Chemical Process Instrumentation
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Lecture – 52
Miscellaneous Measurements: Composition (Contd.)

Welcome to lecture 52. In our previous lecture, we started our discussion on concentration measurement. Specifically, we started our discussion on chromatographic method. So, we have seen the basic principle of chromatography. In this week, in this class we will see the more details of the chromatographic systems. We will talk about the detectors in more details, will also talk about the liquid chromatography.

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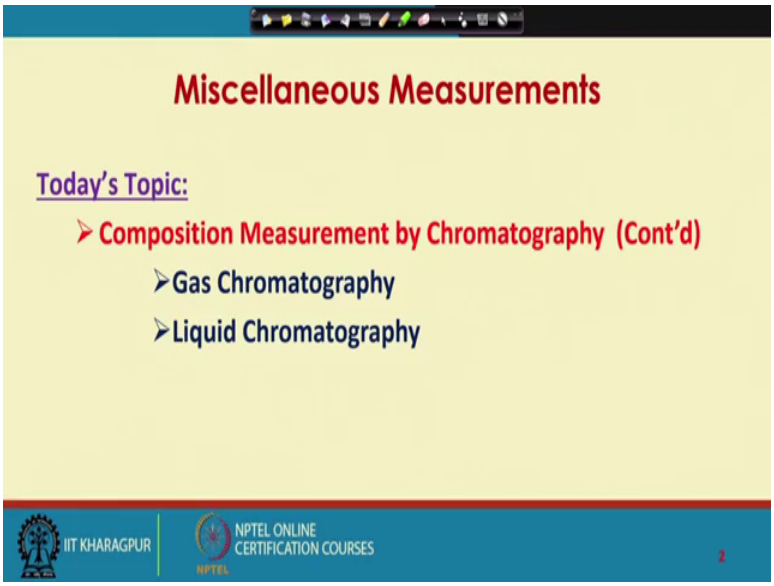

So, will continue our discussion on composition measurement by chromatography.

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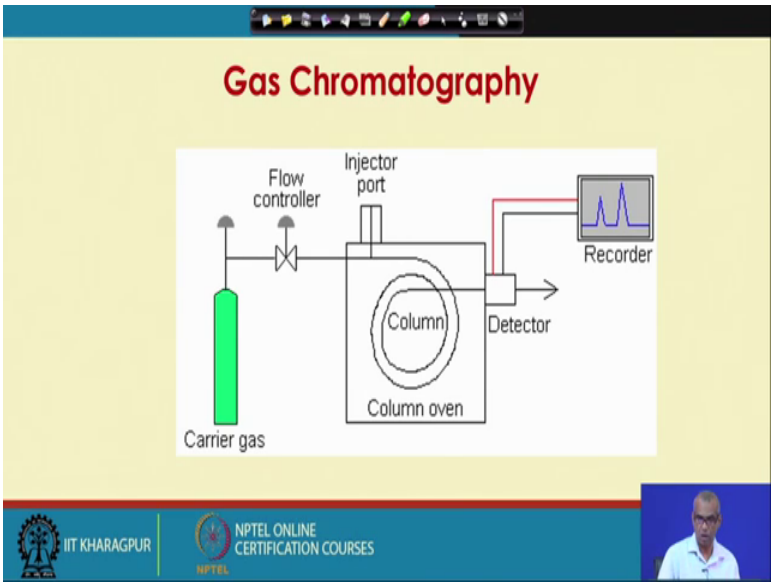

So, these are the various parts of gas chromatography, as we discussed in our previous lecture, you have carrier gas flow controller sample injection system, column you have column oven, detector and finally, the detector gives you the response which is a detector signal versus time graph, which is known as chromatograph.

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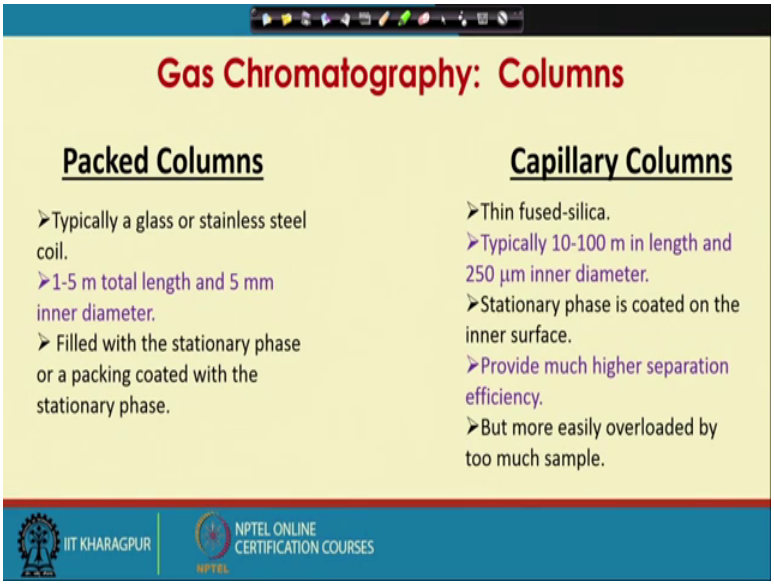

Now, now we will see in more details about the columns as well as detectors, and then we will talk about liquid chromatography. Now there are broadly 2 types of columns, packed columns and capillary columns packed columns. Typically, a glass or stainless-

steel coil. It is length is about 1 to 5 meter, and the inner diameter of the column is typically around 5 millimetre. The packed column is filled with the stationary phase or a packing coated with the stationary phase. The capillary column consists of thin fused silica, typically, the capillary column is of 10-meter 200 meter in length, and 250 micrometre inner diameter.

So, the diameter of capillary column is much less than the diameter of the packed column. In fact, the name also suggests that because it is capillary column. In case of capillary columns, stationary phase is coated on the inner surface. Capillary column provide much higher separation efficiency, but more easily overloaded by too much sample. Please note that the diameter of the capillary column is around 250 micrometre, so small diameter.

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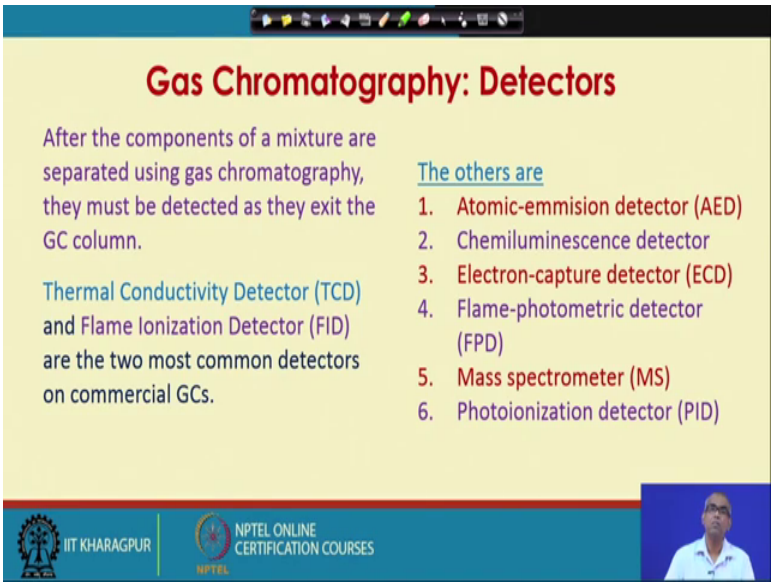

Next, we talk about detectors of gas chromatography. After the components of a mixture are separated using gas chromatography, they must be detected as they exit the gas chromatography column. There are 2 common detectors. Thermal conductivity detector and flame ionization detector are the 2 most common detectors on commercial gas chromatography.

There are other detectors as well, some of the other detectors are atomic emission detector, chemiluminescence detector, electron capture detector, flame photometric detector, mass spectrophotometer, mass spectrometer, photo ionization detector;

however, thermal conductivity detector and flame ionization detector are very common detectors that you find in commercial gas chromatographs, and in this lecture we will very briefly talk about thermal conductivity detector and flame ionization detector.

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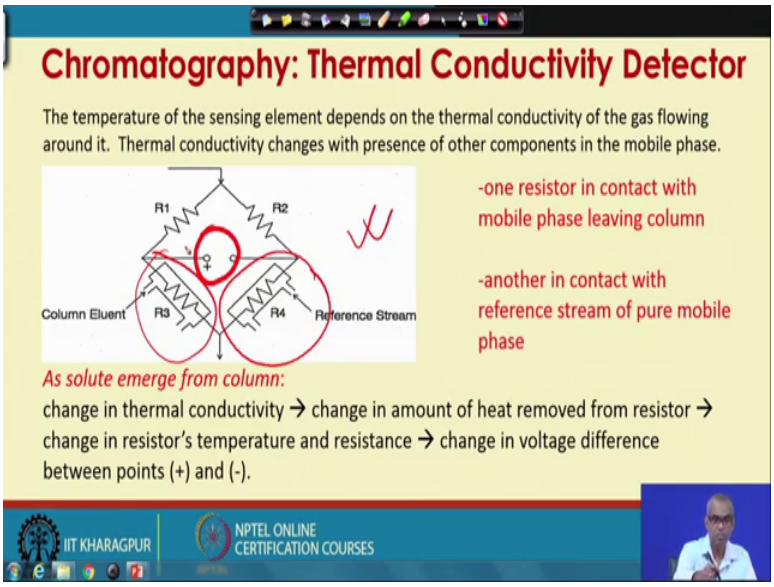

First, let us talk about thermal conductivity detector. The temperature of the sensing element depends on the thermal conductivity of the gas flowing around it. Thermal conductivity changes with presence of other components in the mobile phase. So, first thing to note that the temperature of the sensing element depends on the thermal conductivity of the gas flowing around it. Please note that we have talked about this when we discussed temperature measurement. Thermal conductivity changes with the presence of other components in the mobile phase. So, the mobile phase having different components will have different thermal conductivity.

So, when the mobile phase flows around a sensing temperature sensing element, the temperature of this element will depend on the components of the mobile phase. Because the components in the mobile phase will determine the thermal conductivity of the mobile phase. And depending on the thermal conductivity, the temperature of the sensor will be determined. Look at this diagram working on the wood stone bridge principle. One resistor is in contact to the mobile phase leaving the column. So, this is the resistor which is in contact with the mobile phase that comes out of the column. So, the more the mobile phase comes out of the column that passes through the detector. So, this is the

resistor which is in contact with the mobile phase that leaves the column. And the another is in contact with the reference stream of pure mobile phase. So, this is a pure mobile phase.

As the solute emerge from the column, there will be change in thermal conductivity. Change in thermal conductivity because the pure mobile phase does not does not have any components or from the mixture, but the mobile phase that comes up the out of the column has components of the mixtures that I am separating in it. So, the thermal conductivity of these 2 phases are different thermal conductivity of the pure mobile phase and the thermal conductivity of the column element which is basically mobile phase and the components of the mixture are different. So, as solute emerge from column change in thermal conductivity takes place. So, if there is change in thermal conductivity change in amount of heat removed from the resistor. So, there will be change in registers temperature and resistance. Thus, there will be change in voltage difference between points plus and minus as shown here.

So, when you have pure mobile phase here and if I have pure mobile phase here , both this mobile phases will have the same thermal conductivity. So, they will be able to remove the same amount of heat from the resistor. So, the temperature of the resistor and the resistance will be same. There will not be any voltage difference or the unbalanced current in the circuit. Now I have pure mobile phase here, and I have the column affluent column element here; that means, the mobile phase that comes out from the column and along with it carries components at different times. Now since the components in the mobile phase will change the thermal conductivity of it, there will be change in the thermal so, there will be change in the amount of heat removed from this resistor.

So, there will be change in resistors temperature and resistance, thus there will be a change in voltage difference between these 2 points, or there will be an unbalanced current in this circuit. So, thermal conductivity detector thereby will produce an electrical signal corresponds to the components present in the mobile phase. So, the output of the thermal conductivity detector will be an electrical signal corresponding to the amount of components present in the mixture. Because the different amount of the components and the different components will change the thermal conductivity of the mobile phase differently, thereby, there will be different change in temperature and

resistance which will be reflected in the different voltage difference or different unbalanced current in the circuit.

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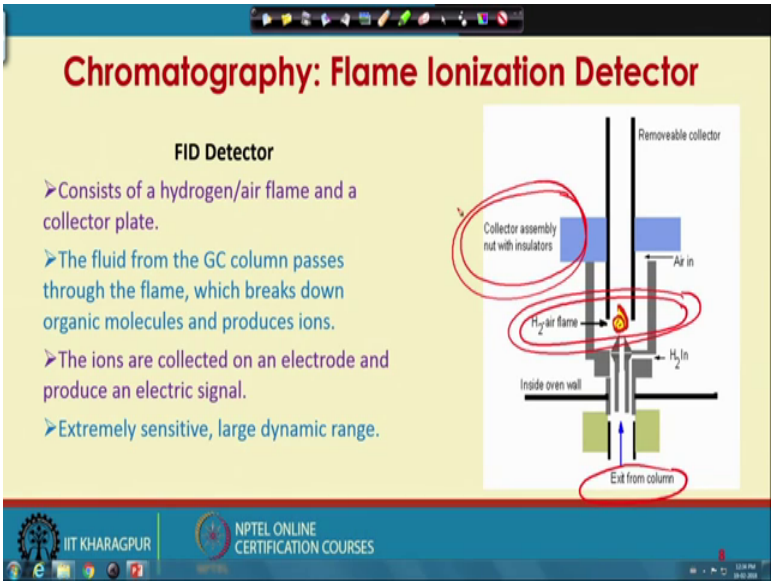

Next let us talk about the other popular detector which is known as flame ionization detector. Flame ionization detector consists of a hydrogen air flame and a collector plate. The fluid from the gas chromatography column passes through the flame, which breaks down organic molecules and produces ions. The ions are collected on an electrode and produces an electric signal. So, this electric signal is a measure of the components in the mixture. It is extremely sensitive and has large dynamic range. So, the flame ionization detector consists of hydrogen air flame and a collector plane.

So, this is hydrogen air flame and this is the collector assembly to collect the produced ions. So, here the fluid from the gas chromatography column enters the flame ionization detector, this is burned here using hydrogen air flame, ions are produced and these ions are collected on this ion collector. So, thereby an electrical signal corresponding to the components in the mixtures is produced. Flame ionization detector is extremely sensitive and has large dynamic range.

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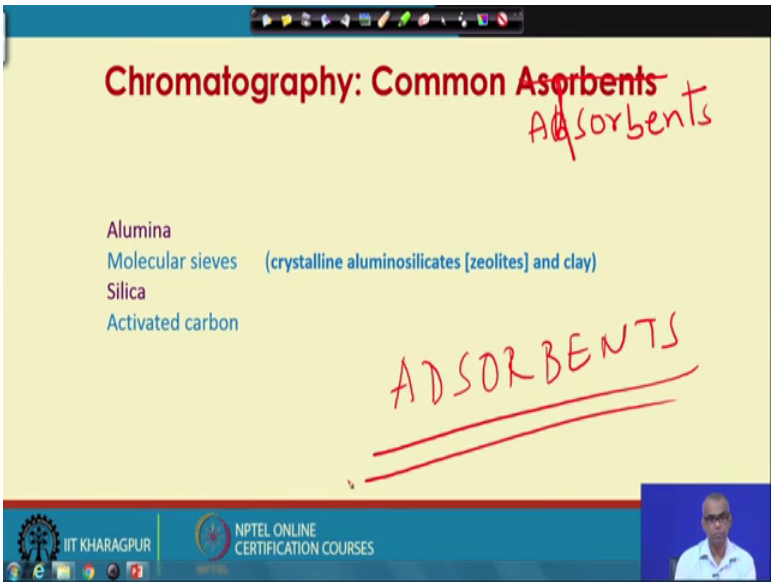

Here some common absorbance, this is absorbance, some common absorbance are or adsorbents, alumina, molecular sieves silica activated carbon. So, alumina molecular sieves this crystalline alumina silicates realized sand clay silica and activated carbons are commonly used adsorbents in the column.

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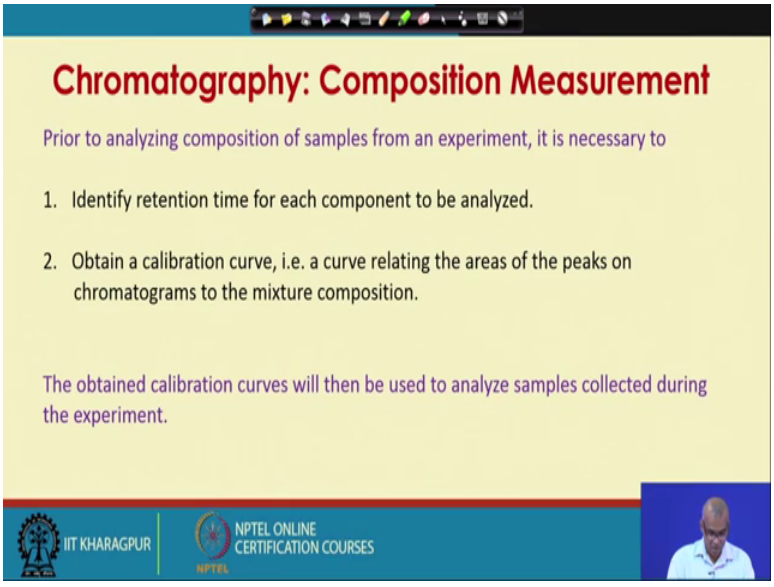

Now, how exactly we measure the composition? Prior to analysing composition of samples from an experiment it is necessary to identify retention time for each component to be analysed and obtain a calibration curve. That is a curve relating the areas of the pigs

on the chromatograms to the mixture composition. So, to import 2 things are important to information import to important information must be known to us. First is we need to identify the retention time for each component to be analysed , and then we need to obtain a calibration curve that is a curve relating the areas of the peaks on chromatograms to the mixture composition. The obtained calibration curves will then be used to analyse samples collected during the experiment.

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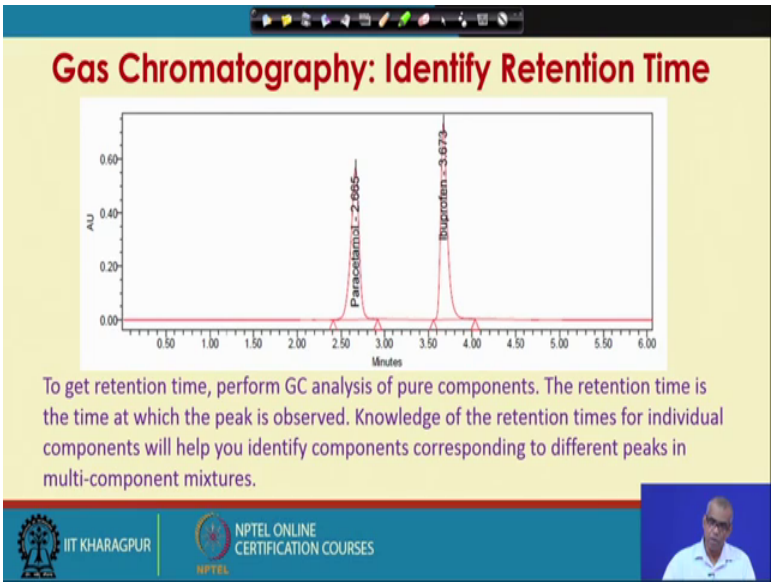

For example, this is the chromatograph we have seen again for paracetamol ibuprofen mixture. So, to get the retention time, we have to perform gas chromatography analysis of pure components. The retention time is the time at which the peak is observed, knowledge of the retention times for individual components will help you identify components corresponding to different peaks in multi component mixtures. So, to know the retention time for paracetamol and ibuprofen; what I will do is, I will first perform experiments with pure paracetamol and pure ibuprofen. So, I will take pure paracetamol and inject it with the carrier with the carrier gas it will pass through the column it will be detected by the detector, and it will produce the chromatogram.

So, the chromatogram will tell me the retention time for paracetamol. Similarly, I will repeat the experiment for pure ibuprofen. So, I will take pure ibuprofen mixture injected with the carrier gas, it will be passed through the column detected by the detector, and then detector produces the chromatogram which tells me the retention time for the

ibuprofen. So, I know now the retention time for ibuprofen retention time of paracetamol. So now, when I have a mixture of paracetamol and ibuprofen, and I want to know how much of paracetamol and how much of ibuprofen is there, the experiment I do now is I take this mixture send it through the chromatography column along with the carrier, the detector produces the chromatogram. Now the chromatogram will have 2 peaks. Now since I know the individual attention times, I can find out which peak corresponds to paracetamol which peak corresponds to ibuprofen.

So, this is what is shown in the chromatograph on the screen. You have a paracetamol peak, you have a ibuprofen peak. Note that they are retention times are different. So, you will first carry out experiments with pure components to have an idea of the retention time of individual components. So, this information will help you in identifying the components in multi component mixtures.

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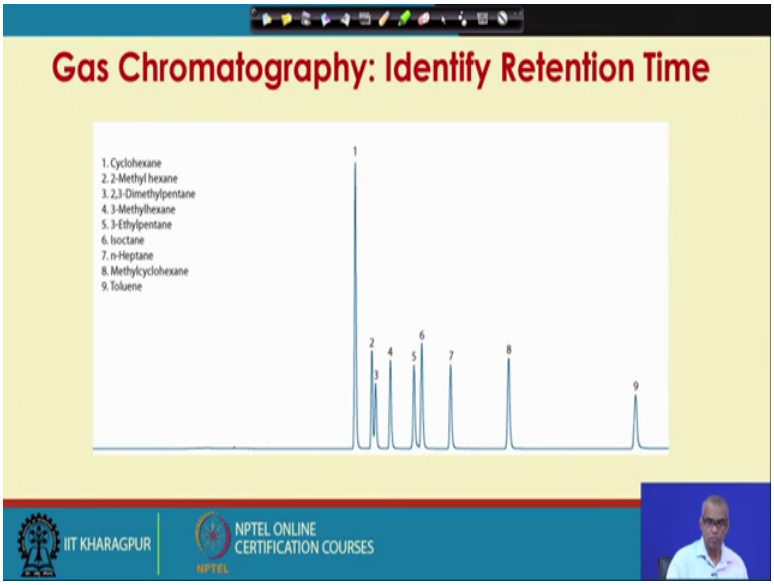

For example, here you have several components. So, you must first perform experiments, with the pure components to know about their retention times. So, then you know that one is pro cyclohexane 2 is for 2 methyl hexane so on and so forth.

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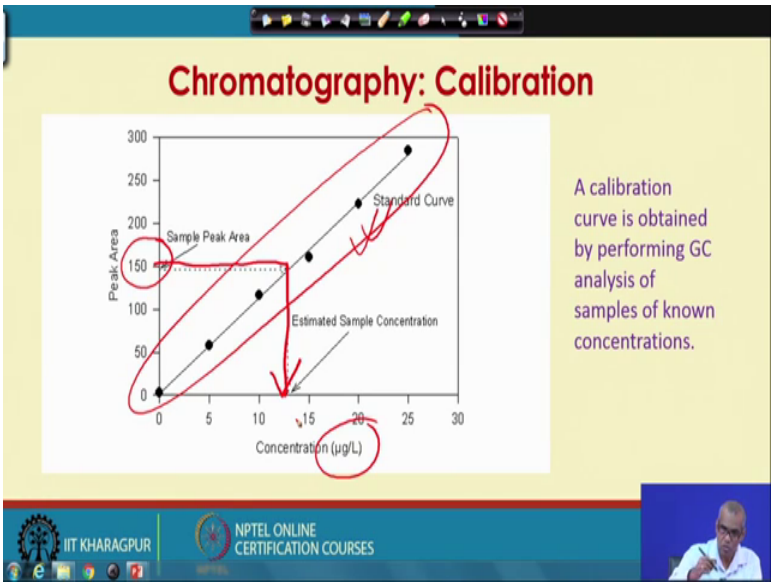

Next step is to obtain a calibration curve. To obtain the calibration curve you have to perform gas chromatographic analysis of samples of known concentration.

So, first thing is to know the retention type, second thing is to perform experiments or calibration experiments of samples with known concentration. So, let us say I take 5, 6 samples. Each of these samples concentration is known. So, you prepare say ibuprofen and paracetamol mixture of known concentrations, 5 such samples I prepare. So, in these 5 different samples of paracetamol and ibuprofen mixtures, the compositions are different. Now I perform gas chromatographic analysis for these 5 samples. So, a 5 chromatograms, retention times I know, because I have performed previously the experiments on pure ibuprofen and pure paracetamol.

So, I now relate the area under the individual curve with the amount of the component. Suppose the first experiment you do you get first experiment with a known concentration, or known mole fraction of paracetamol and ibuprofen, you have the chromatograph. So, from this coma program? You know, which area corresponds to paracetamol which area corresponds to ibuprofen from the knowledge of the retention time. So, you know that this much of area for paracetamol corresponds to this much of mole fraction. And this much of area for ibuprofen corresponds to this much of mole fraction of ibuprofen. So, you have these informations. Once you have these informations, you can draw a graph.

So, peak area, area under the curve versus concentration you can draw. So, on the figure on the screen you see that I have plotted the area under the curve versus concentration. This has been done for a particular component. So, for a particular component, the area under the curve or the peak area and the concentration plot is the calibration curve. So, once you have this calibration curve, so, this is the calibration curve. So, this calibration curve has been obtained by performing experiments with known concentrations. If you are mixing a single component if you are a mixture of multi component, you will do the same strategy. When you have mixtures, you make sure that there is no overlapping between these area under the curve.

So, you choose the flow rate of the mobile phase suitably. So, that the components are completely separated. So, they do not appear in the chromatograph as overlapped areas. So, that is important. In that case so, when the components are completely separated they will come as there you can identify the individual areas. So, these individual areas are related to the concentration. So, if we are finding out the concentration of only one component you will have one area under the curve. So, because you have one peak so, no problem. So, known concentrations, samples if prepared say 5 6 samples we prepare and draw a curve where you plot area under the curve versus concentration.

Now, you perform gas chromatography analysis, for the sample whose concentration is not known. So, get the chromatograph, determine the area under the curve and look at the figure find the corresponding concentration. For multi component mixture you can follow the same strategy. But make sure that you have chosen the operating conditions such as proper mobile phase and the flow rate of the mobile phase are suitable such that the components are completely separated so that you get individual peaks. Those are not overlapped with each other. So, areas under the peak should not be overlapping with each other. In that case, you know which p corresponds to which component get their area and do the same analysis, area under the curve versus concentration you plot that will give you a calibration curve, and the unknown samples concentration can be found out by doing the gas chromatography analysis, get the chromatogram get the area under the curve, now look at the curve, say our calibration curve to find out the concentration.

So, if you look at the screen so, this is the calibration curve or standard curve. Now, I perform the gas chromatographic analysis of an unknown sample, and I see let us say the area under the curve or the peak area is 150, 150 unit. So, I cancel the calibration curve

from 150, I find out the concentration is around say 12.5 or 13-unit microgram per litre. So, this is how you will set up a calibration for measurement of composition of concentration. The liquid chromatography let us discuss now..

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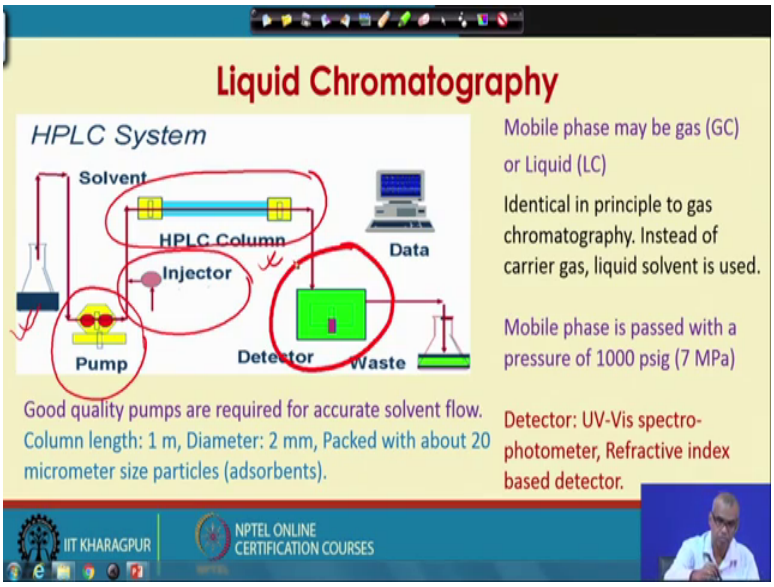

Liquid chromatography is identical in principle to gas chromatography, instead of carrier gas, liquid solvent is used. Please recall that we discussed the mobile phase in case of chromatography may be gas or as well as liquid. It is always a fluid, when the mobile phase is gas we call gas chromatography, when the mobile phase is liquid we call it liquid chromatography.

So, the liquid chromatography is identical in principle to gas chromatography instead of carrier gas we use liquid solvent as mobile phase. So, the mobile phase is passed with a pressure of 1000 psi g, which is 7 mega pascals almost. So, if you look at liquid chromatography or this high-pressure liquid chromatography HPLC as it is known. You have the column as you have seen in case of gas chromatography, you have the mobile phase which is solvent. You now need a pump to send it through the column. You have sample injection system. So, the solvent or the mobile phase is being pumped through the column at a high pressure which is 1000 psi g.

Now, you also have in between a sample injection system. So, sample injecter is there, where you inject the liquid sample to the mobile phase, and the mobile phase carries this injected sample through the HPLC column. So, it is similar to gas chromatography.

When the mobile phase comes out from the other end of the chromatographic column, it passes through the detector. The detector produces the chromatograph which is again a plot of detector response versus time. So, a liquid, liquid chromatography and gas chromatography works in identical principle, the only difference is in case of gas chromatography you had a gas carrier as mobile phase, here I have liquid solvent as a mobile phase. Here the mobile phase is passed with a pressure of 1000 psi g which is about 7 mega pascal.

The detector that are used in case of HPLC or high-pressure liquid chromatography systems are UV spectrophotometer or refractive index-based detector. So, 2 common detectors are UV-Vis or UV visible spectrophotometer-based detector or refractive index-based detector. The good quality pumps are used with liquid chromatography, because the solvents needs to be forced through the liquid force to the HPLC column with accurate flow rate at high pressures. So, very good quality pumps are required for liquid chromatography, because the mobile phase which is solvent has to be passed through the column with a very accurate flow rate at high pressures.

The typical column length is one meter, diameter is 2 millimetre, and packed with about 20 micrometre sized particles, which are adsorbents.

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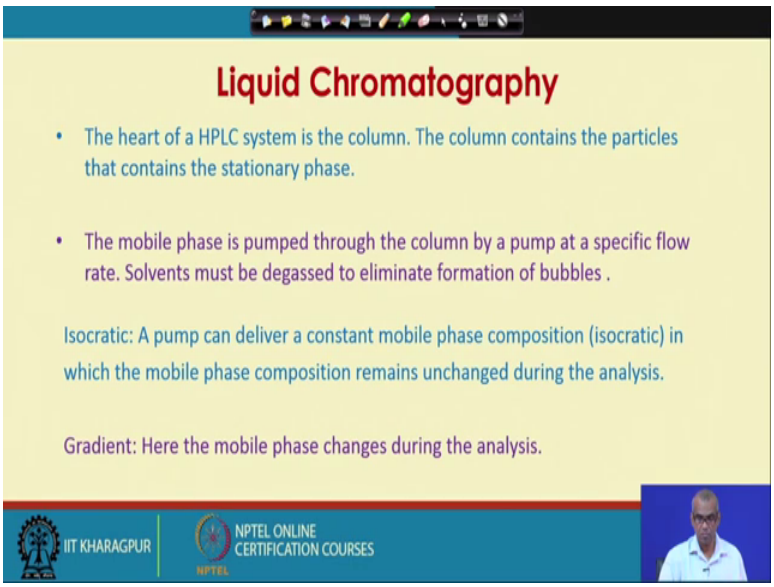

The heart of HPLC system is the column the column contains the particles that contains the stationary phase. The mobile phase is pumped through the column by a pump at a

specific flow rate solvents must be degassed to eliminate formation of bubbles. A pump can deliver a constant mobile phase composition in which the mobile phase composition remains unchanged during the analysis. This is known as isocratic condition. In case of gradient condition, the mobile phase changes during the analysis. So, in case of isocratic condition, the mobile phase composition remains unchanged during the analysis in case of gradient condition, the mobile phase changes during the analysis.

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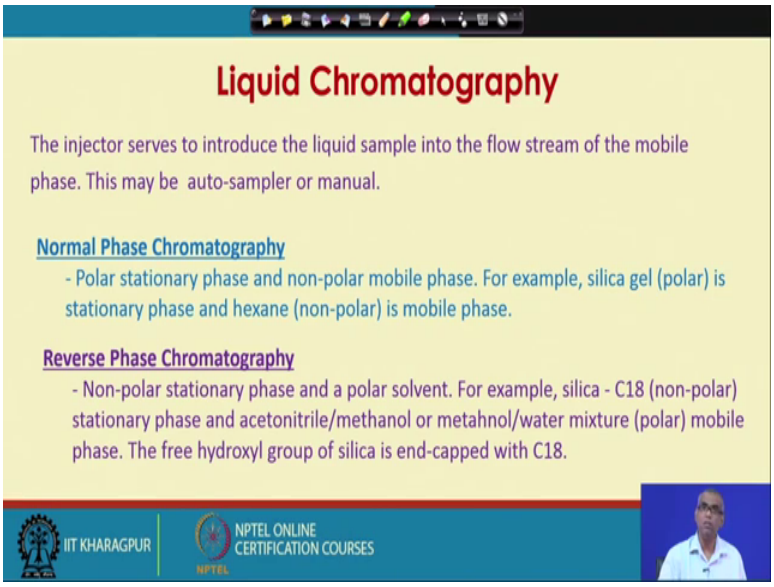

The injector serves to introduce this liquid sample into the flow system of the mobile phase, this may be auto sampler or manual. There are 2 important terminologies one is normal phase chromatography, other is reverse phase chromatography. In case of normal phase chromatography, polar stationary phase and nonpolar mobile phase are used. So, normal phase chromatography means, you have a polar stationary phase and nonpolar mobile phase.

For example, silica gel which is polar is stationary phase and hexane which is nonpolar is mobile phase. In case of reverse phase chromatography, it is just opposite. Non-polar stationary phase and polar solvents are used. In case of reverse phase chromatography non-polar stationary phase and polar solvent are used. For example, silica c 18 silica carbonate tin which is non-polar is used stationary phase. And acetonitrile methanol or methanol water mixture which a polar are used as mobile phase. The free hydroxyl group

of silica is intact with c 18, and thus now you get silica c 18 as non-polar stationary phase.

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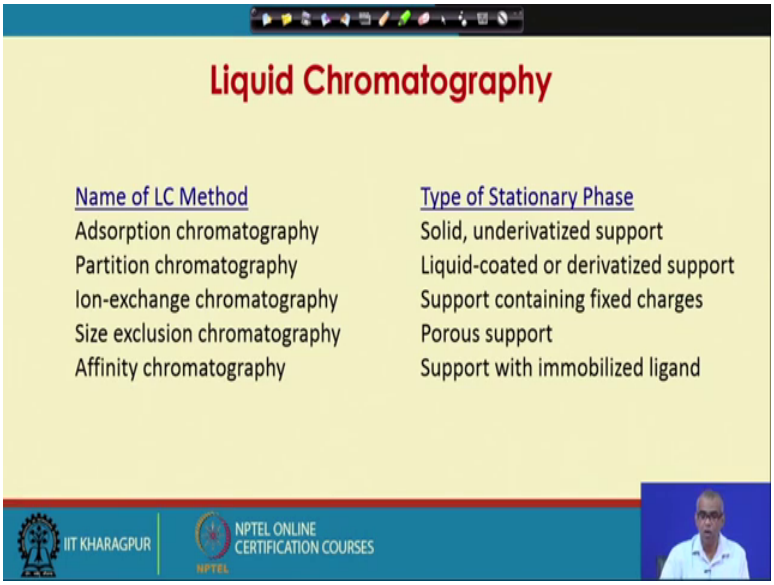

Again, depending on various stationary phases. We have different names for liquid chromatography method. Adsorption chromatography, the stationary phase is solid, underivatized support. Partition liquid chromatography, the stationary phase is liquid coated or derivatized support. Ion exchange chromatography, the stationary phase is support containing fixed charges. Size exclusion chromatography stationary phases porous support, affinity chromatography stationary phases support with immobilized ligand.

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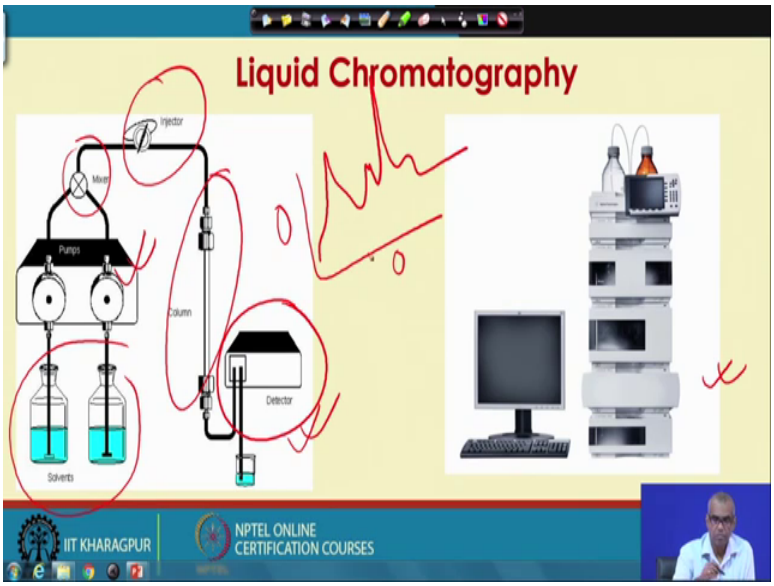

So, this is a schematic of the liquid chromatography, and this is a real photograph. So, you have the solvents, you have the palms. So, they are mixed, you have the sample injection system, you have the column through which the mobile phase is passed at high pressure, and then from the other end of the column the mobile phase or enters the detector which produces a chromatograph, which is detector signal versus time plot.

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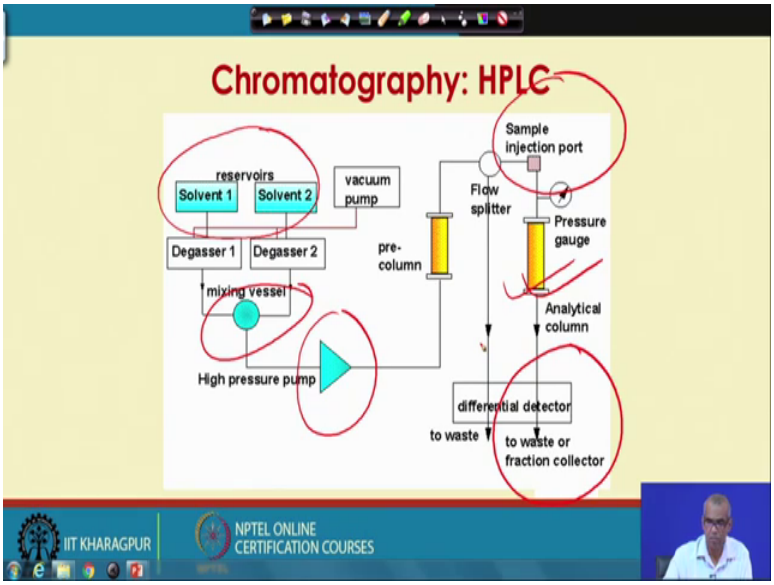

Again, another schematic, you have the reservoirs for the solvents. It is passed to degassers solvents must be degassed, you mix them, you have the high-pressure pump,

because the mobile phase is first at high pressure. You have the sample injection port and this is the column, the output of the column goes through the detector.

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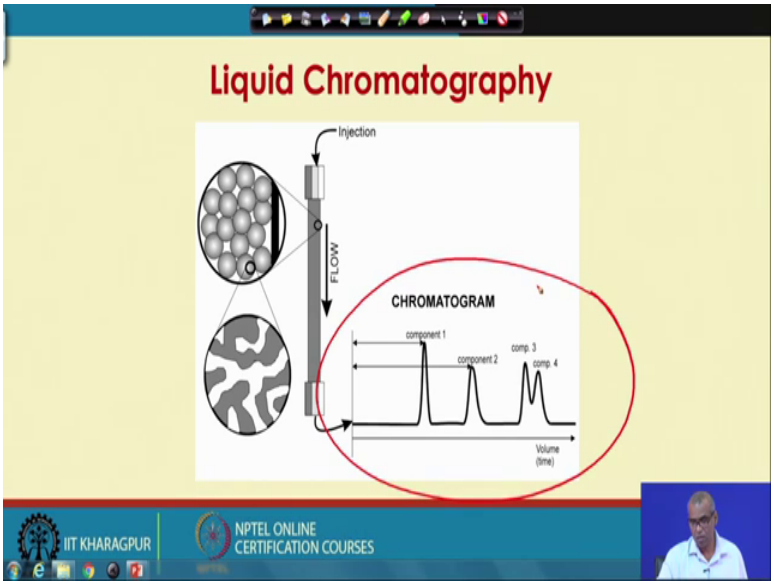

So, the detector produces chromatogram, exactly the same way as we have seen in case of gas chromatograph. The retention time for different components is different. And the mole fraction of the components; that means, the amount or the concentration of the component depends on the area under the curve. Or the area under the curve is a measure of the concentration or the amount of the components.

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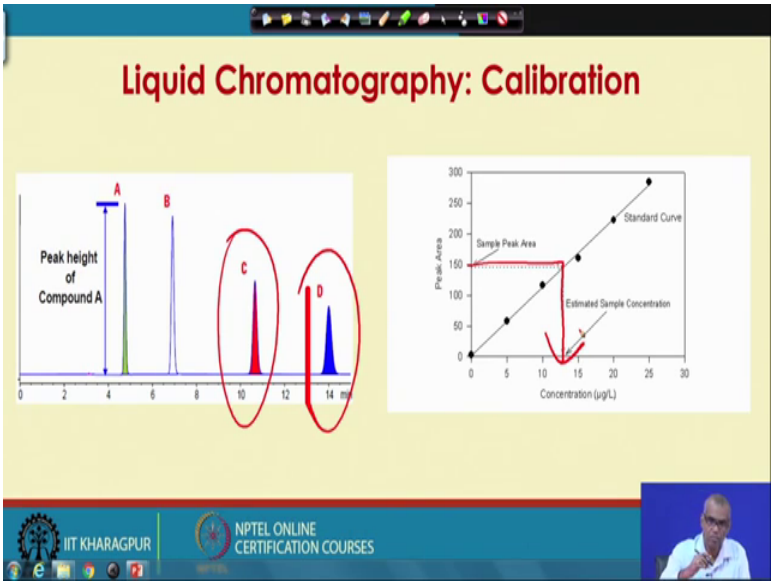

So, again the calibration can be set up by performing experiments with samples of known concentration, and the area under the curve can be related to the concentration. So, when you want to measure the concentration of an unknown sample, you take the chromatograph, find the area under the curve and then look at the calibration curve, and if you know the area you now know the concentration. So, the principles and the working of gas chromatography and liquid to gas chromatography is almost identical. If the principle is identical, the mobile phases are different, most of the components are also very much similar.

So, these 2 concentration measuring devices gas chromatography and liquid chromatography are extensively used for separation, quantification and analysis of liquid and gas samples, in laboratory, research laboratories, as well as in laboratories in industries or in case of quality management departments in industries.

So, we stop our discussion on chromatographic method here. In our next class, we will talk about concentration measurement by UV-Visspectrophotometer.