Industrial Instrumentation
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Lecture - 36
Chromatography – II

Welcome to the lesson 36 of industrial instrumentation. This lesson is a continuation of the lesson 35 where we have discussed the chromatography 1. In this lesson, we will discuss chromatography 2. So, let us look at the contents, this is the chromatography 2 as I told you.

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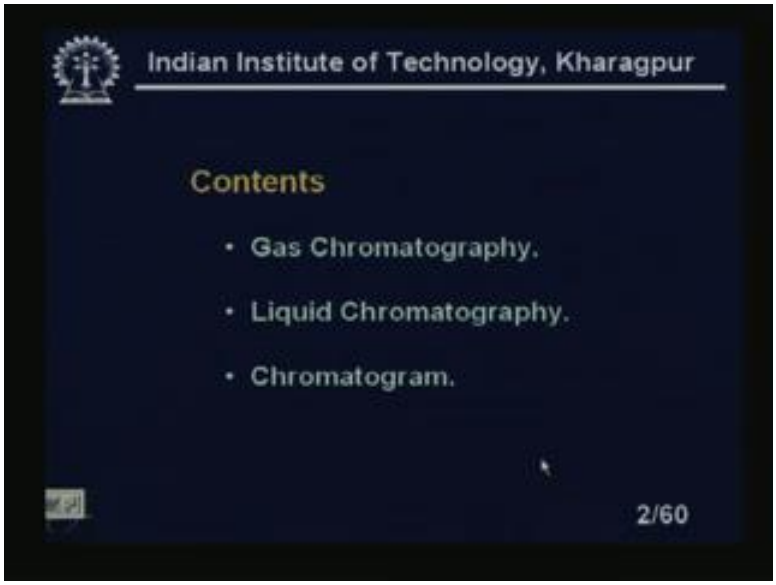

The contents are gas chromatography liquid chromatography chromatogram. So, we will see that in these particular lessons, we will discuss the actual the how the chromatogram should look like. And what are the, if the multiple peaks comes and what are the problems, what exactly I mean the shape of the chromatogram should be? I mean that depends actual the finding the concentrations of the different components of the gas and liquid precisely.

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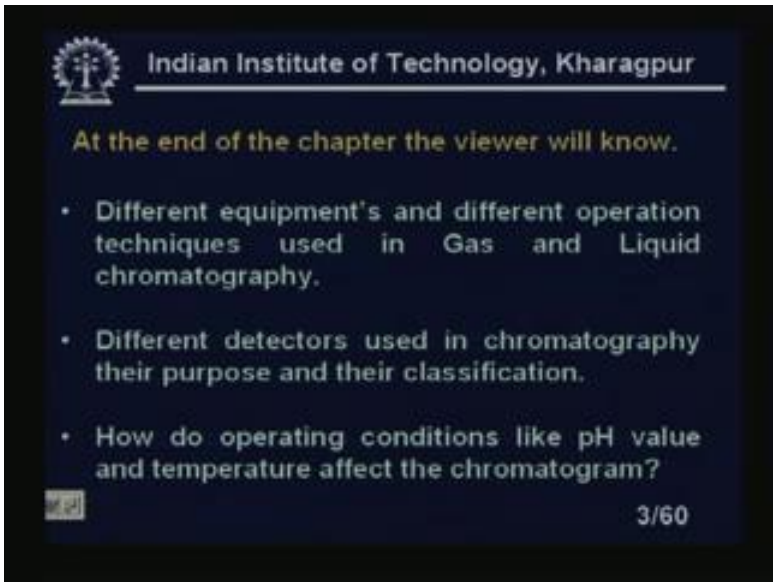

At the end of the lesson or end of the chapter the viewer will know different equipment's and different operation techniques used in gas and liquid chromatography. Different detectors used in the chromatography and their purpose and their classification. We will find there are different types of detectors available. There are thermal conductivity detector; there are film ionization detector; there are electron capture detector. So, there are different I mean the principles are I mean somewhat different.

And though the thermal conductivity detectors are the oldest one and most widely used detectors in the gas chromatography. But we will discuss the other detectors also where you will find that is we will be making a derivatives of the particular elements of the mixtures. So, that to find the concentration accurately, how do operating conditions like pH value temperature affect the chromatogram. So, we will find these things also the pH value though I mean it is I mean not very importance in the case of gas chromatography. This is very important in the case of liquid chromatography.

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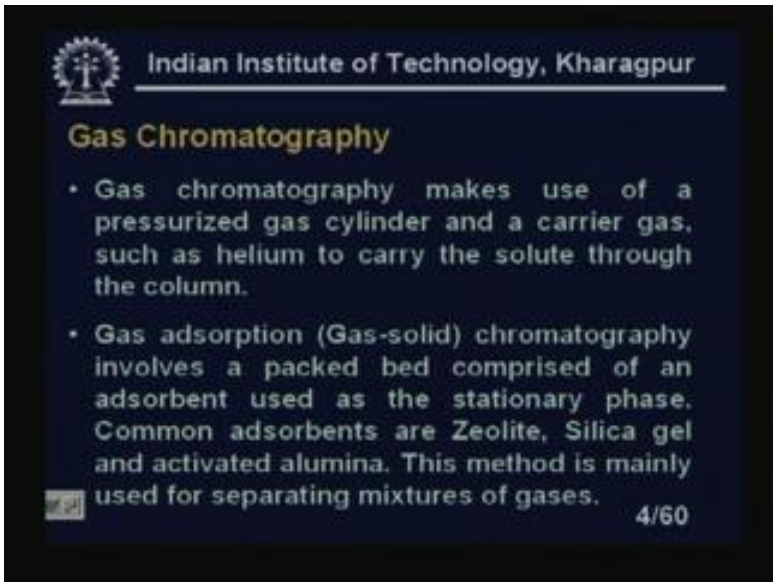

Gas chromatography; already that we have discussed that gas chromatography makes use of a pressurized gas cylinder and a carrier gas such as helium which is inert in nature to carry the solute through the column. Because we will find we have discussed these things that there is a column and through column these the gas would flow right and can there is a packing material inside the column, so which will adsorb or absorb and elute it after sometime one by one. Gas adsorptions that is gas solid chromatography involves a packed bed comprised of an adsorbent used as the stationary phase. Common adsorbents are Zeolite silica gel and activated alumina these are the most common sort of I mean adsorbents are used in a chromatography. This method is mainly used for separating mixture of gases. We will find this is basically used for the mixture of gases right.

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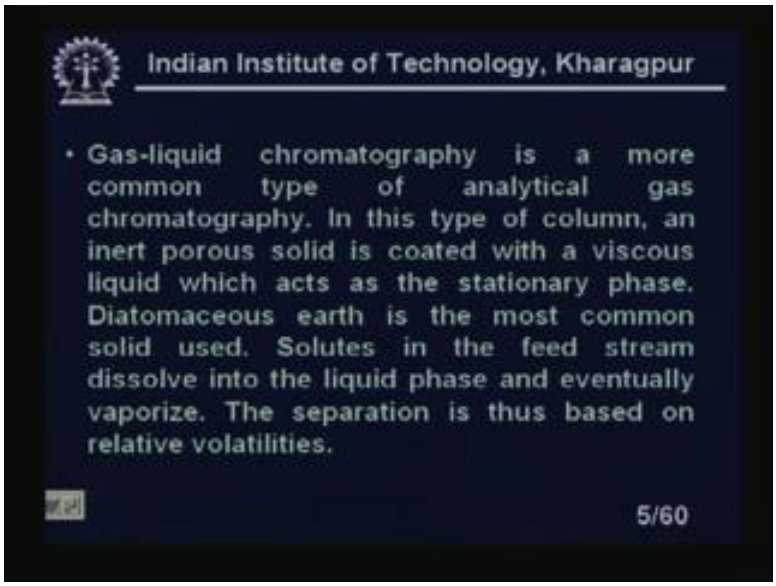

Gas liquid chromatography is a more common type of analytical gas chromatography. In this type of column an inert porous solid is coated with a viscous liquid which acts as a stationary phase. And diatomaceous earth is the most common solid used and solutes in the feed stream dissolved into the liquid phase and eventually vaporize and the separation is thus based on the relative volatilities. So, these you can see that these are all different these already we have discussed in the lesson 35. Now, we are discussing in more details you see the last line which is most important the separation is thus based on the relative volatilities.

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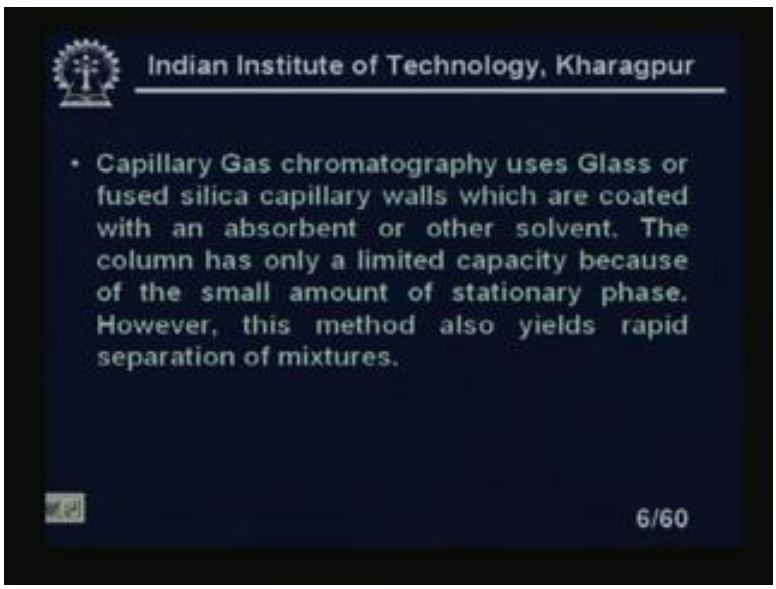

Now, capillary gas chromatography uses a glass or fused silica capillary walls which are coated with an absorbent or other solvent. And the column has only a limited capacity because of the small amount of stationary phase. However, this method also yields rapid separation of the mixtures right.

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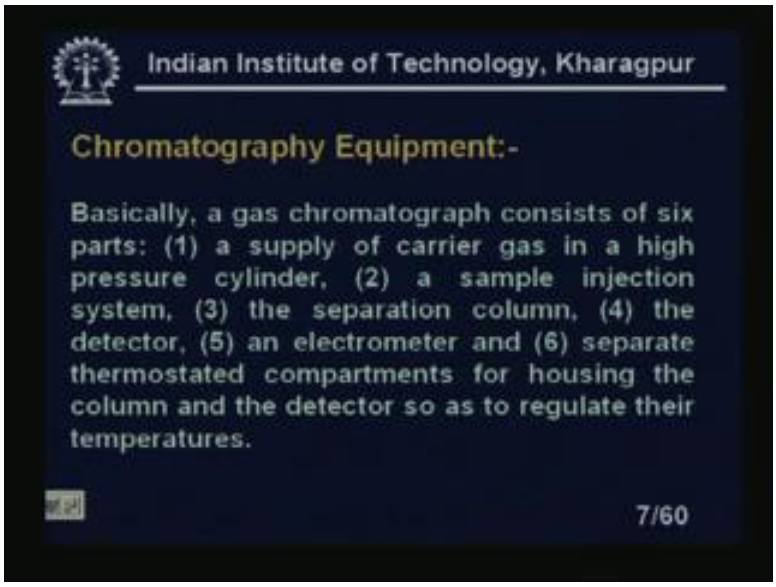

Now, chromatography equipment's, what we need and the entire chromatography equipment? Let us look at that. Basically we will see that an figure in the next diagram. In the next slide basically a gas chromatograph consists of six parts a supply of carrier gas in a high pressure cylinder, a sample injection system. Number 3 is a separation column where we have the packing materials and all those things. The detector which is most important in this particular lessons we will discuss a detectors in very much details.

There are various kinds of detectors we will discuss the detectors and we are already we have discussed about the the packing materials in the case of. I mean in the case of gas chromatography we will discuss also some to some extent that thing in this particular lesson. An electrometer; that means, and a separate thermostated compartments for housing the column and detector. So, as to regulate their temperatures right, so detector usually followed by a strip chart recorders or any other electronic recorders where we will I mean record the peak. In the strip chart recorders we will get a hard copies. It can be computerized also where you will get the actual plot on the screen itself.

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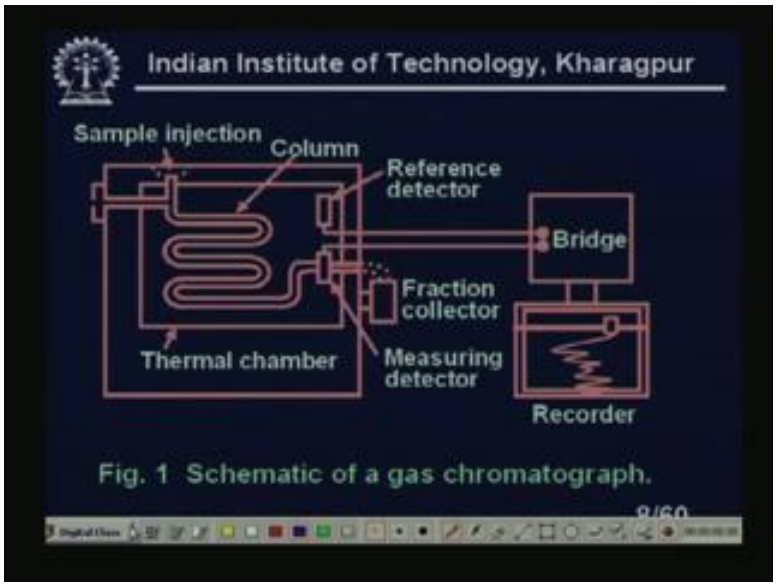

Now, see this is the symmetric of a gas chromatography you see can we can see here this is the sample injections systems. We have a column here you see the columns are made like this. Columns are these are the columns we can see here these are the columns. Then it is going out and this entire thing placed in a thermal chamber. You can see this is the thermostatic chambers we has used and there is a bridge what is the bridge. And there is a reference detector we will find that to make the I mean other things nullified, because you see these types of measurements already we have did in the case of Wheatstone bridge. We will find the same principles is utilized especially in the case of I mean thermal conductivity detector this is used and we have a measuring detectors and we have a recorder.

This is the reference detectors it has no column nothing is there only detector is there and there is a measuring detector. Because measuring detector I mean suppose the detector if the temperature I mean if I want to make the temperature independent or ambient temperature radiation independent. So, in that case I should use a reference detectors. Reference detector has no column nothing but it has a same I mean same material I mean what are the insight the components. So, that, so that temperature variation everything will be nullified and I will get the only the only the, this will go to the two offset offs of the Wheatstone bridge. So, that it can be nullified we will see these thing in the next I mean in the subsequent slides.

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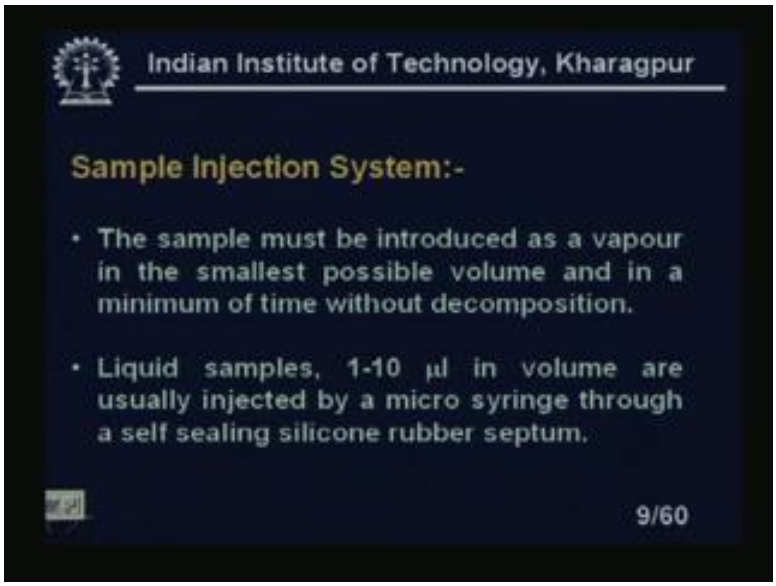

Now, sample injection system; let us look at the sample must be introduced as a vapour in the smallest possible volume sample should be very very small. So, the smallest the, I mean smallest amount that is better for our detection, the peak will be better that is most important thing right. So, the sample should be, because ultimately we are measuring the relative concentrations. So, it does not matter I mean if it is small or large, so if it is small, so it is good for us. The detection will be easy the peak will be more separable we will see the, that type of things. The sample must be introduced as a vapour in the smallest possible volume and in a minimum of time without decomposition. It should not be decomposed that is most important thing. Liquid samples; it is order of 1 to 10 micro liters in a volume are usually injected by a micro syringe through the self sealing silicone rubber septum. So, it is a very small amount that we can see 1 to 10 microliters and it should be it should have micro syringe. So, that a very small amount can be injected.

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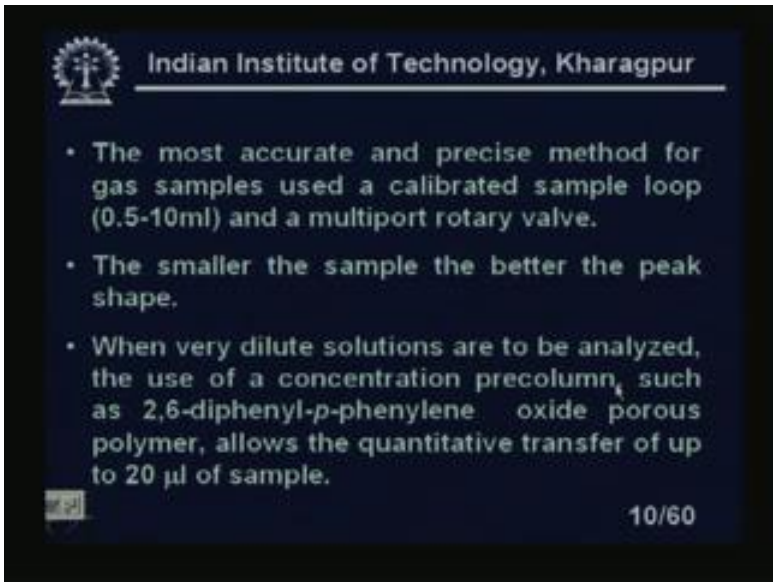

The most accurate and precise method for the gas sample used a calibrated sample loop of 0.5 10 milliliters and a multi port rotary valve. So, different types of rotary valves will be used in the case of in the case of this type of sample injections because sometime we need a valve. So, the precise amount can be injected. The smaller the sample the better the peak shape that I told you the peak shape should be better and better and how the peak should look like let us look at that.

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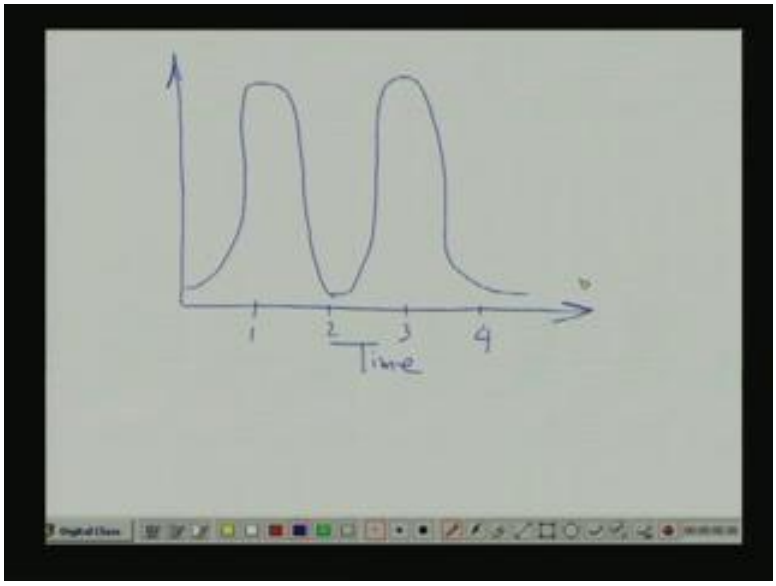

You see that peak should the ideal peak should be suppose I have a suppose I have 2 components. So, time it is in 1 2 3 4. So, it is coming like this the 2 peak is coming like this right. It is a good separation, separation of the 2 components the 2 peaks are coming like this. It might be sharp also it may not necessary.

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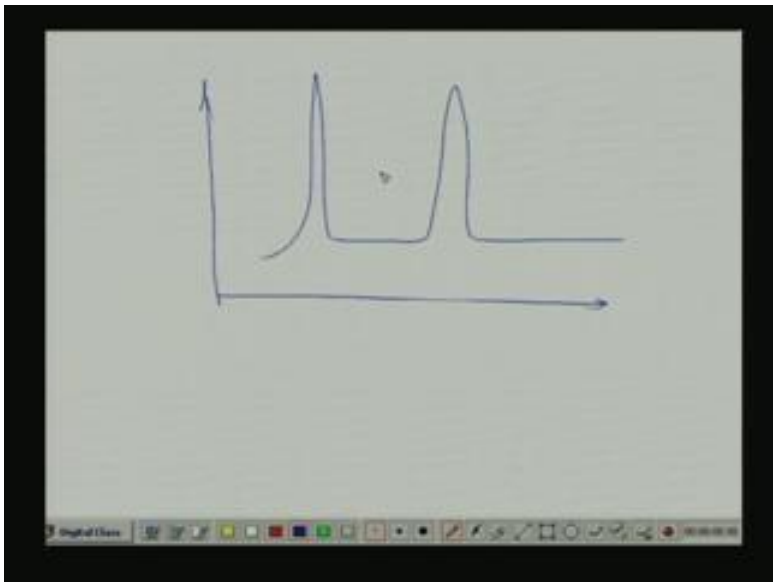

It will be like this when it can be I mean sharp also it can be like this one. It can be very sharp like this one also. Now, if the, this is always possible if the sample quantity is very, very small.

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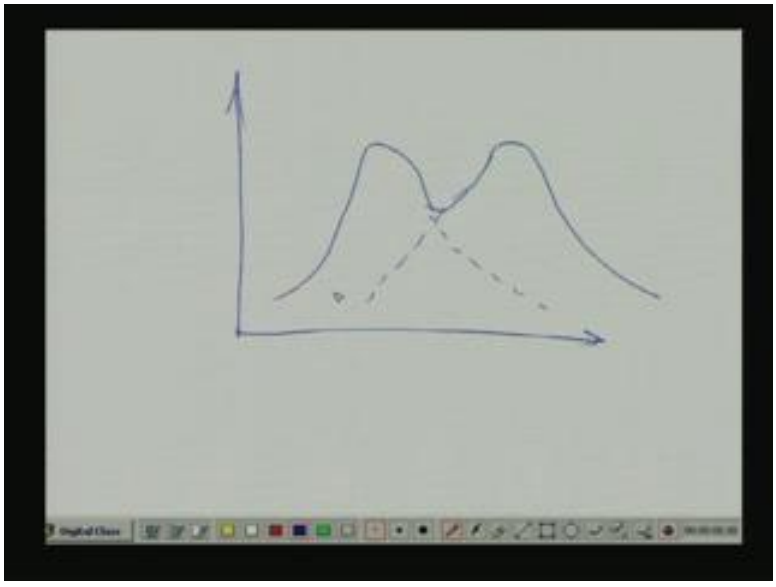

If the sample quantity is large you can see that what type of peak you will get? You will may get a peak which looks like this. Actually do it actually look like this one. So, it is very difficult to find the area under the curve from this graph. So, this is another way the why we I mean give so much emphasis on the small quantity of the sample right. The smaller the sample the better the peak shape when a very dilute solutions are to be analyzed the use of a concentration precolumn such as two six diphenyl-ah p- phenylene oxide porous polymer allows the quantitative transfer of up to twenty micro liter of sample. So, this is a precise transfer of the particular amount of liquids we are considering here.

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Now, derivative formations derivative play an important role in gas chromatography for analysis of polar compounds such as fatty acids steroids drugs biological amine and phenols etcetera. Derivatives make a polar compound less polar improve quantitation and increase the volatility of high molecular weight compounds.

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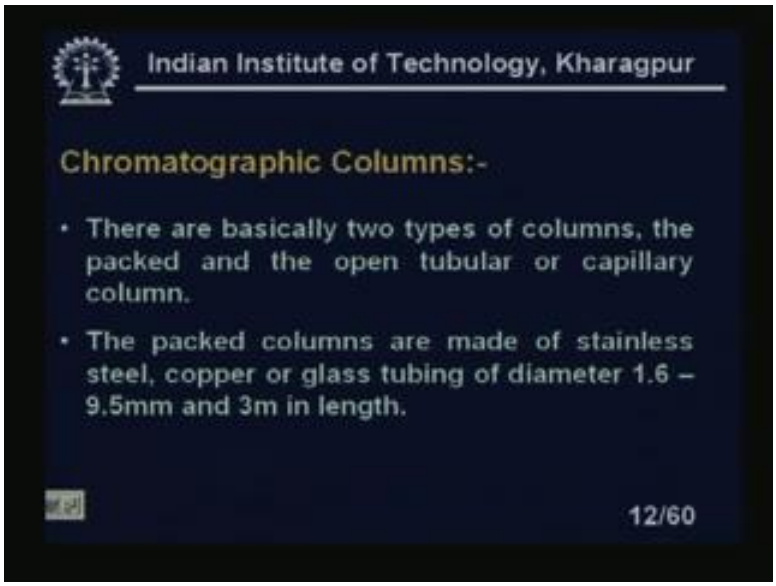

Chromatographic columns, so let us look at the columns first there are basically 2 types of column the packed bit column open tubular or capillary column that is all we have already discuss in the lesson 35. The packed columns are made of stainless steel copper or glass tubing of diameter 1.6 to 9 millimeter, and typically is 3 meter in length. We have seen that it is a zigzag fashion, because there is a large column length 3 meter of length you can see you cannot accommodate. So, it should be zigzag phase.

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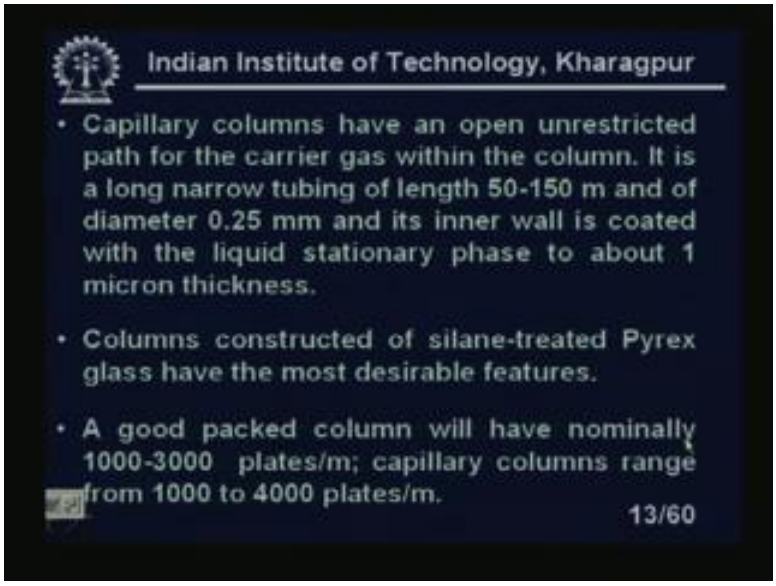

Capillary columns have an open unrestricted path for the carrier gas within the column. And it is a long narrow tubing of length 50 to 150 meters extremely long and of diameters 0.25 millimeter extremely small. And it is inner wall is coated with a liquid stationary phase to about one micron thickness right. Columns constructed of silane-treated Pyrex glass have the most desirable features. A good packed bed column will have a nominally one thousand to three thousand plates per meter already we have seen that this concept actually came from the distillation columns. So, more number of plates better is a separation. Capillary column is range from 1000 to 4000 plates per meter this is the theoretical value right.

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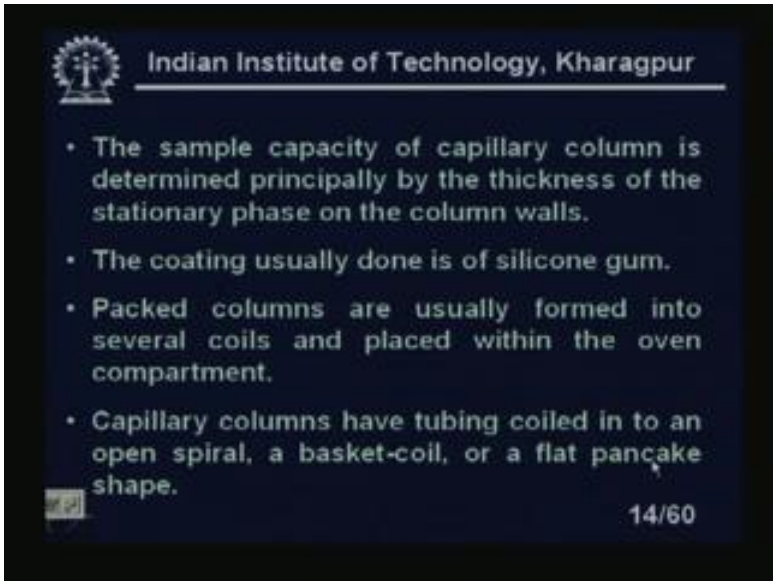

The sample capacity of capillary column is determined principally by the thickness of the stationary phase on the column walls. The coating usually done is of silicon gum. Packed columns are usually formed into several coils and placed within the oven compartments.

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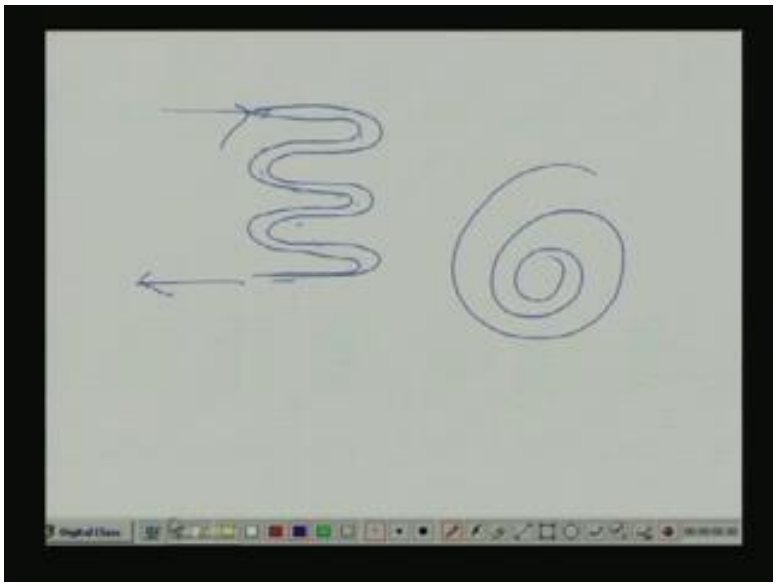

Its several coils we have seen its coil looks like this that we already we have seen. It looks like this right, its looks like this it can be coiled like this one also, but it is better and if you this. So, it will get it will come and it will go out like this one. So, the packing materials are inside this column. So, the packed column are usually formed into several coils and placed within the oven compartment and oven compartment. It is nothing but a temperature I mean is a thermostat based or more precisely controlled temperature environments. Capillary columns are tubing coiled into an open spiral and basket coil or a flat pancake shape.

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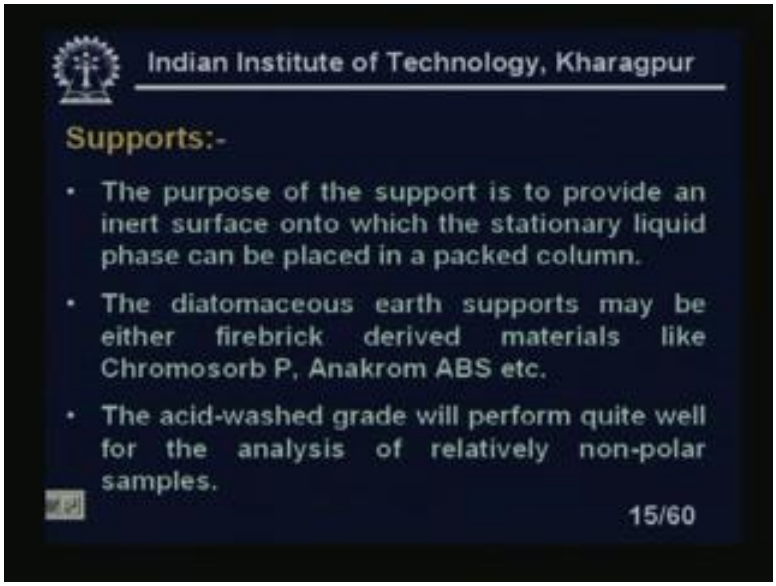

Supports; the purpose of the support is to provide an inert surface onto which the stationary liquid phase can be placed in a pan in a packed column. The diatomaceous earth supports may be either firebrick derived materials like Chromosorb P and Anakrom ABS etcetera. This is special materials the acid-washed grade will perform quite well for the analysis of relatively non polar samples.

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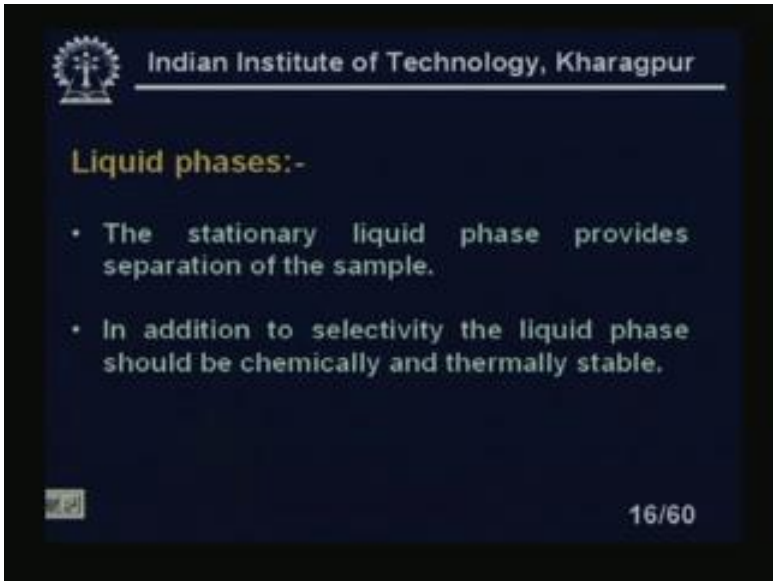

Liquid phase the stationary liquid phase provides the separation of the columns. In addition to selectivity the liquid phase should be chemically or thermally stable, that is most important thing.

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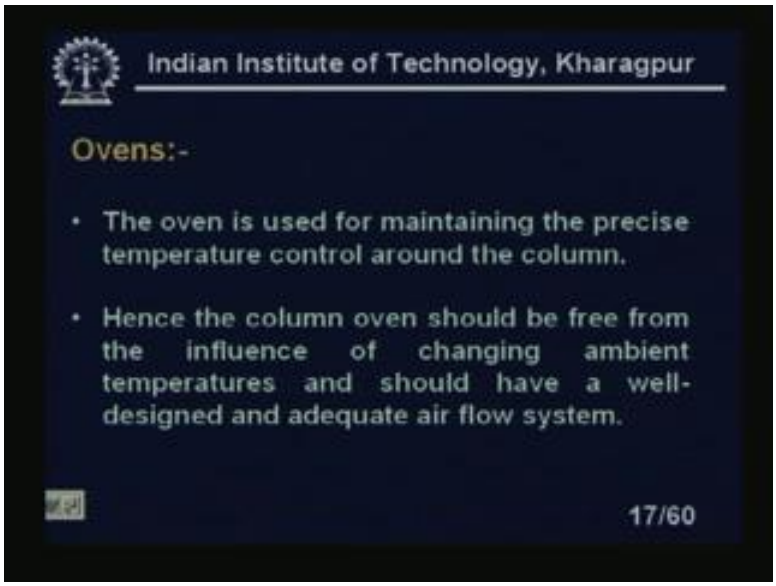

Now, ovens as I told you the oven is used for maintaining the precise temperature control around the column. Hence the column oven should be free from the influence of changing the ambient temperature and should have a well designed and adequate air flow systems. There should be good air flow systems otherwise temperature control will be difficult.

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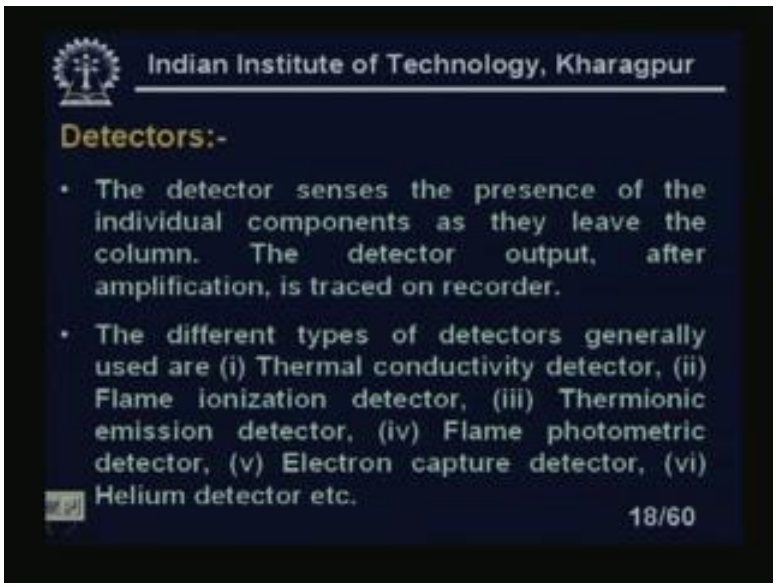

Detectors now detectors will discuss in very much detail as I told you. The detector senses the presence of the individual components as they leave the column as they leave

the column. So, we will have, so all the detectors we have injection systems we have carrier gas we have a solvent pump everything is there. So, it will go to the column from the column it will come out it will elute depending on the absorbent material absorbent material and ultimately it is come to the. So, all the columns will be followed by detector and detector will be followed by another recorder. The detector output after amplification is traced on a recorder as I told you right. The different types of detectors generally used are thermal conductivity detectors flame ionization detectors, thermionic emission detector, flame photometric detector, electron capture detector and helium detector. So, we will discuss some of the things. So, we will discuss some not all we will discuss in this case. So, that anybody can refer to some standard book on the gas chromatography for this.

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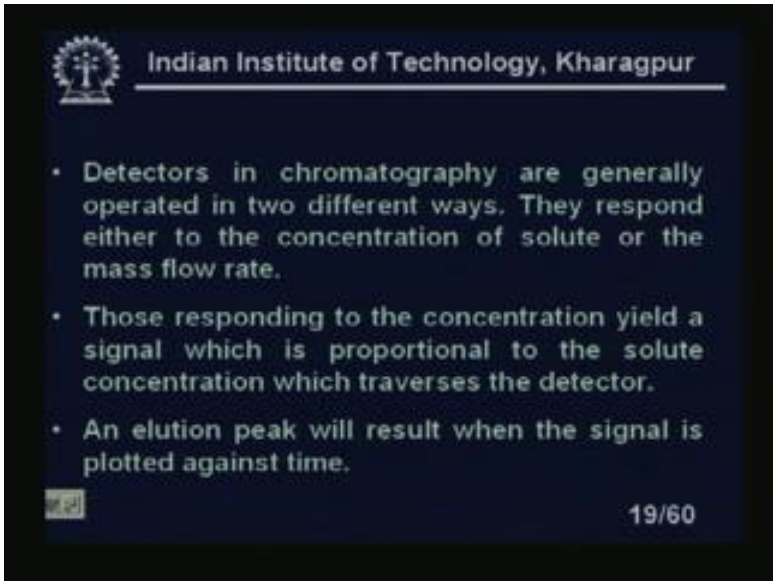

Detectors in chromatography are generally operated in 2 different ways they respond either to the concentration of the solute or the mass flow rate. Those responding to the concentration yield a signal which is proportional to the solute concentration which traverses the detector. An elution peak will resolve will resolve when the signal is plotted against time right. So, there can be different components. So, how does it look? Let us look at.

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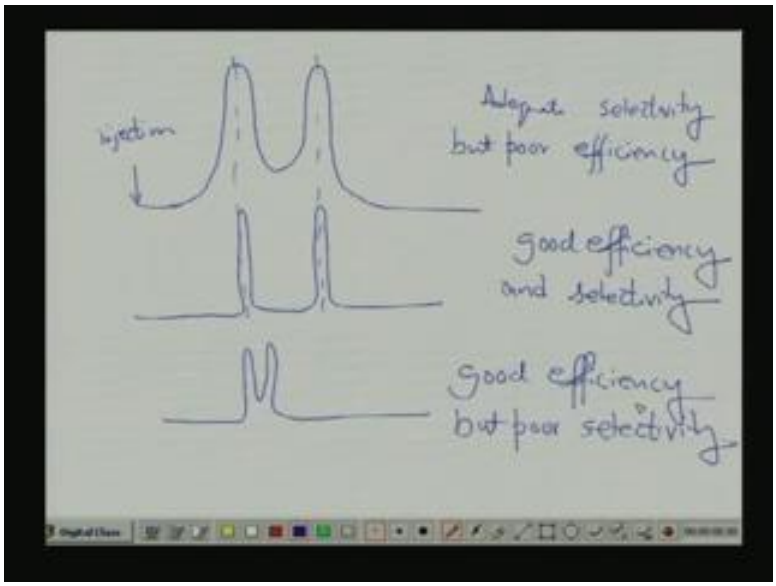

You see here that the selectivity efficiency this is very important in the case of the peak right. So, those are the things we should discuss what is the selectivity? What is the efficiency? Let us look at, I have you see here this is a injections I have made here this is adequate selectivity, but poor efficiency. You see here good efficiency and selectivity. This is the desired characteristics and selectivity and you see this another one where you can see like this one. This is a good efficiency, but poor selectivity. So, selectivity efficiency I mean everything we have discussed for the 2 columns in this particular slide right.

So, these are the desired characteristics for any I mean the peak should be look like this one. So, who will decide this one actually this will be decided in the detector itself efficiency of the detector selectivity of the detector. So, the two most important thing for the detector is the selectivity and efficiency and also the partition ratio right. So, the elution peak that I just drawn elution peak the peak is that what I we have drawn is for the elution peak right. So, this is the peak which we have drawn talked about. So, this is the suppose…

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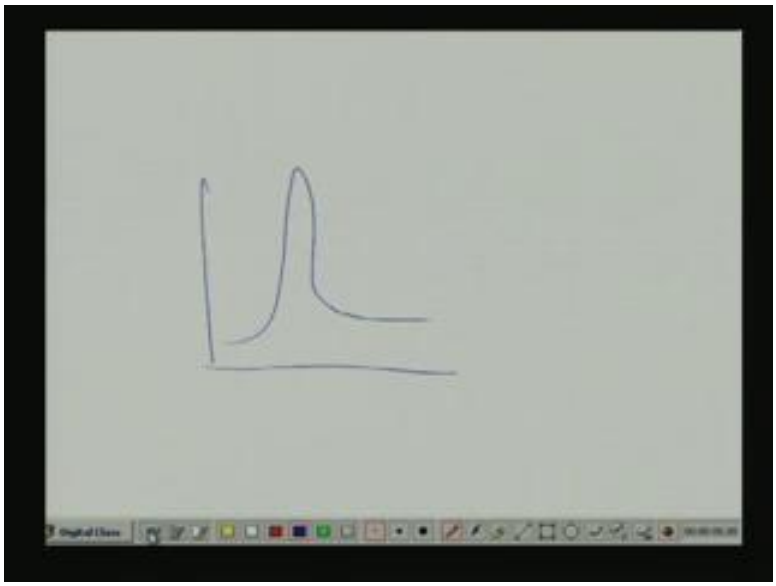

So, this peak is called the elution peak, because it will come out of the gas and suddenly it will detect at the output.

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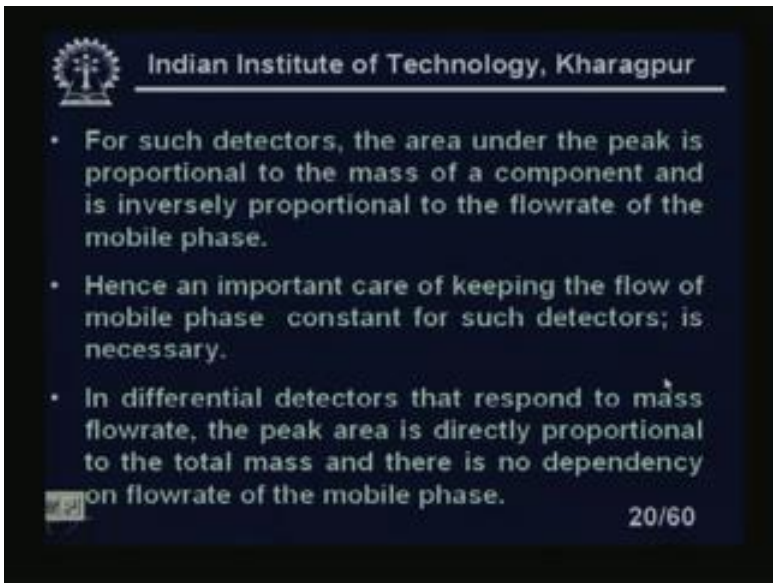

For such detectors the area under the peak is proportional to the mass of a component and the inversely proportional to the flow rate of the mobile phase. Hence an important care of keeping the flow of mobile phase constant for such detector is necessary. In differential detectors that responds to the mass flow rate. The peak area is directly proportional to the total mass and there is no dependency on flow rate of the mobile

phase, you see let us go back first of all. Now, you see when I talked about the thermal conductivity detector. Thermal conductivity detector is the one of the oldest detector and its steel is used because of it is simplicity of the system. It is very simple system and it is very widely used. Data's are available over the years peoples are using these things and it is nondestructive. This is the most important thing for the many of the gas chromatography right.

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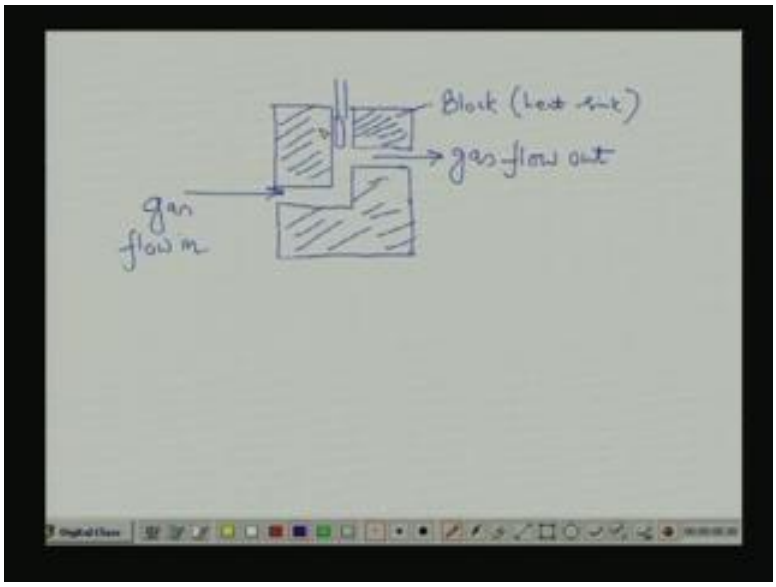

So, let us look the, how it looks? I have a block looks like this. We will take a new page block is there and here actually I will put the detector. So, here it will look like this then it will go like this. So, this is our block, so this is the gas flow in and this is the gas flow out and this is the block. It will work as a heat sink please note work as a heat sink, because there is a some sort of heating here and these actually, if I want to make the it is independents of the detector. Independents of the other parameters of the detector because detector output should be should be solely depends on the, I mean different components of the or the elution peak. So, in that type of cases the, it should not influence by the any other I mean any other component any other components of the other detector itself. So, that is the reason we are using 2 detectors. So, let us look at how the 2 detectors methods are using.

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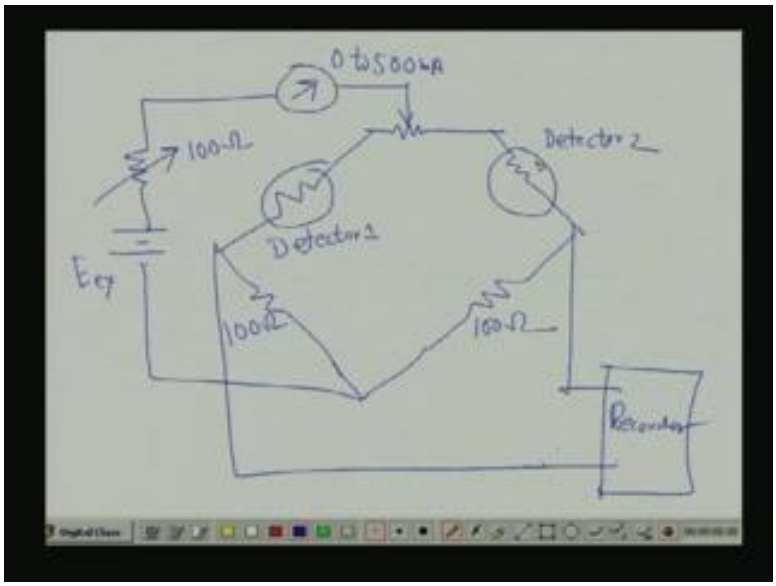

It looks like this we have a one detector here. Then we have a here 100 ohm. This is 100 ohm which balanced though there is an ammeter 0 to 500 milliampere. This is coming through a resistance 100 ohm it is coming here. So, this is excitation voltage Ex; this is excitation. So, this is coming to the recorder, because this is the unbalanced voltage. So, this is the detector 1 and this is the detector 2. So, same carrier gas is passing through both the detector. So, in both the cavity; that means, cavity means actually we have showing that you can have a four same sensors also. So, it looks like this you see this is the thing let me let me first explain then I will go to that, see 2 detectors are there. So, same carrier gas is passing through the both the detectors, but there is no column or anything on the detector 2.

Initially what we will do? I will balance with the by varying this potentiometer I will balance the bridge. So, whenever the detector I mean I mean elution peaks comes; that means, when the gas is coming out of the elution peaks. So, detector output will change because the there is a thermal conductivity there. So, it will change, so automatically if it changes I will get a unbalanced voltage that will be recorded in the recorder right. Now, again you see the in the hydrogen flame ionization detectors for high sensitivity analysis of organic compounds. Hydrogen flame ionization detector is used that is different thing we will discuss that thing also. So, 2 detectors are using you see we can have 4 detectors also 4 detectors in one block itself, right.

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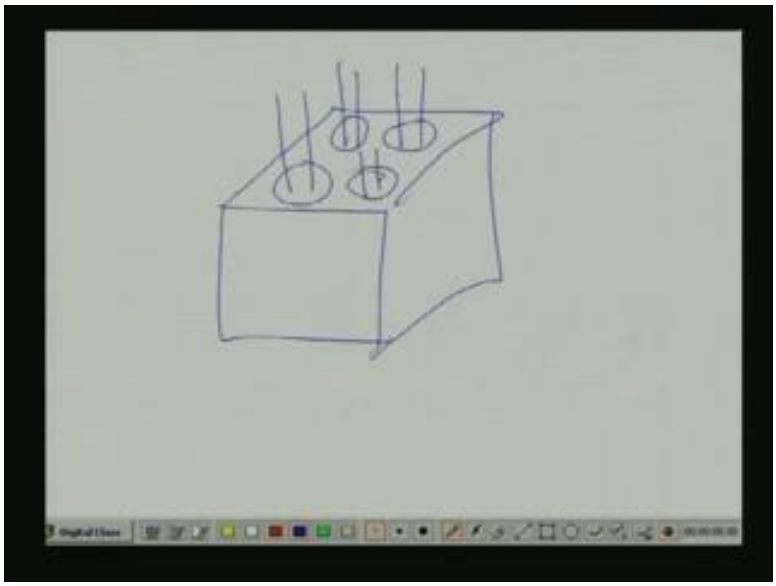

It looks like this, you see... So, what they have? We have 1 4detectors is a block. I can have 2 detectors and I can have 4 detectors also. It is coming like this one right. Let us go back now.

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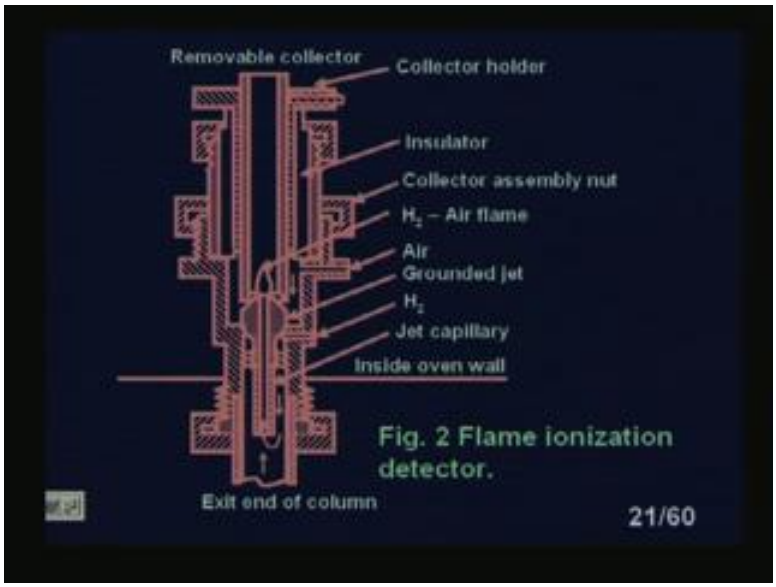

Now, you see this is the flame ionization detector as I told you this flame ionization detector is one of the detectors since the different components in the flame ionization detectors. This is a exit end of the column let us look at you see here a jet capillary is there it is the I mean flowing through this one. This is the hydrogen air flame and air is

coming through this one and this is with the air and hydrogen. So, it is I mean. So, we have a collector assembly nut. We have insulators and we have a collector holder. We are putting across that high voltage inside the detectors plates. So, what is this? Let us explain the flame ionization detector is currently is one of the most popular detectors, because of its high sensitivity wide range and great reliability. As shown in the figures I mean this particular figure; figure 2 I think figure two the column affluent enters the nut base through the multiple filters.

So, through this one this is enters and it is mixed with the hydrogen gas and mixture bonds at the tip. This is the tip of the hydrogen you see this is the tip let me. So, this is the tip you can see here this is our tip hydrogen tip. The jet air with the air or oxygen, because through air it is coming. So, it is going down and is bonding. Ions and the free electrons are formed in the flame and these enter the gap between the 2 electrodes. We have 2 electrodes and there is a high voltage and the flame jet and the collector which may be a parallel plate or might be a cylindrical. Now, this is mounted around 0.5 to 1 centimeter 1 centimeter above the flame tip. So, above the, around 0.5 either 2 0.5 to 1 centimeter half centimeter to 1 centimeter above the flame tip and across the 2 electrodes a high voltage of 400 volt is applied right. And this lowers the resistance across the gap and a causes a current to flow, right.

Normally an externally bucking voltage is produced to balance the potential generated by the ions and free electrons generated in the pure hydrogen air flame. This ensures that a net current flows only when ionized materials enters the gap and the net current flows when the ionized material enters the gap. Thus enhance enhancing the differential sensitivity of the detector the current flow across the external register is sensed with a voltage drop and is amplified and displayed on a recorder. Now, hydrocarbon groups are introduced into a flame and a complex process takes place in which positively charged carbons spaces and electrons are formed. Now, the current is greatly increased. Now, this flame ionization detector responds only to the substance that produce charged ions when bond in hydrogen air flames that is most important thing. In an organic compound the response is proportional to the number of oxidizable carbon atoms. So, this is basic principles of the flame ionization detector.

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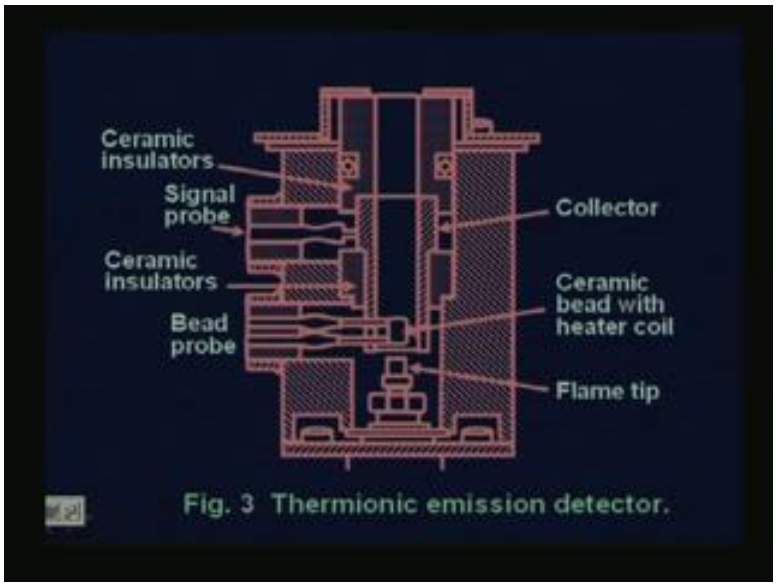

Now, if I look at the thermionic emission detector you see this is thermionic emission detector it is principle is something different. It employs a fuel pore hydrogen plasma. This low temperature source suppresses the normal flame ionization response of a compounds not containing hydrogen and phosphorous. Although the response to carbon is not entirely eliminated a nonvolatile rubidium silicate bead here you see here nonvolatile rubidium silicate bead centered about one point two centimeter above the plasma jet is electrically heated by variable current supply to between six hundred to eight hundred degree centigrade.

This arrangement permits the fine adjustments of the beads temperature an independent of the plasma as a source of thermal energy with a very small hydrogen flow, the detector response to both nitrogen, phosphorous compounds. And enlarging the plasma the changing the polarity between the plasma tube and the collector, and detector response only to the phosphorous compounds. So that I can make it nitrogen I can make it sensitive to the phosphorous compounds also. Now, compared to the compared with the flame ionization detector; the thermal emission detector. Thermionic emission detector is about 50 times more sensitive for nitrogen and about 500 times more sensitive for phosphorous.

So, any traces of phosphorous any traces of nitrogens can be better detectors in the case of I mean a thermionic emission detector compared to the flame ionization detector.

Though flame ionization detector also is comparatively new compared to the thermal conductivity detector. The minimum detectable limit is around 0.06 Pg I mean per second for nitrogen. So, this is the thing you can see here that there is a flame tip which is one point around 1.2 centimeter above the plasma jet. So, this is our, I mean thermionic emission detector. Last we will discuss the electron capture detector.

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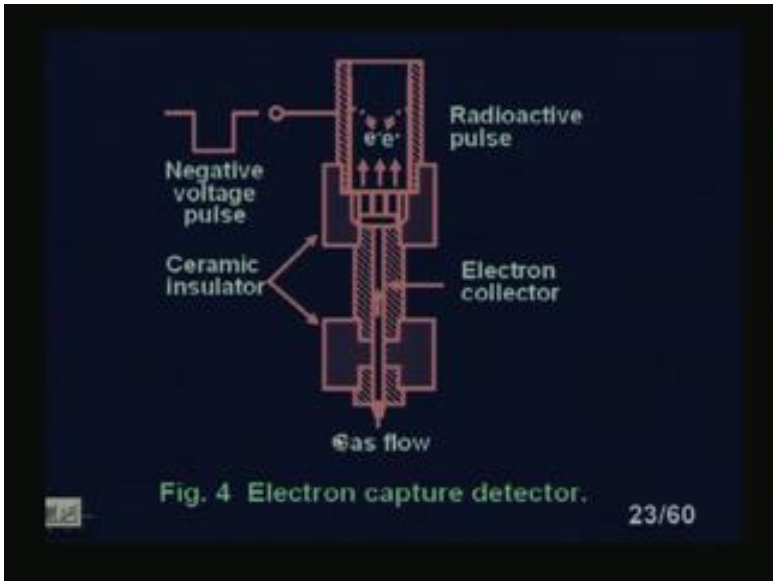

You see this is a electron capture detector. Now, electron capture detector has two electrodes within the, with the column affluent passing between one of the electrodes is treated with radioisotopes. That emits high energy electrons as it decays and this emitted electrons produced. I mean amounts of large amount of low energy thermal secondary electrons in the gas chromatography carrier gas. All of which are collected by other positively polarized electrode either of the positive polarized electrode. Molecules that have an affinity for thermal electrons captured electrons as they pass between the electrodes. And deduce the steady current thus providing a electrical reproductions of the gas chromatography peak of the 2 general designs. The plane parallel and the concentric cell and the lateral design is preferred since it is easier to construct a small low voltage volume cell in this form.

Now, particular radioactive sources which are used that the tritium adsorb in the titanium or scandium and nickel 63 as a foil or plated on the interior of the cathode chamber. Tritium sources have a high specific activity giving a large standing current and high

sensitivity, but the beta energy is. So, low that the sources extremely susceptible to contamination. The maximum working temperature is 225 degree centigrade. Now, nickel 63 is a higher energy sources that can be used up to 400 degree centigrade. So, this you see this is the, I mean we are talking the electron capture detectors. Let us now go back. Now, adsorbent in cases of gas solid chromatography

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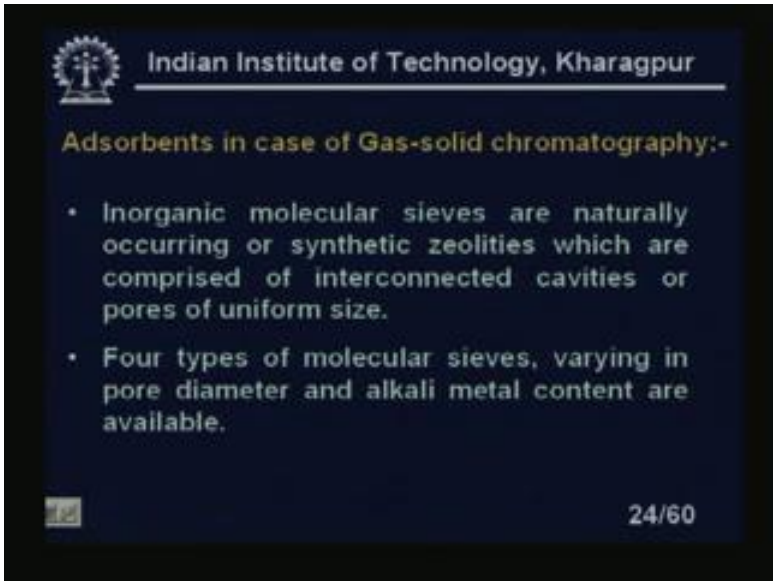

Inorganic molecular sieves are naturally occurring for synthetic or synthetic zeolites which are comprised of interconnected cavities or pores of uniform size. Four types of molecular sieves are varying in pore diameters. This is the absorbent because is different types used are the dimensions of the pores are. So, that the I mean it is a Armstrong level, so that the some of the larger cannot enter the pore some will enter which are smaller in diameter. Varying pore diameter and alkali metals contents are available.

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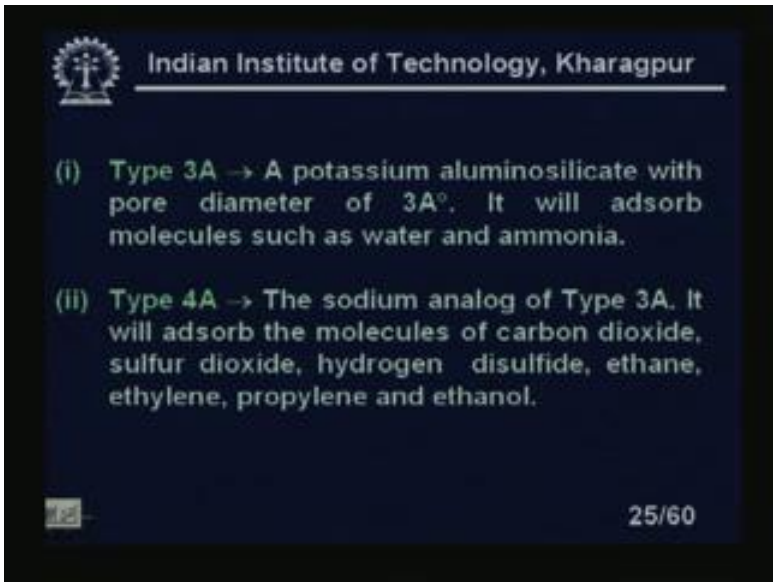

Type 3 A; a potassium aluminosilicate with the pore diameters of 3 Armstrong. It will absorb the molecules such as water and ammonia. It will absorb the molecules of water and ammonia. Type 4 A; it is a sodium analog of type of 3 A; it will absorb the molecules of carbon dioxide, right. Sulfur dioxide, hydrogen disulfide, ethane ethylene, propylene and ethanol, right.

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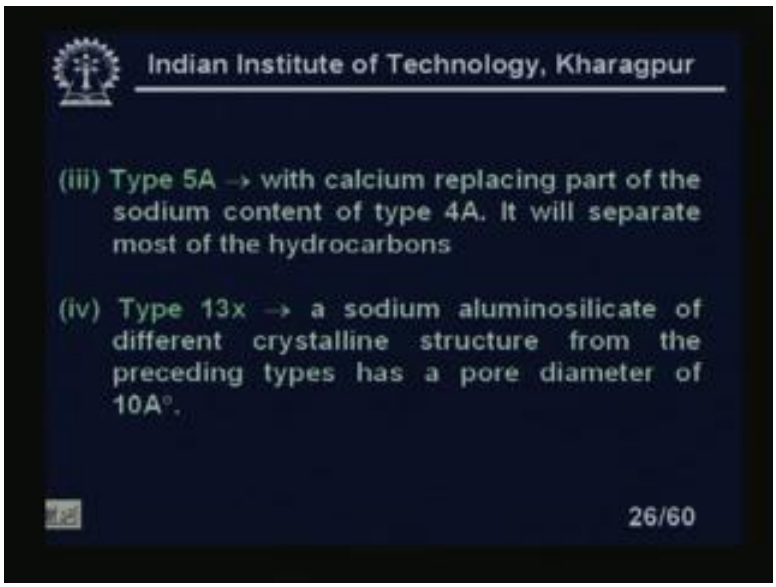

Type five A; with calcium replacing part of the sodium content of type four A and it will separate most of the hydrocarbons. In many I mean this particular industry this is very

important. Type 13 x; a sodium aluminosilicate of different crystalline structure from the preceding types has a pore diameter of 10 Armstrong.

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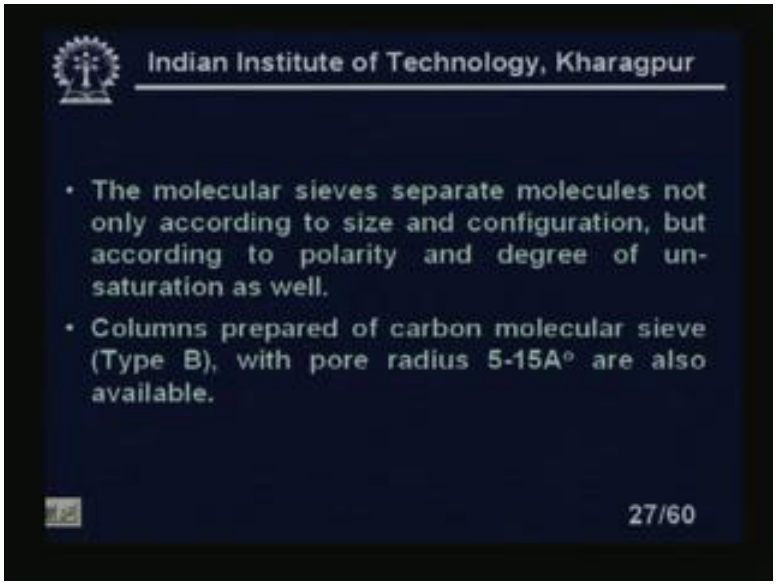

The molecular sieves separate molecules not only according to size and configuration, but according to the polarity and degree of unsaturation as well. Columns prepared from carbon molecules sieve type B with pore radius of 5 to 15 Armstrong are also available.

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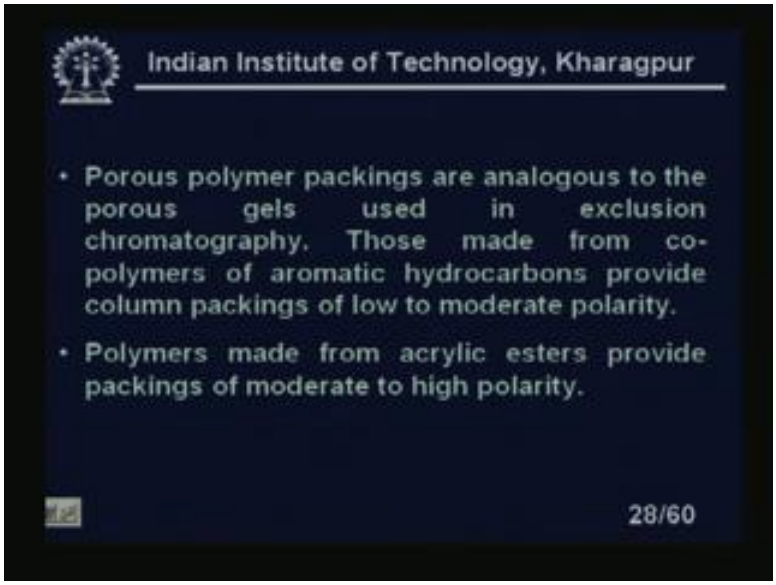

Porous polymer packings are analogous to the porous gels used in the exclusion chromatography. Those made from the copolymers of aromatic hydrocarbons provide

column packings of low to moderate polarity. Polymers made from the acrylic esters and provide the packing of moderate to high polarity.

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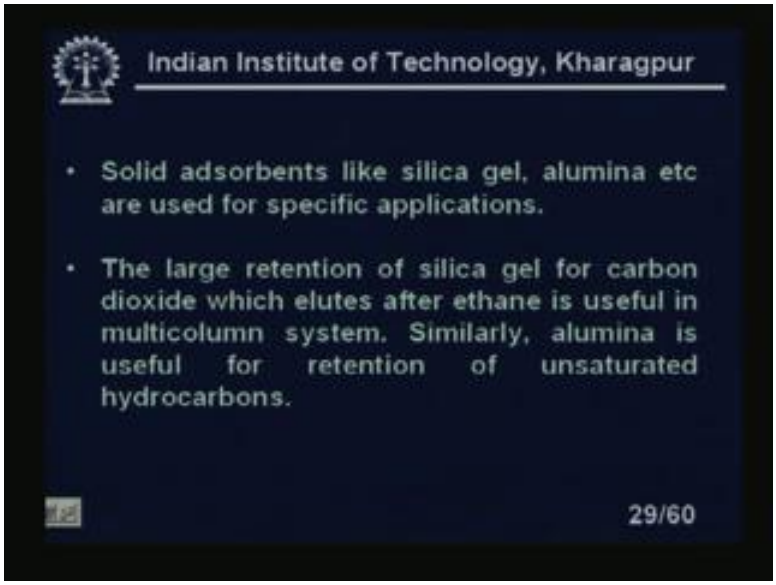

Solid adsorbents like silica gel alumina etcetera are used for specific applications right. So, these are the some specific applications we will used silica gels alumina etcetera right. It is also these are also basically adsorbent please note. The large retention of silica gel for carbon dioxide which elutes after an ethane is useful in multicolumn systems because there is a large retention which is it will retained it for a longer time. So, it will; obviously, help to get a better peak. So, the large retention of the silica gel for carbon dioxide which elutes after ethane is useful in the multicolumn systems. Similarly, alumina is useful for retention of unsaturated hydrocarbons, because unsaturated hydrocarbons. So, it will; obviously, helpful for retention, because we have to retain right. So, that is the thing of the packing materials.

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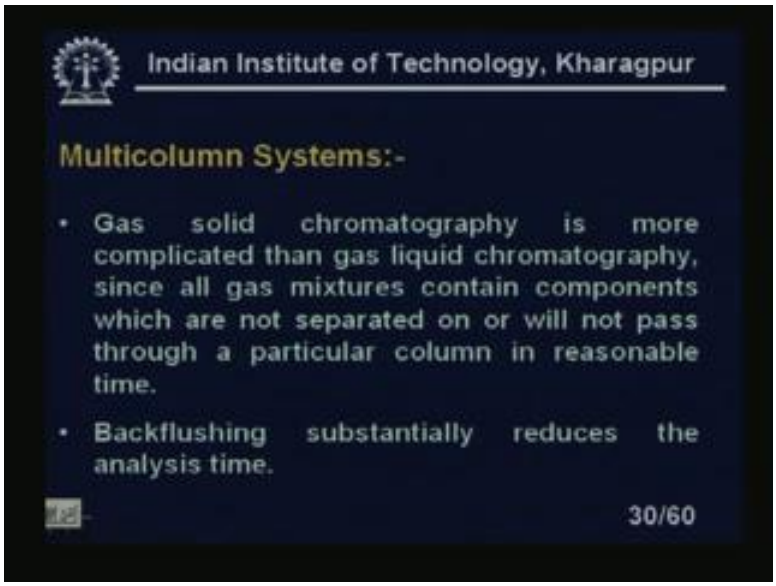

Multicolumn systems gas solid chromatography is more complicated than the gas liquid chromatography. Since all gas mixtures contain components which are not separated on or will not pass through a particular column reasonable time. Backflushing substantially it reduces the analysis time.

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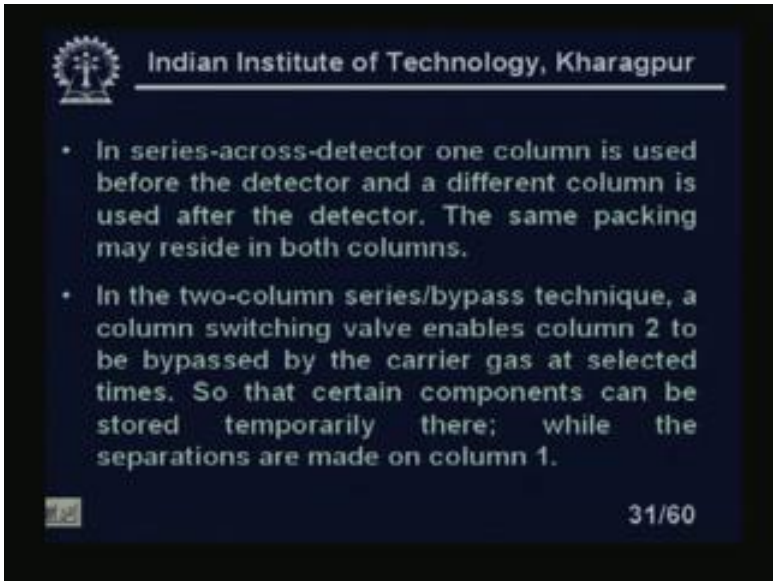

In series-cross-detector across detector one column is used before the detector and a different column is used after the detector. The same packing may reside in both the columns. In two column series bypass technique a column switching valve, valve enables

a column 2 to bypass by the carrier gas and at the selected times. So that the certain components can be stored temporarily there while the separations are made on column 1.

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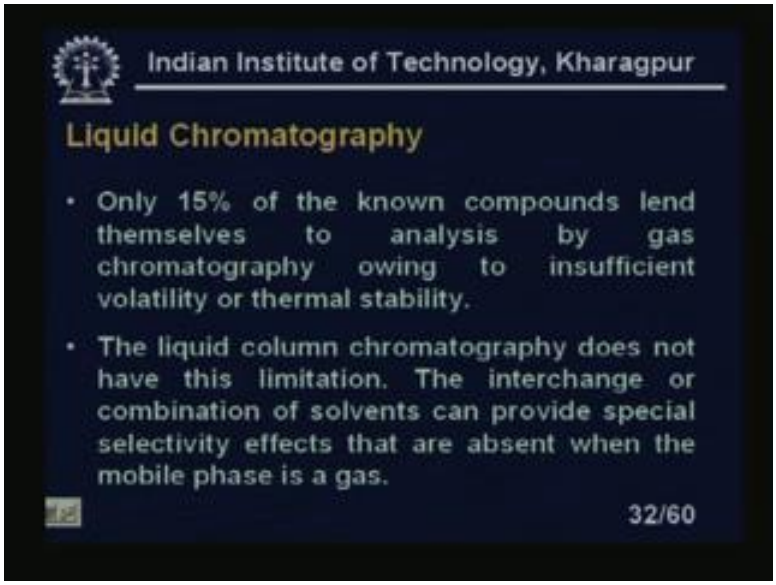

Liquid chromatography let us know only 15 percent of the known compounds lend themselves to the analysis by gas chromatography owing to the insufficient volatility of thermal stability. This is the most important the gas should not vaporize every time we have said right. The liquid column chromatography does not leave the limit this limitation. The interchange or combination of the solvents can provide special selectivity effects that are absent when the mobile phase is a gas.

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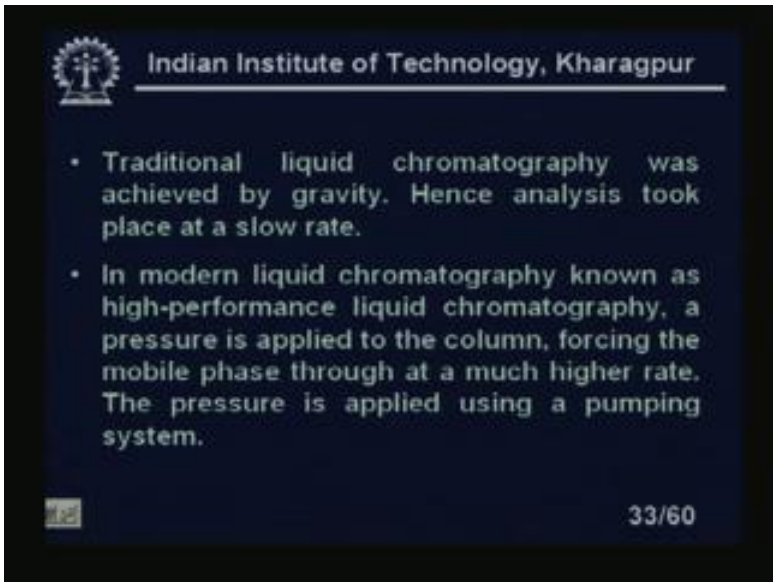

Traditional liquid chromatography was achieved by gravity hence analysis took place at a slow rate. In modern liquid chromatography known as the high performance liquid chromatography, a pressure is applied to the column forcing the mobile phase through the through at a much higher rate and the pressure is applied using a pumping systems. Instead of gravity we are using pumping system.

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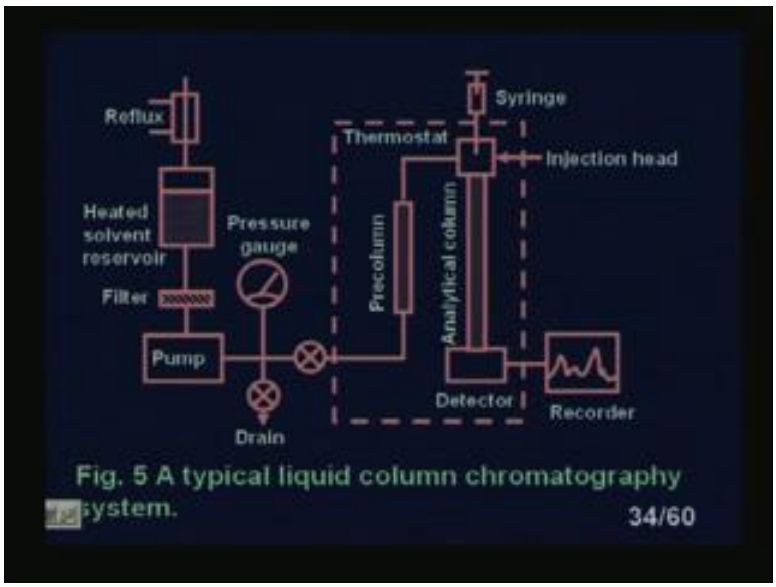

You see this is a typical liquid column chromatography the entire instrumentations we are showing here. We have a reflux systems we can see here. I am sorry you see here.

So, we have a pressure gauge here a filter heated solvent reservoir pump and this is a precolumn and this is analytical column. So, it is a injection head here syringe and there is a detector and this is come to the detector. So, this is entire instrumentation system. So, this thing should be put in a thermostatic path. So, that the constant temperature can be achieved, right.

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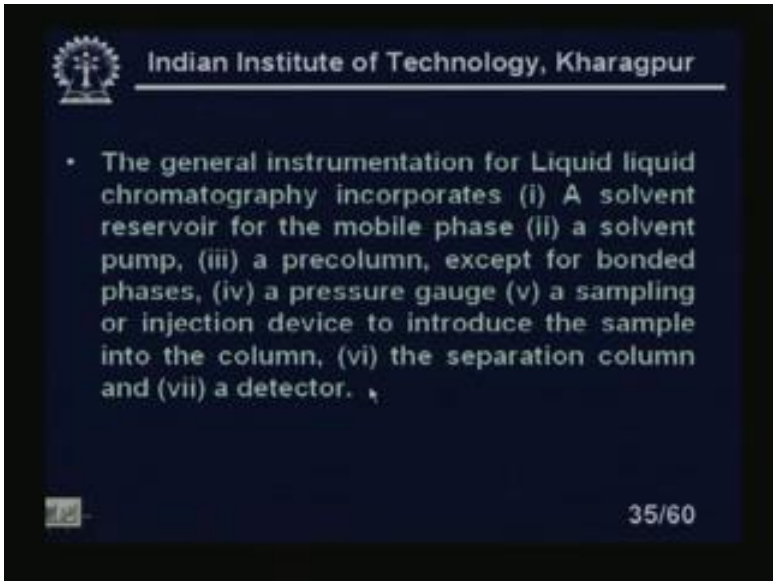

The general instrumentation for liquid liquid chromatography incorporates a solvent reservoir for the mobile phase a solvent pump, a precolumn except for the bonded phases; a pressure gauge; a sampling or injection device to introduce a sample in to the column; a separation column and a detector.

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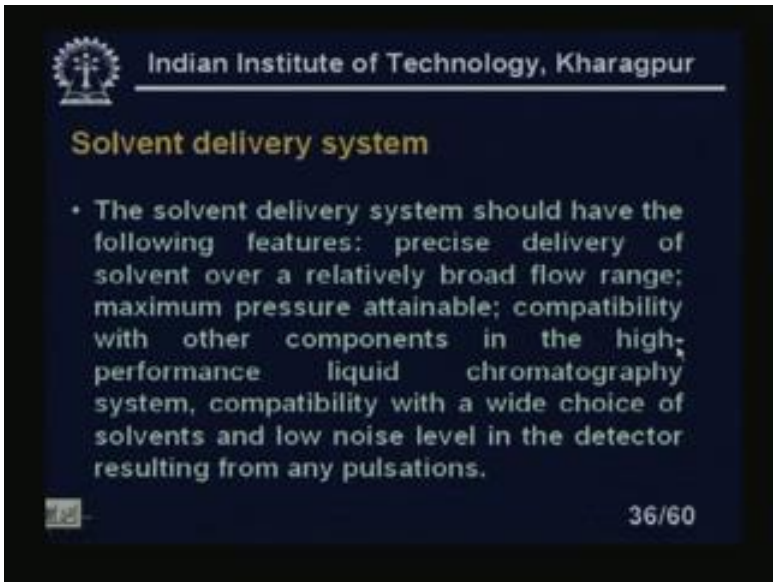

Solvent delivery system; the solvent delivery system should have the following features; precise delivery of solvent over a relatively broad flow range; maximum pressure attainable. Compatibility with other components in the high; performance liquid, chromatography system, compatibility with the wide choice of the solvents and low noise levels in the detector resulting from any pulsations.

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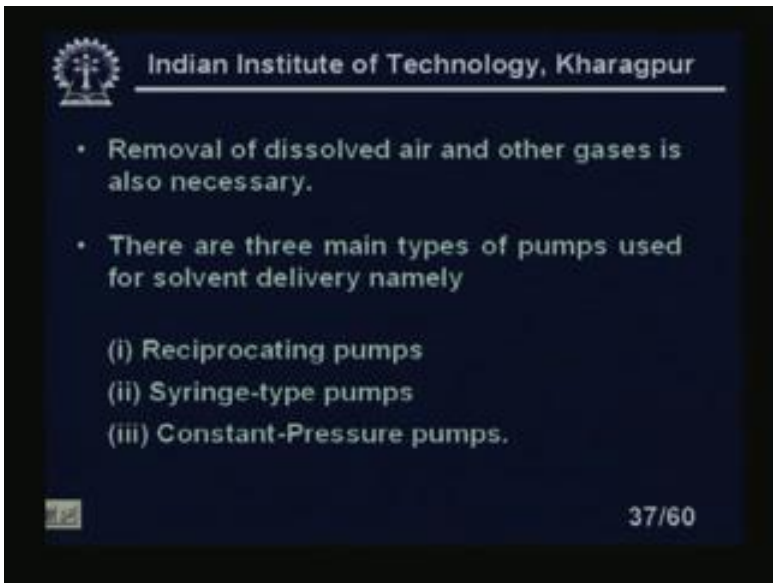

Removal of the dissolved air and other gasses is also necessary. So, there are three main types of delivery pumps used for solvent delivery system namely reciprocating pumps, syringe-type pumps and constant-pressure pumps.

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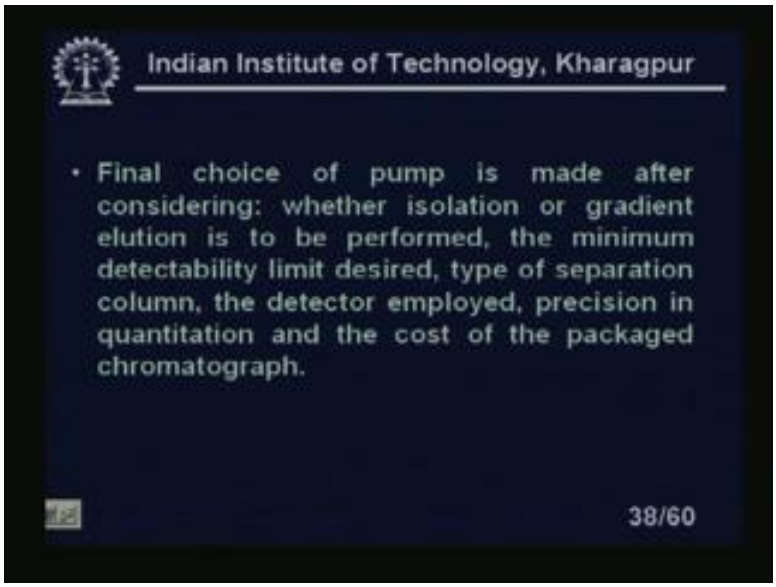

Final choice of pump is made after the considering; whether the isolation or gradient elusion is to be performed and the minimum detectability limit desired type of separation column. The detector employed precision in quantitation and the cost of the packaged chromatograph.

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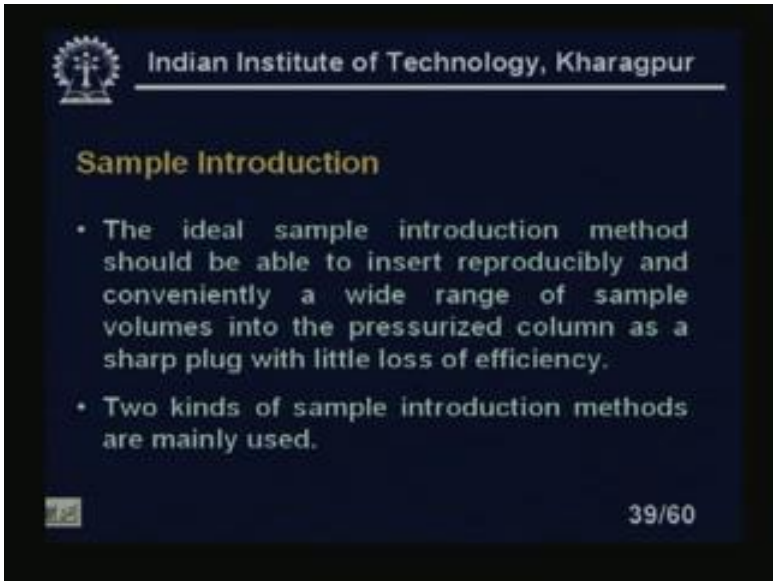

Sample introduction; the ideal sample introduction method should be able to insert reproducibly and conveniently a wide range of sample volumes into the pressurized column as a sharp plug with little loss of efficiency. Two kinds of sample introduction methods are mainly used one is the syringe injection.

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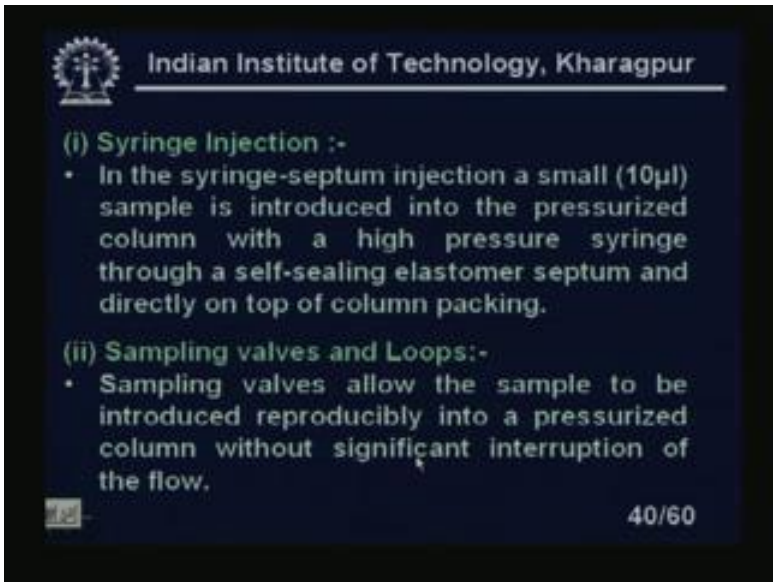

In the syringe septum injection a small 10 microliters already we discussed the smaller the amount better is the peak sample is introduced into the pressurized column with a high pressure syringe to a self ceiling elastomer septum and directly on top of the column packing. It is to be given on the top of the column packings we have seen that thing. If you look at the figures you will find you see syringe it is at the top of the column packings we are giving here. You see here top of the column packing we are giving sample introduction right. Now, sampling valves and loops sampling valves allows the sample to be introduced reproducibly into a pressurized column without significant interruption of flow.

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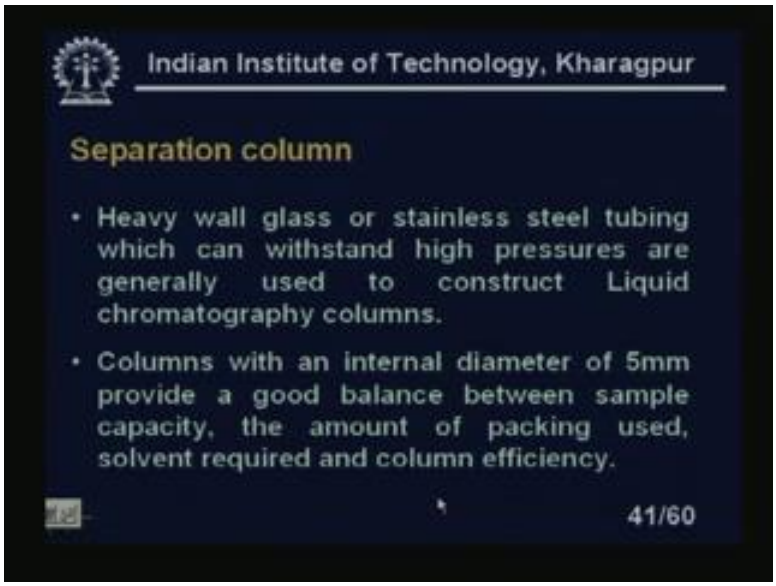

Separation column; heavy wall glass or stainless tubing which can withstand high pressures are generally used to construct liquid chromatographic columns. Columns with an internal diameter of 5 millimeter provide a good balance between the sample capacity. The amount of packing used the solvent required and column efficiency.

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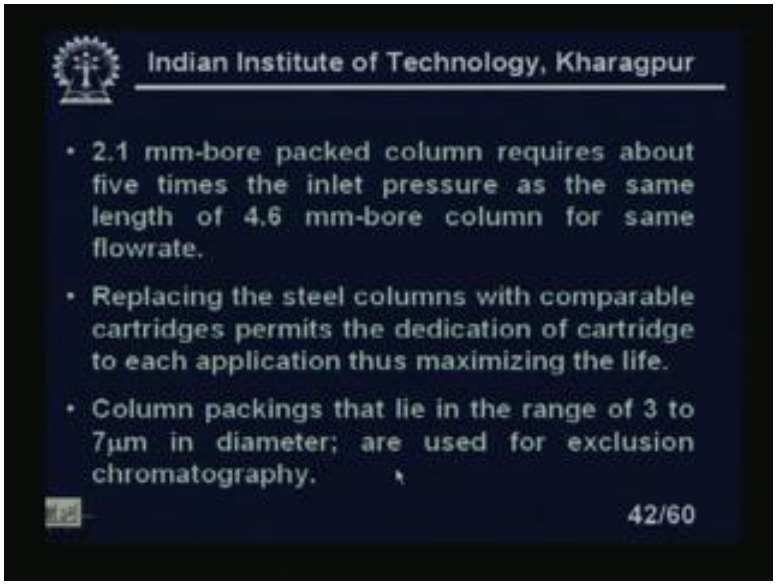

2.1 millimeter bore packed column requires about five times the inlet pressure or as the same length of the 4.6 millimeter bore column for the same flow rate. Replacing the steel columns excuse me steel columns with comparable cartridges permits the cartridges

permits the dedication of the cartridge to each application thus maximizing the life. Column packing that lie in the range of three to seven micrometre in diameter are used for exclusion chromatography.

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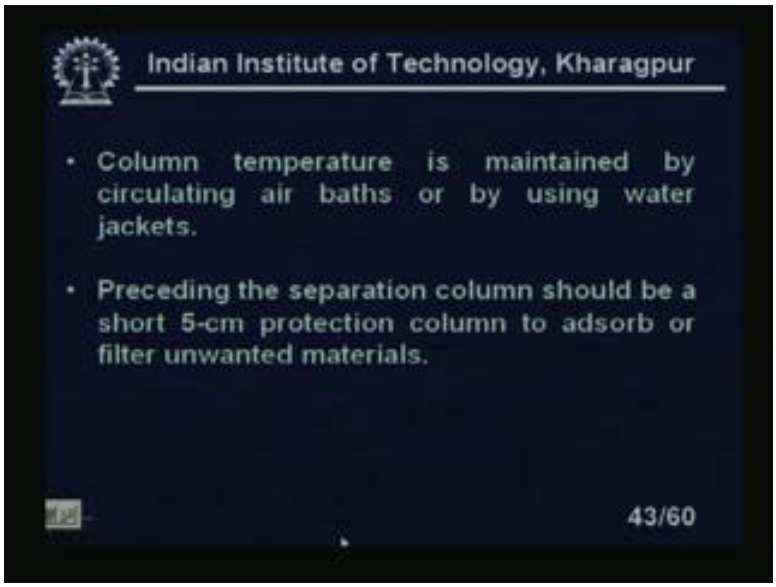

Column temperature is maintained by circulating air baths or by using water jackets or thermostats, that is already we have discussed it. Oven is nothing but a thermostat to add it is constant temperature is maintained or you can we can make I mean instead of oven we can circulate with the jackets where the constant temperature. And you can by controlling the circulating waters we can control the temperatures of the oven also of the column. Preceding the separation column should be short five centimeter protection column to adsorb or filter unwanted materials. We can see there in the, if you go back to the initial slide you will find on the instrumentation systems we have a filter. So, this will be I mean filter all the unabsorbed unwanted materials.

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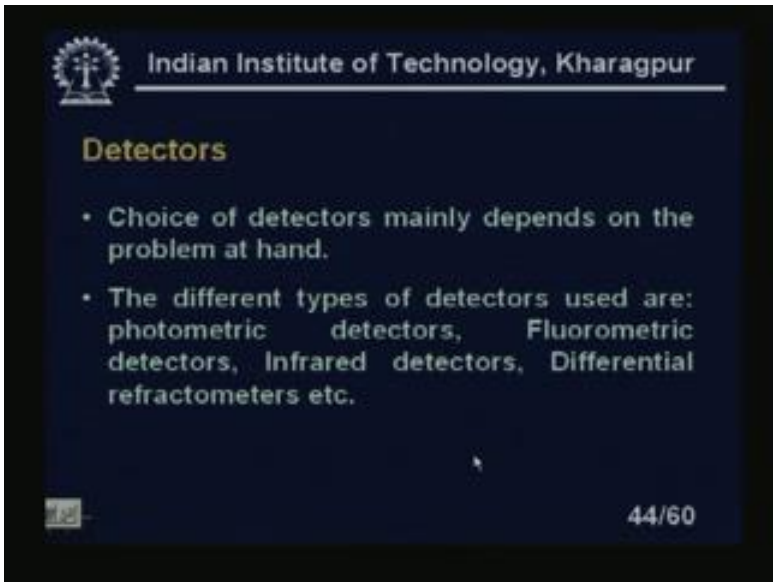

Detectors choice of detectors mainly depends on the problem at hand. The different types of detectors used we have photometric detectors, fluorometric detectors, infrared detectors, differential refractometers.

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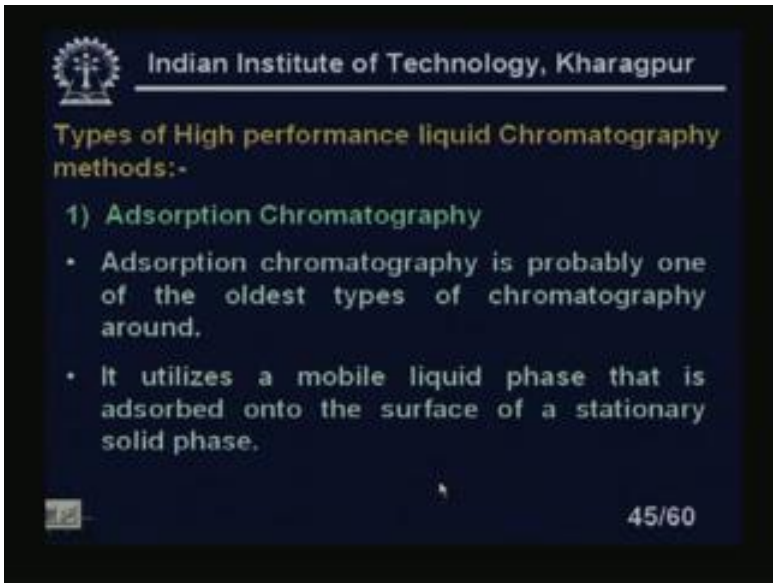

Types of high performance liquid chromatography methods; adsorption chromatography; adsorption chromatography is probably one of the oldest type of chromatography around. It utilizes the mobile liquid phase that is adsorbed onto the surface of the stationary solid phase.

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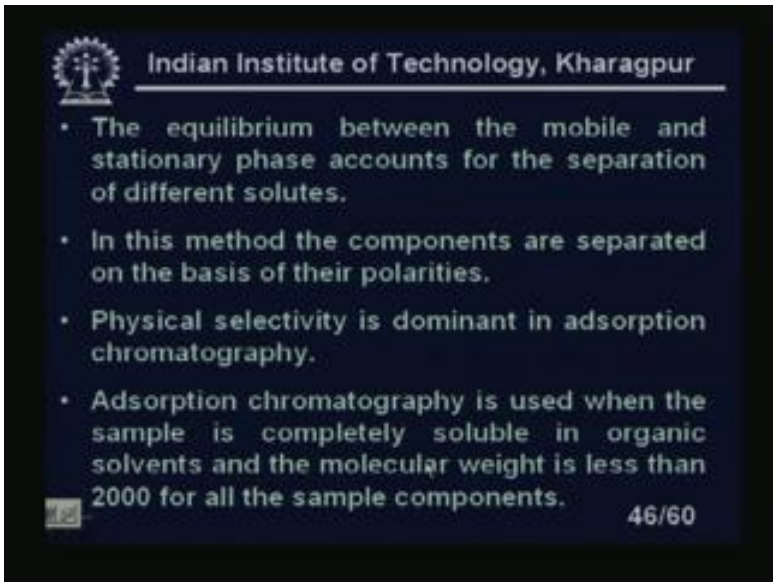

The equilibrium between the mobile and the stationary phase accounts for the separation of the different solutes. In this method the components are separated on the basis of their polarities. Physical selectivity is dominant in adsorption chromatography. Adsorption chromatography is used when the sample is completely soluble in organic solvents and the molecular weight is less than 2000 for all the sample components. We have for more than 2000 we have some different methods.

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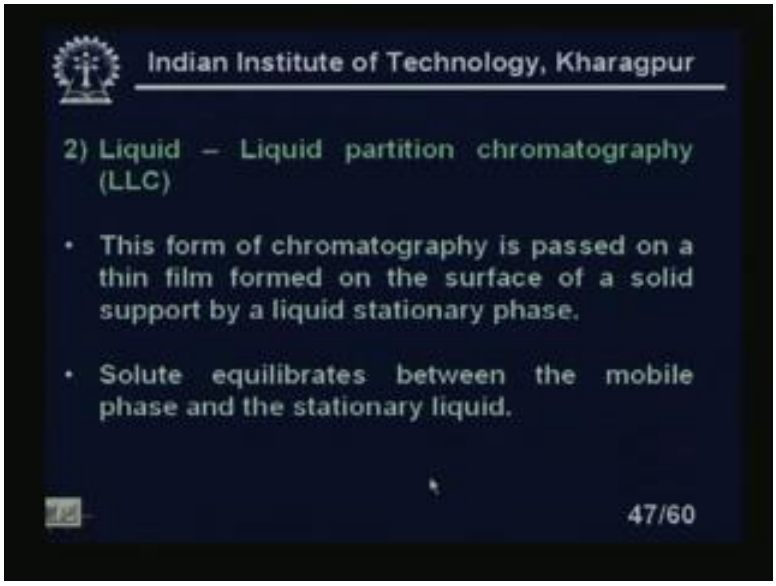

Liquid-liquid partition chromatography; this form is chromatography is passed on the thin film formed on the surface of a solid support by a liquid stationary phase clear? Solute equilibrates between the mobile phase and the stationary liquid.

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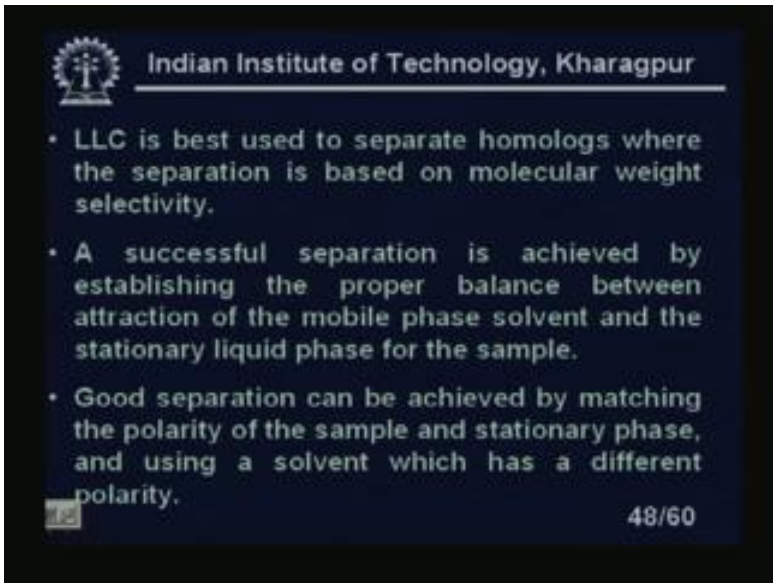

LLC is best used to separate homologs where the separation is based on the molecular weights selectivity right. A successful separation is achieved by establishing the proper balance between the attraction of the mobile phase solvent and the stationary liquid phase for the sample. Good separation can be achieved by matching the polarity of the sample and the stationary phase and using a solvent which has different polarity.

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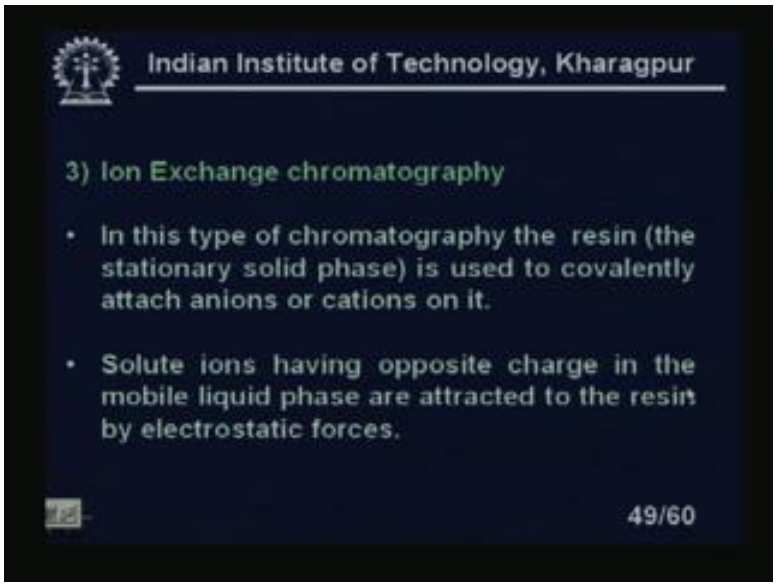

Ion exchange chromatography; here in this type of chromatography the resin the stationary solid phase is used to covalently at attach anions or cations on it, right. Solute ions having opposite charge in the mobile liquid phase are attracted to the resin by electrostatic forces right.

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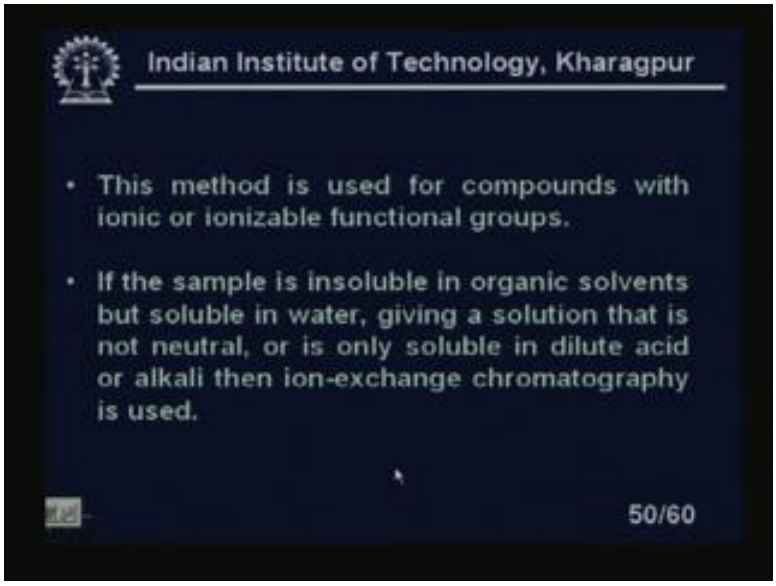

This method is used for compounds with the ionic and ionizable functional groups. If the sample is the insoluble in the organic solvents, but soluble in water giving a solution that

is not neutral or is only soluble in dilute acid or alkali then ion-exchange chromatography is used.

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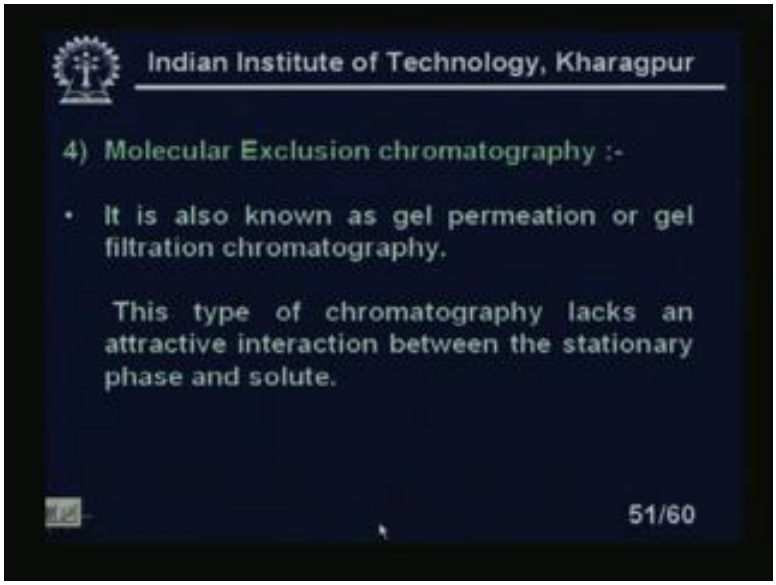

Molecular exclusion chromatography this is also known as known as a exclusion chromatography sometimes it is a simple exclusion chromatography. But actually it is a molecular exclusion chromatography let us look at it is also known as the gel permeation or gel filtration chromatography. This type of chromatography lacks an attractive interaction between the stationary phase and the solute sometimes there is a, this desirable properties.

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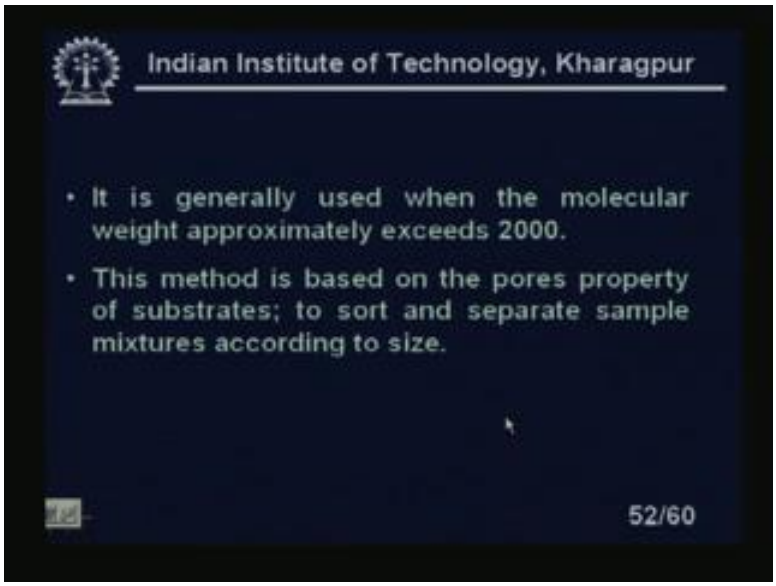

It is generally used when the molecular weight approximately exceeds 2000s in case where it is less than 2000 we have adsorbent method here we have a molecular exclusion. I mean gas chromatography which is used for the molecular when the molecular weights more than, approximately exceeds 2000. This method is based on the pores property of the substrate to sort and separate sample mixtures according to size.

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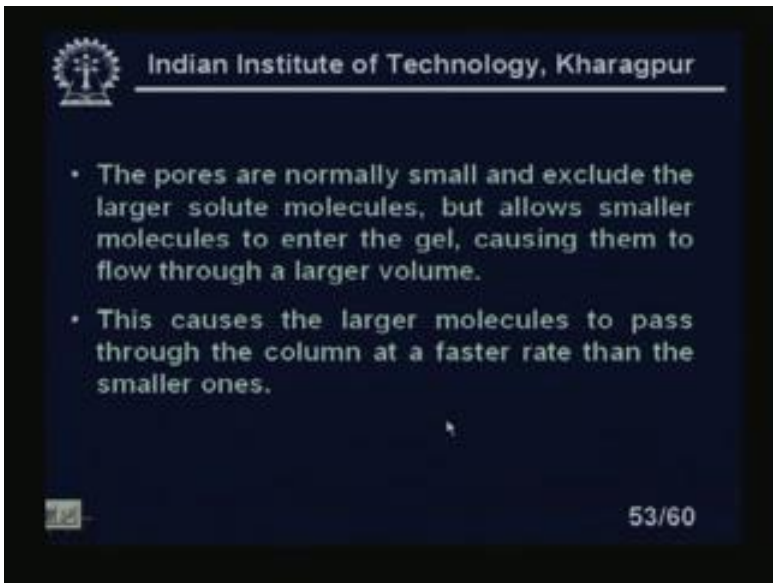

The pores are normally small and exclude the larger solute molecules, but allows smaller molecules to enter the gel causing them to flow through the larger volume. This causes

the larger molecules to pass through the column at a faster rate than the smaller ones right. So, the pores are normally small and exclude the larger solute molecules, but allows smaller molecules to enter gel causing them to flow through the larger volumes. This causes the larger molecules to pass through the column at a faster rate than smaller ones.

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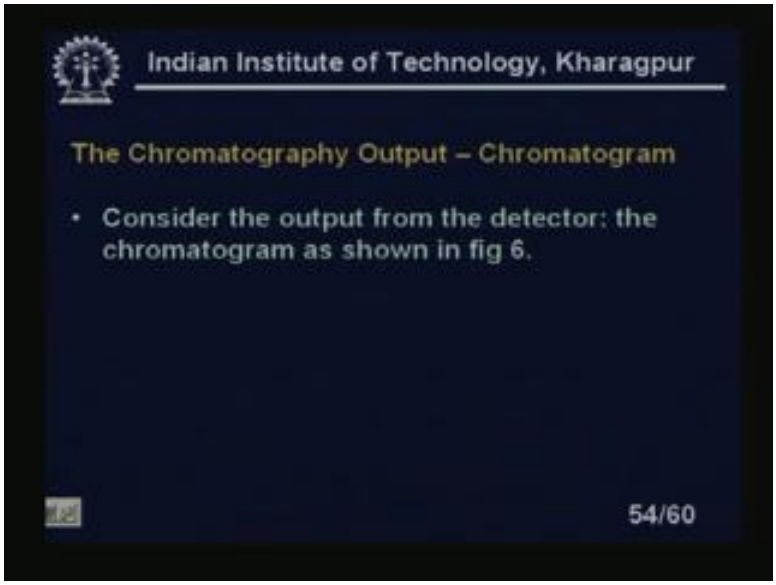

The chromatograph output or which is called the chromatogram. So, let us look at consider the output from the detector that is chromatogram as shown in figure 6.

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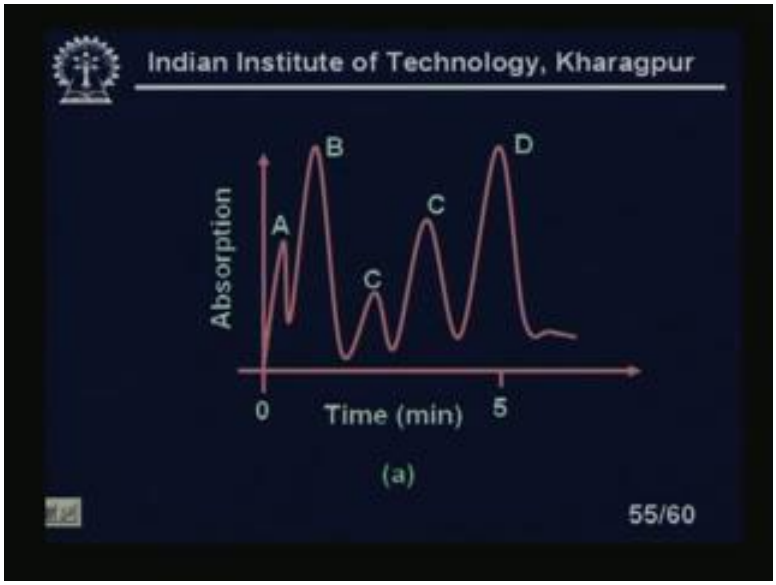

You see this is a typical chromatograph which we got at the detectors or the strip chart recorders although we have different components, we have a gas we have a components of A B C D, this will be I am sorry. So, this will be A B C; this will be D different components and this will be E right.

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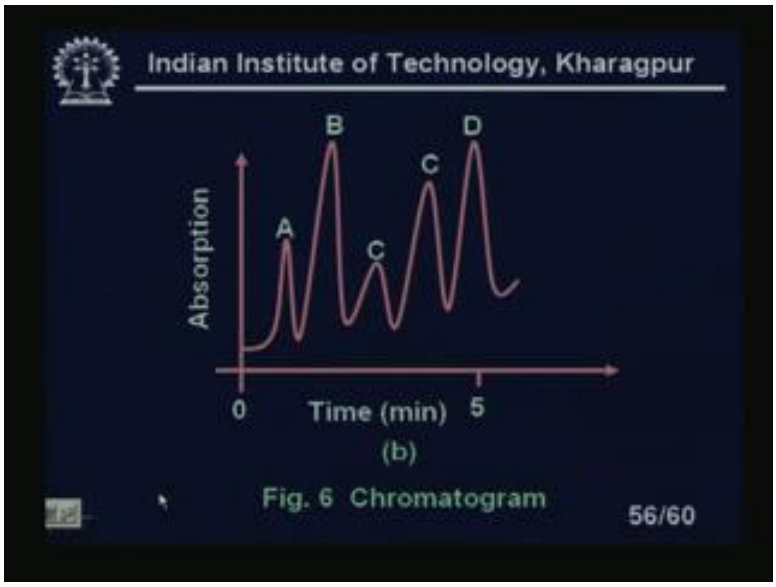

So, this is the chromatogram for the, another one see it is a absorptions as absorptions this is also you see here.

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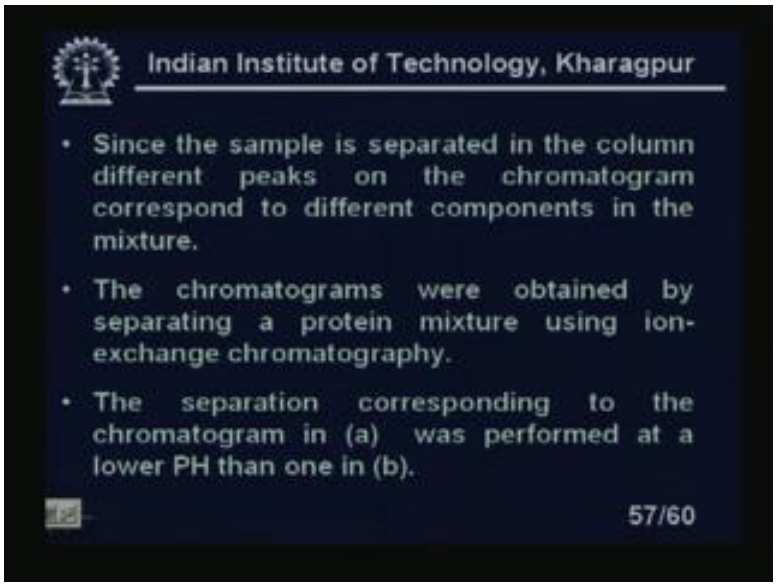

Since, the sample is separated in the column different peaks on the chromatogram corresponds to different components in the mixture clear? Since the sample is separated in the column itself. So, when it is either adsorptions it elutes and come to the detectors. So, it is one by one is detector. Different peaks on the chromatogram corresponds to different components in the mixture this I told you several times. The chromatograms were obtained by separating a protein mixture using ion exchange chromatography depends on the what type of... So, in ion exchange chromatography you can use a protein you can separate a protein mixture. The separation corresponds to the chromatogram and was performed at a lower pH value than 1 in b. If you look at you see the pH value is was performed at lower value in a then b, if a has a lower pH value than b. You see the; obviously, the separation is better in a.

So, this will tell you that is actually the how the pH will affect the actual my chromatogram. So, pH should be maintained to a particular value. So, it should be neither low or neither high like that this about this chromatography we are talking about. Since the sample is separate in the column different peaks in the chromatogram corresponds to different components in the mixture. The chromatograms were obtained by separating a protein mixture using a ion exchange chromatography. The separation corresponding to the chromatogram that is the a was performed at a lower pH value than one in b this actually should like this PH we should not write like this. It should like this PH should like this.

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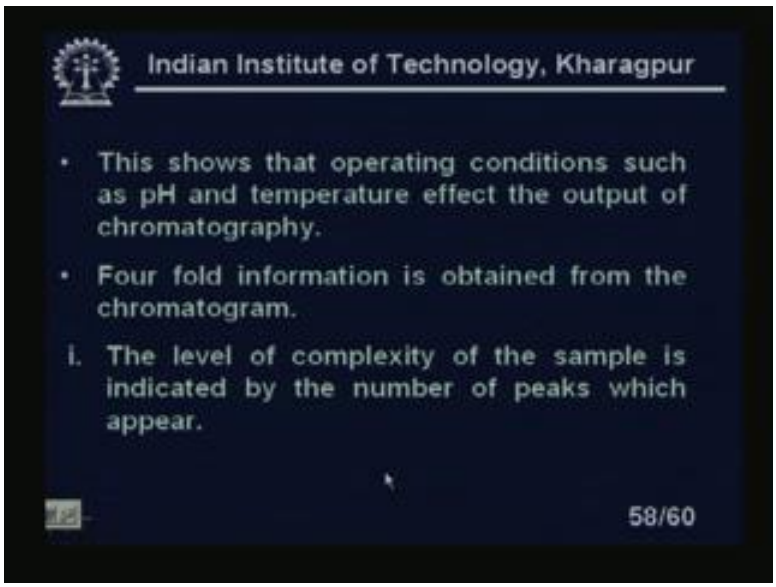

This shows that the operating conditions such as pH and temperature affect the output of the chromatography if the pH changes. So, your chromatograph will change so; obviously, they if the chromatogram change efficiency selectivity everything will. So, this is to be. So, preside whatever the desired value of the chromatogram that is to be maintained right. Four fold information is obtained from the chromatogram, what are the different I mean components I am getting from the chromatogram. What are the different I mean information's I am getting from this chromatogram. Already we have discussed, but let us downright, what one by one. The level of complexity of the sample is indicated by the number of peaks. How many what is the complex mixtures I mean that we can detect from this one. So, level of complexity of the sample is indicated by the number of peaks which appear.

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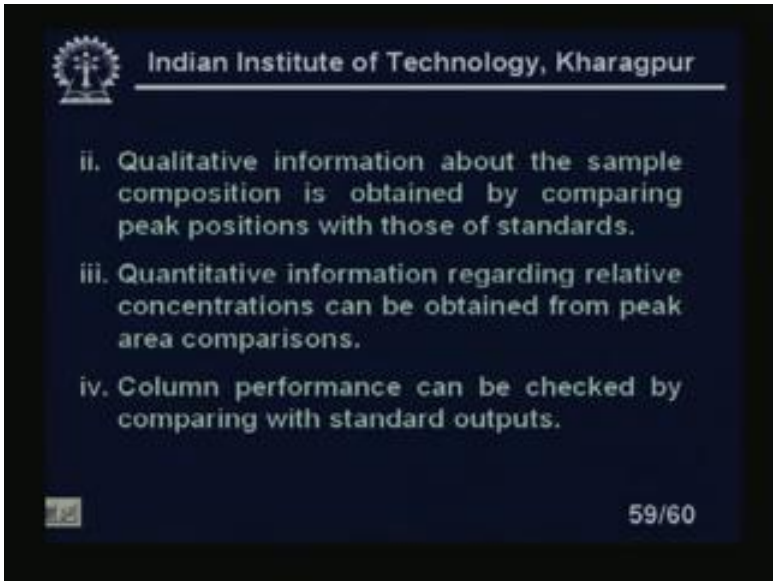

Qualitative information about the sample of composition is obtained by comparing peak positions with those of the standards. Qualitative information about the sample composition is obtained by comparing the peak positions with those of standards. Quantitative information regarding the relative concentrations this is most important. So, we are measuring the relative, because the sample if the we are not absolute measuring we are making relative comparison relative concentrations actually we are measuring the gas chromatography. Because otherwise, we cannot measure, because we need the injections and the so many tin microliter, that is the best smaller the sample better is the detection sorry.

Quantitative information regarding the relative concentration can be obtained from the peak and the area comparison. Column performance can be checked by comparing with the standard outputs. This column performance also because if I have if I know if I knew the particular. Suppose if I want to calibrate the column performance I know the particular gas with which actually we are injecting. So, that time when an unknown gas is coming if I want to calibrate the how the column actually working. So, the column efficiency can also be known I mean I mean can be known by looking at the.., So, these are the 4 I mean features I mean which we will get from the gas chromatography.

And the most important thing is the relative I mean first of all the, what are the different components of the gas present in the mixture? Second thing is the relative this is the most prime importance. Second thing is the relative concentrations of the gas or liquid or whatever it may be in the mixture. So, with this I come to the end of the lesson 36 of industrial instrumentation. Preview of next lecture; welcome the lesson 37 of industrial instrumentation. In this lesson we will study the pollution measurement when we were talking on the pollution. Basically we are talking about the environmental pollution or the air pollutions or the type of gases that brings the carbon monoxide and the nitrogen oxide. All these of type of gases how it is making the air polluted and we must measure it.

Because you know the certain I mean if it crosses some limit that is and it is not safe it is hazardous for the human being. Pollution will be there we cannot in industrial industrializations you know there will be pollutions you cannot avoid pollutions. You have to be with pollutions, but what is the level we must know, because if you I mean stay in some in forest or there is; obviously, the pollution will be less. But if you leave in a city; obviously, there will be some pollution, because of the exhaust of the cars and truck and all those things burning of the fuels burning of the waste. Because if you know the waste is the big problems in a I mean cities or metropolis. They cannot dump it and they usually they burn it, because that will reduce the volume of the waste.

So, that will cause the pollutions? The factories; the power stations all these thing will make the air polluted. Now, we must know what is the concentrations of the different gases? And if it is within the safe limit fine otherwise we have to check it and we have to warn. Our goal is to warn the public or the. So, that they will also know that what type of pollution they are going through and what is the, what should be the safe level of

contents? Now, in some countries we will find that the forest fire that also will cause the pollutions some countries where there is where if the I mean like Australia when it is very dry weathers.

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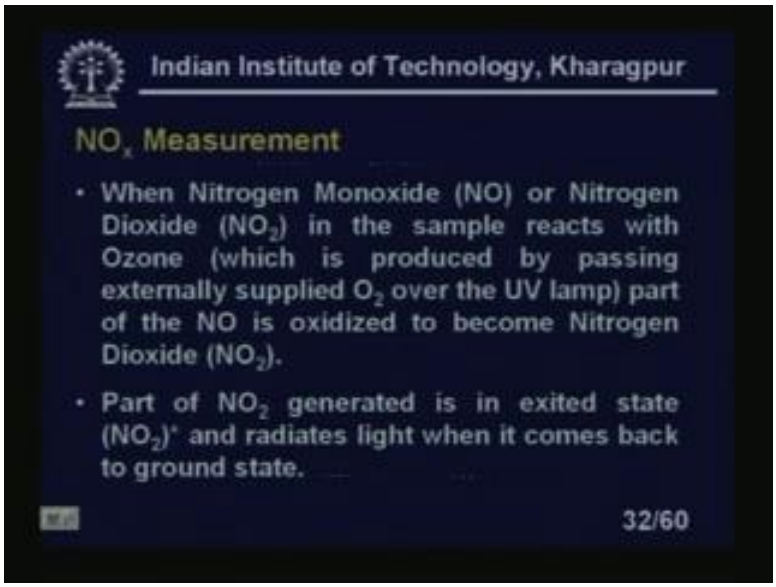

When nitrogen monoxide or nitrogen dioxide in the sample reacts with the original, which is produced by passing externally supplied O2 over UV lamp part of the NO is oxidized to become a nitrogen dioxide right. Part of the NO2 generated in is in the exited state exited states actually we are defining it by this please note.

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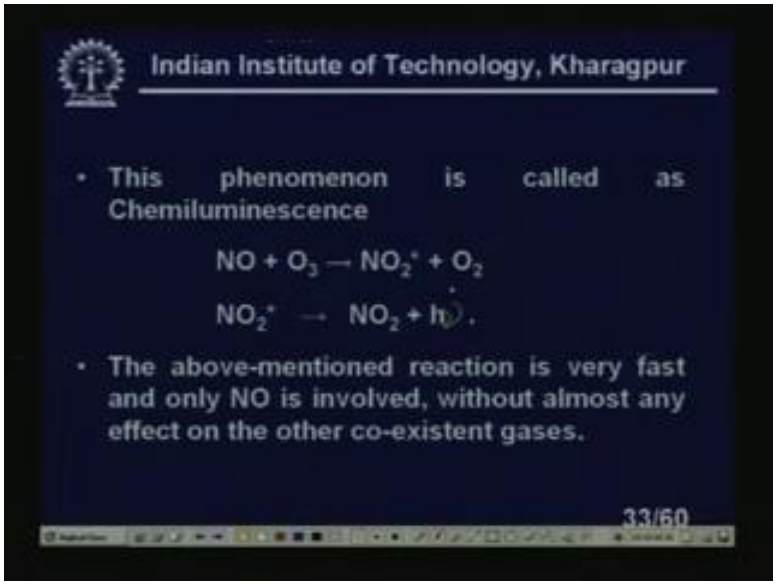

This phenomena is called the chemiluminescence actually you see here. So, we are defining this NO2 and this asteric means it is in exited state. I am sorry it is in exited state NO2 asteric you see it is exited state plus O2. So, it is NO2 exited state it is liberating the actually this will be h mu. I am sorry this will be h mu NO2. Second equations if you see NO2 asteric which goes to NO2 plus h mu right. You can this will be this will be h mu right. So, the light will be emitted, so above mentioned reaction is very fast and only NO is involved without almost any effect of the other co-existent gases. So, this also you should remember because it should not react with the other gases. So, the other concentrations or the other gases cannot be detected by that type of method. So, the phenomena is called the chemiluminescence. So, by this it is a nitrogen oxide reacts with ozone. It is making nitrogen dioxide which is in exited state it will go back to the normal nitrogen dioxide.

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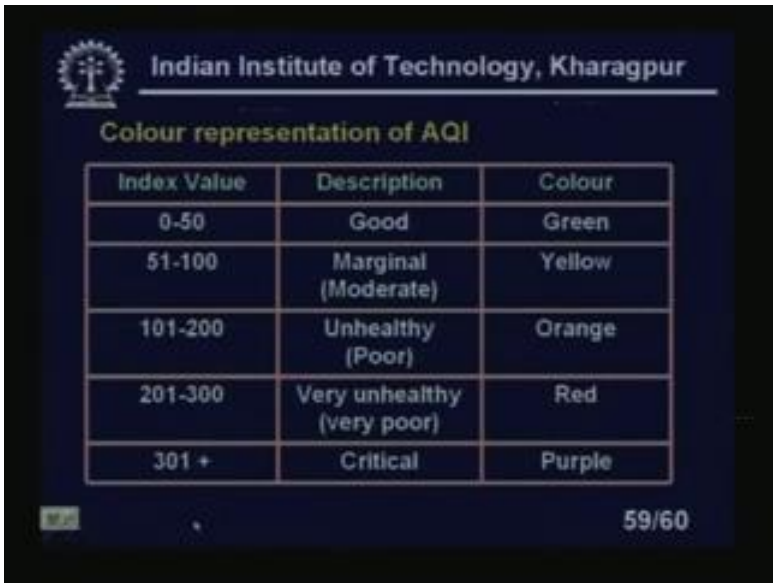

Now, you see we have them now this is a color code of representations of AQI. We have given the color code representations. That is anybody can understand that is in green means always friendly as you know the environment the green environments we are talking about always is a good environment. So, we have given a color of green, so 0 to 5 and my AQI values. So, it is green color it is good 51 to 100 marginal moderates we have yellow. Well, I cannot justify why it is yellow and it is some standard is to be followed. Obviously, the green means that is our environment its green always is better

for us. Then we have a 100 and 1 to 200 unhealthy poor I mean orange. Then we have a 201 to 300 AQI value very unhealthy very poor which is on a red alert.

Red means always alert and 301 last is a critical I mean there is no more for consumptions of the in a I mean for that air is consumption for the human being or any living animals. So, these are things which we have considered in this I what we mean to AQI. So, because if you give the color because it is very difficult to remember all this numbers. So, instead of giving the numbers, so we will measure this one, but once we define when to the public. So, give with this some colors, so that with green it is better yellow is fine orange is not that good. Red is quite bad and if the purple it is extremely bad right. So, with this AQI color coding we can explain to the general public. So, what that, what the color they should have in their particular area of residence or the particular area where they are working? So, with this I come to the end of the lesson 37 of industrial instrumentation.