these techniques.

Thank you very much for your time.